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LETTER TO THE EDITOR

Immune Dysregulation and Lymphoma Risk in Deficiency of Adenosine Deaminase 2

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Dear Editor,

Deficiency of adenosine deaminase 2 (DADA2) is a rare monogenic autoinflammatory disorder characterized by vasculitis, cytopenias, immunodeficiency, and early-onset stroke, with a wide spectrum of clinical manifestations [1]. A recent study on T and B cell repertoires in DADA2 supports the presence of hypogammaglobulinemia and cytopenia in both vasculitic and hematological phenotypes [2]. The authors' comprehensive analysis of the adaptive immune system in patients with DADA2 provides critical insights into the immunogenetic signatures associated with this rare monogenic autoinflammatory disease. An important point raised by Schultheiss et al. is the persistence of elevated Tumor Necrosis Factor (TNF), B-cell Activating Factor, and sCD40L levels despite anti-TNF therapy, which underscores the complexity of immune dysregulation in DADA2. Similarly, they observed that serum immunoglobulin (Ig) levels remained largely unchanged with treatment.

A few years ago, we conducted a study to evaluate the patients with DADA2 in our vasculitis center [3]. Similar to the findings of Schultheiss et al., we observed significant immunological and hematological findings, including hypogammaglobulinemia and cytopenias in patients with DADA2 [2, 3]. Seventeen patients with DADA2 met the Ankara 2008 classification criteria (EULAR/PRES/PRINTO), while fifteen patients fulfilled the American College of Rheumatology criteria for polyarteritis nodosa. Among these patients, seventeen were homozygous (G47R, R381S and R306X) and one was heterozygous (G47R) for *ADA2* mutations. *ADA2* enzyme activity was assessed in seven patients and was consistently low, with a median value of 0.55 U/L.

All patients in our cohort presented with a vasculitic and inflammatory phenotype. Furthermore, two of them exhibited a hematologic phenotype in addition to the inflammatory phenotype and presented with transfusion-associated autoimmune hemolytic anemia (AIHA) prior to diagnosis. The median age at diagnosis was 5.5 (3.5–8) years. Anemia was the most common hematological manifestation ($n = 13$, 72.2%), followed by lymphopenia ($n = 11$, 61.1%). Notably, hypogammaglobulinemia was observed in 55.5% ($n = 10$) of patients, all of whom had selective IgM deficiency (See in Table S1). Seventeen (94.4%) patients were in complete remission under etanercept and only one (5.5%) patient was in remission under adalimumab. The median follow-up was 8 (3–10) years. Long term follow-up demonstrated that none of our patients developed any type of lymphoma.

Genotypic variation in DADA2 correlates with variable levels of residual *ADA2* enzyme activity, which tends to be markedly reduced or even absent in patients with the hematological phenotype, such as those presenting with Diamond-Blackfan anemia or bone marrow failure, compared to those with the vasculitic phenotype [4].

The near-absence of ADA2 enzyme activity appears to contribute to the development of severe hematological presentations. Furthermore, persistently elevated levels of TNF-alpha even in the context of ongoing treatment may have a role in the pathogenesis of autoimmune manifestations and lymphoproliferative disorders. These findings raise the question of whether TNFi, traditionally considered less effective in the hematological phenotype, might still have a therapeutic role.

TABLE 1 | Deficiency of Adenosine Deaminase 2 patients with lymphoma in the literature.

Publication	Patient age (years)/ sex	Disease duration (years)	Intron-exon	*ADA2* mutation (zygosite)	*ADA2* enzyme activity	Phenotype	Clinical findings	Laboratory findings	Treatment	TNFi naïve before malignancy (yes or no)	Type of malignancy	Observation period	Outcome
Manhal et al. [5]	35/male	13	Exon 9	Likely pathogenic homozygous c.1353G>T L451F	Not measured	Immunodeficiency, hematological	Maturity onset diabetes of the young type 2, mediastinal lymphadenopathy, otitis media, upper respiratory tract infection, rash, herpes virus infection, hepatitis B	Hypogammaglobulinemia, anemia, lymphopenia	IVIG, antivirals, oral hypoglycemic drugs	Yes	HL	10 years	Patient's prognosis remains uncertain due to concurrent serious conditions, including relapsed HL and DADA2; however, clinical symptoms and laboratory parameters have shown improvement with treatment
Gardner et al. [6]	38/female	10	Exon 9	Pathogenic homozygous variant c.1397_1403del K466Tfs*2	Minimal	Vasculitic, immunodeficiency, hematological	Thalassemia, depression, alopecia, small joint inflammatory arthropathy, sicca symptoms, Raynaud's phenomenon, hepatitis	Hypogammaglobulinemia, anemia thrombocytopenia, neutropenia	IVIG, corticosteroids	Yes	EBV positive diffuse large B-cell lymphoma	6 years	Remission after 6 cycles of chemoimmunotherapy
Alabbas et al. [7]	17/male	12	Exon 10	Likely pathogenic homozygous c.1447_1451del S483Pfs*	Absent	Immunodeficiency, hematological	Recurrent infections, growth retardation, hepatosplenomegaly	Hypogammaglobulinemia, anemia, thrombocytopenia, lymphopenia, neutropenia	IVIG, corticosteroids, anti-TNF, HL treatment, G-CSF, splenectomy	No	HL	7 years	Patient had corticosteroid-dependent pure red cell aplasia and anti- TNF–refractory cytopenia, which responded to a combination of splenectomy, cyclosporine therapy, and monthly IVIG
Alabbas et al. [7]	15/male	11	Exon 10	Likely pathogenic homozygous c.1447_1451del S483Pfs*	Absent	Immunodeficiency, hematological	Hepatosplenomegaly	Hypogammaglobulinemia, neutropenia	HL treatment	Yes	HL	6 years	Under follow up with G- CSF
Alabbas et al. [7]	12/male	9	Exon 10	Likely pathogenic homozygous c.1447_1451del S483Pfs*	Not measured	Autoinflammatory, immunodeficiency	Myalgia, oral infections, aphthous ulcer	Hypogammaglobulinemia, neutropenia	HL treatment	Yes	HL	2 years	Under follow up with G- CSF
Springer et al. [8]	43/male	8	Intron 6/Exon 9	Likely pathogenic mutation missense mutation (c.973-2A> G); and likely pathogenic mutation p.Val458Asp (c.1373T> A). V458D	Minimal	Vasculitic hematological	Bilateral sensorineural hearing loss, polyarthritis, heart failure, pulmonary hypertension (presumed secondary to chemotherapy), bloody stools, progressive fatigue myalgia, superior vena cava occlusion (presumed secondary to a port catheter) and esophageal varices	Slightly low CD8 count	HL treatment, adalimumab, etanercept	Yes TNFi started about 30 years after being diagnosed and treated for HL	HL	Not mentioned	Demonstrated marked improvement in inflammation with no progression of neurological disease
Bulte et al. [9]	28/female	5	Exon 9	p.Tyr456Cys/p. Tyr456Cys	Minimal	Hematological	HL	Severe neutropenia after chemotherapy	Methylprednisolone	Yes	HL	Not mentioned	Prolonged neutropenia after HL treatment requiring HSCT

Abbreviations: ADA, Adenosine Deaminase 2; EBV, Epstein- Barr Virus; G-CSF, Granulocyte-colony stimulating factor; HL, Hodgkin Lymphoma; HSCT, Hematopoietic stem cell transplantation; IVIG, Intravenous immunoglobulin; TNF, Tumor necrosis factor.

In our cohort, two patients presented with DADA2- associated severe AIHA, both requiring regular transfusions and long-term corticosteroid therapy for at least 2 years. Following initiation of TNFi therapy, both patients achieved complete remission, maintained through the third and eighth years of follow-up, respectively.

In the current literature, six cases of Hodgkin Lymphoma (HL) and one case of Epstein–Barr Virus (EBV) associated B- cell lymphoma have been reported in patients with DADA2 [5–9]. Notably, three of the six HL cases were under 18 years of age. Of the seven patients affected by lymphoproliferative malignancies, six had a hematological dominant phenotype, with two displaying overlapping vasculitic and hematological features, consistent with their respective genotypes. Importantly, six of the seven patients had not received TNFi therapy prior to the diagnosis of lymphoma. Hypogammaglobulinemia was identified in five of the seven cases (Table 1). While the presence of pathogenic *ADA2* mutations and markedly reduced *ADA2* enzymatic activity are consistent findings across these cases, the absence of prior TNFi exposure supports the hypothesis proposed by Schultheiss et al., which highlights lymphomagenesis in DADA2 may arise from an intrinsic disease mechanism rather than being driven by immunosuppressive therapy.

This finding demonstrates the importance of adding TNFi to medical therapy when stem cell transplantation is not considered, especially in patients with genotypically and phenotypically predominant hematologic features. However, randomized controlled studies would be necessary to confirm the role of TNF blockade in this context.

Another key observation, as highlighted by Schultheiss et al., is the co- occurrence of hypogammaglobulinemia with*ADA2* mutations associated with the vasculitic phenotype, suggesting a potential link between immune dysregulation and specific genotypic variants in DADA2. In our cohort, hypogammaglobulinemia was observed in patients presenting with vasculitic manifestations, most prominently characterized by a selective deficiency of IgM. We propose that isolated low serum IgM levels may serve as a useful diagnostic clue for DADA2 diagnosis with vasculitic phenotype.

Author Contributions

Concept – M.A., M.K.C.; Design – M.A., M.K.C.; Supervision – Y.B., S.O.; Materials – M.A., M.K.C.; Data Collection and/or Processing – M.A., M.K.C.; Analysis and/or Interpretation – M.A., M.K.C.; Literature Search – M.A; Writing – MA, M.K.C.; Critical Review – Y.B., S.O.

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The authors have nothing to report.

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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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Descriptive characteristics and laboratory values of DADA2 patients.

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GUIDELINE

The Asia-Pacific League of Associations for Rheumatology Consensus Recommendations on the Management of Systemic Juvenile Idiopathic Arthritis (Juvenile Still's Disease)

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ABSTRACT

Background: The unique characteristics of the Asia Pacific called for the development of pragmatic and viable guidelines for the management of juvenile idiopathic arthritis (JIA). These consensus recommendations represent the second part of the guideline intending to offer an updated and regionally relevant framework for the management of systemic JIA (sJIA)/juvenile Still's disease.

Methods: A multidisciplinary task force of 35 members from 14 countries, including pediatric and adult rheumatologists as well as patient representatives, was convened. The guideline development followed the GRADE, ADAPTE, and AGREE II frameworks. Relevant international guidelines were critically appraised, and a systematic literature review was performed to address 10 PICO questions. Draft statements were discussed and voted upon using a modified Delphi process, with consensus defined as $\geq 80\%$ agreement.

Results: Four overarching principles and eighteen statements, including five statements addressing macrophage activation syndrome (MAS) were formulated. The recommendations align with international guidelines, advocating early use of IL-1/IL-6 inhibitors, avoidance of glucocorticoid monotherapy in sJIA without MAS, promoting tapering of glucocorticoids once inactive disease is achieved, alongside class switching for biologic refractory cases and a treat-to-target approach akin to EULAR/PReS guideline. Additional features include recommending conventional synthetic DMARD when biologics are unavailable and listing the options, acknowledging regional health system heterogeneity in the Asia Pacific, mentioning tapering strategy, discouraging NSAIDs as initial monotherapy, highlighting non-biologic salvage options, and placing less emphasis on sJIA-associated lung diseases.

Conclusion: These recommendations provide direction for practitioners caring for sJIA patients in limited resource areas to avoid treatment delay, hence improve overall outcomes. A shared decision approach and treat-to-targets are emphasized.

For affiliations refer to page 16.

1 | Introduction

Juvenile idiopathic arthritis (JIA), one of the most common childhood- onset rheumatic diseases, was categorized into 7 subtypes according to the International League of Associations for Rheumatology (ILAR) classification criteria [1]. Systemic JIA (sJIA) represents a distinct subtype of JIA characterized by systemic inflammation, quotidian fever, evanescent rash, and potential internal organ involvement with arthritis manifesting either initially or later in the disease course [2]. Unlike other JIA subtypes, sJIA possesses significantly higher morbidity due to complications like macrophage activation syndrome (MAS), lung disease, growth impairment, and long- term disability. While overall mortality in JIA is rare, sJIA carries a disproportionately elevated risk of death, largely attributable to MAS and severe systemic disease activity [3]. Compared to the 10%–15% found in the West, the prevalence of sJIA is notably higher in various regions in the Asia Pacific, accounting for up to 30%–50% of all JIA cases [4–6]. Similarly, the reported prevalence of MAS is higher in this region (15%–20% and in some JIA cohorts, up to 50%) than in the West (10% or less) [2, 7, 8]. Over the past decade, there has been a notable improvement in the mortality rate associated with MAS, decreasing from 20%–30% to 8%–12%, primarily attributed to early recognition and the prompt implementation of biologics [9]. However, such improvement has not been observed in our region, where the mortality rate remains elevated at 15%–20% and in some areas, up to 33% [8, 10]. In addition to restricted availability of care and medication, particularly biologic therapies, delayed diagnosis and inadequate disease management contribute to unfavorable outcomes, as the majority of cases are managed by non- pediatric rheumatologists [11]. The international recommendations advocated for the prompt initiation of biologic therapy, such as IL- 1 inhibitors (IL- 1i) or IL-6 inhibitors (IL-6i), in the therapy of sJIA [12, 13]. Nevertheless, findings from our recent regional survey indicate that IL- 1i were unavailable in the majority of participating countries, with over half reporting no access to these agents [11]. Additionally, IL- 6i were not universally accessible across all surveyed nations [11]. A practical and regionally relevant treatment guideline is necessary to enhance sJIA outcomes in the Asia Pacific, accounting for unique regional characteristics such as healthcare access disparities, cultural diversity and health perceptions, medication adherence and significant variation in clinical practice, especially among the non- pediatric rheumatology healthcare providers.

In response to region- specific issues, the Asia-Pacific League of Associations for Rheumatology (APLAR) formulated evidence-based consensus recommendations aimed at revising and refining the guidelines published in the prior 5 years. These recommendations constitute the second segment of the three- part APLAR consensus guidelines for the management of JIA, focusing on sJIA with and without MAS. This document is designed to provide guidance for healthcare practitioners in the Asia-Pacific region who directly manage patients with JIA. The intended audience encompasses specialists, general practitioners, specialty nurses, and other allied health professionals, who will collectively be referred to as "practitioners". In addition, the recommendations are relevant to other stakeholders including patients, their caregivers, and health policy makers. It is crucial to understand that these consensus recommendations are not intended to prescribe a definitive course of care for any individual patient. Instead, therapeutic decisions must be informed by the unique clinical circumstances of each patient and involve shared decision- making between the treating practitioner and the patient/caregiver, ensuring that all choices are made with the patient's best interests in mind.

2 | Materials and Methods

This consensus recommendation development process followed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology (www.gradeworkinggroup.org), which involves the process of rating the quality of the best available evidence and developing health care recommendations following the approach proposed by the GRADE Working Group [14] and adhered to the Appraisal of Guidelines for Research and Evaluation II (AGREE II) criteria [15] for consensus recommendation quality and ADAPTE Collaboration methodology for existing guideline adaptation (www.g-i-n.net) [16].

2.1 | Task Force Working Group Organization

The APLAR Pediatric Rheumatology Special Interest Group (PR SIG) established the Task Force (TF) working groups in October 2023 (Appendix S1). There were five working groups: (1) *Steering Committee (SC)*, chair (TA), nominated by PR SIG convenor (SFR), formed TF SC, consisting of 10 expert pediatric rheumatologists and one adult rheumatologist from 10 countries (median years of experience 19.0 years [interquartile range (IQR) 16.0–25.0]). This committee identified and appraised existing guidelines, formulated and finalized clinically relevant Patient/Population, Intervention, Comparison, and Outcomes (PICO) questions, ranked outcomes, supervised and coordinated the entire development process (Figure 1) and drafted recommendation statements and manuscripts; (2) *Guideline Development Methodologist* (LD), advised and guided methodology/technical process, checked evidence summaries for correctness of interpretation of evidence, and provided technical assistance to other working groups; (3) *Evidence Review Panel (ERP)*, consisting of 13 pediatric rheumatologists, searched and identified relevant literature, critically appraised and rated the certainty of evidence; (4) *Consensus Panel (CP)*, consisting of 14 pediatric rheumatologists, three adult rheumatologists (median years of experience 16.5 years [IQR 11.3–18.8]), and two JIA patient and parent panel (PPP) representatives from 14 countries, who assisted in formulating and refining PICO questions, identified and prioritized the critical and important outcome measures prior to finalizing the questions, such that stakeholders' values and preferences could be incorporated, reviewed evidence summaries and voted on the recommendation statements for which the PPP representatives votes carried equal weight to those of other panel members.; (5) *Patient and Parent Panel (PPP)*, consisting of nine adult JIA patients and seven parents from six countries, provided input and opinion on benefits and adverse events of drugs, reviewed evidence summary and assisted in rating outcomes and provided input on patient values and preferences related to treatment options, outcomes and evidence. All task force members declared conflict of interest prior to all consensus statement development activity

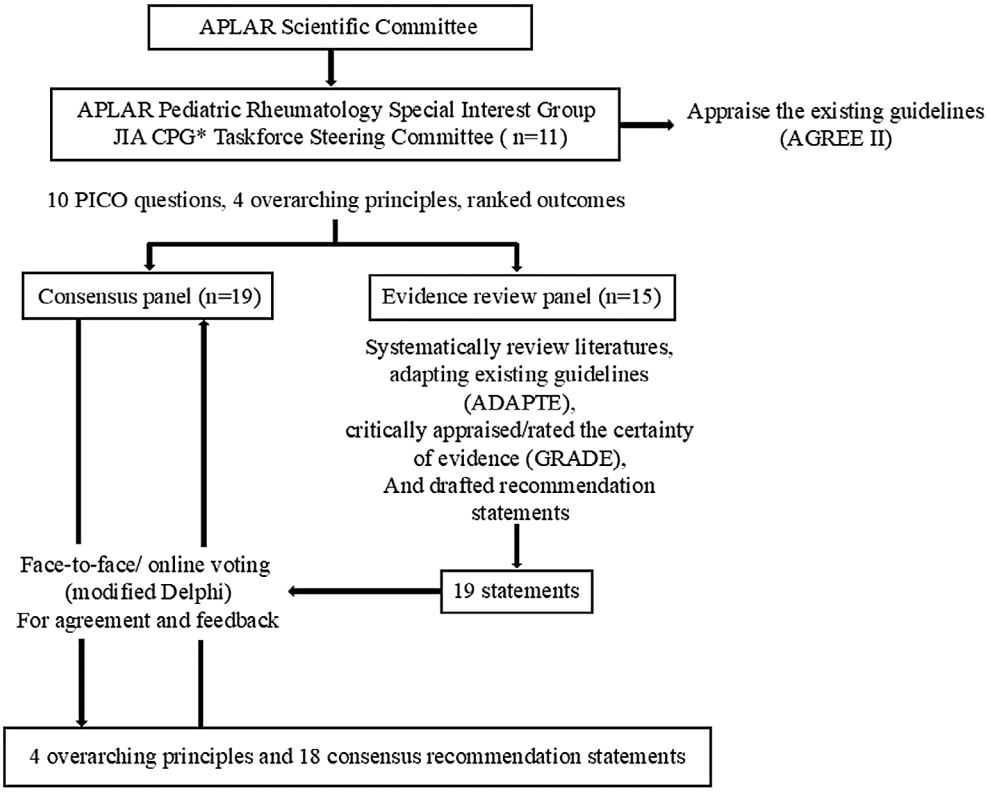

FIGURE 1 | Flowchart of consensus recommendation statement development. Briefly, The Steering committee (SC) defined the scope, drafted PICO questions and appraised the existing guidelines. The consensus panel (CP) and evidence review panel (ERP) reviewed and refined the PICO questions. The ERP reviewed and graded the certainty of evidence and drafted recommendation statements for CP members to vote on using modified Delphi process with a final of 18 consensus recommendations. APLAR, Asia Pacific League of Associations for Rheumatology.

and > 95% of members had no conflict of interest. Panel members with conflicts of interest shall be excluded from all related activities, including evidence appraisal, group discussions, voting sessions, and leadership roles concerning the medications in question.

2.2 | Existing Guidelines Adaptation

The ADAPTE framework was adopted by the SC with the intention to adapt international guidelines for the current consensus recommendations [16]. The Appraisal of Guidelines for REsearch & Evaluation (AGREE) II instrument (www.agreetrust.org) was used to appraise the quality of two international guidelines which were published in the past five years [12, 13]. Three SC members appraised each guideline. It was decided by SC to set the prioritized domain (domain 3: rigor of development) score of > 70% as the threshold for high- quality guidelines to be adapted for the current project. Both guidelines were selected for adaptation with modification [12, 13].

2.3 | Consensus Development

Figure 1 summarizes the consensus recommendation development process. In brief, SC drafted the overarching principles and the first version of the PICO questions (based on predefined clinical priorities, unmet regional needs and stakeholder surveys) and determined and ranked the outcomes prior to the CP and PPP review and then finalized. The scope of these consensus recommendations was decided to be limited to sJIA by EULAR/PReS recommendations' definition as follows [13]:

- Fever is typically spiking with temperature ≥ 39°C (102.2°F) for at least 7 days.
- Rash is transient and often coincides with fever spikes, preferentially involving the trunk. It is typically erythematous (salmon pink), but other rashes (e.g., urticaria) may be consistent with the diagnosis.
- Musculoskeletal involvement is usually present with arthralgia/myalgia. Overt arthritis is supportive but not necessary for diagnosis and may appear later.
- High levels of inflammation are typically identified by neutrophilic leucocytosis, increased serum C- reactive protein (CRP), and ferritin.

It includes patients with and without MAS, both overt and subclinical disease, and excludes patients from other ILAR categories and children with uveitis that may influence treatment decisions. The MAS is defined using Ravelli criteria as follows [17]:

A febrile patient with known or suspected sJIA *is classified* as having MAS if the following criteria are met:

- Ferritin > 684 ng/mL and any 2 of the following:
- Platelet count $\leq 181 \times 10^9$/L

- Aspartate aminotransferase > 48 Units/L
- Triglyceride > 156 mg/dL
- Fibrinogen ≤ 360 mg/dL

Moreover, persuaded by the EULAR/PReS recommendations that sJIA and adult- onset Still's disease are the same disease supported by compelling evidence, the TF shall henceforth utilize the term 'Still's disease' to refer to sJIA throughout the document [13]. Additional information regarding the PICO structure is detailed in Appendix S2.

A systematic literature review was performed by the ERP members, following Preferred Reporting Items for Systematic Reviews and Meta- Analyzes (PRISMA) guidelines and reg istered on PROSPERO (CRD420251055826) [18]. The literature searches for each PICO question were conducted across MEDLINE, Embase, Web of Science, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception through 1 April 2025, with updates on 1 September 2025 (Appendices S3 and S4; Figure S1). Because the current consensus recommendations were adapted from existing international guidelines approved by the SC using the AGREE II tool, the final evidence review excluded studies that had been incorporated into previous guidelines. The risks of bias and certainty of evidence (CoE) assessment were performed following the Cochrane Risk of Bias 2 (RoB2) tool and the GRADE approach, respectively [14, 19]. The ERP drafted recommendation statements (RS) considering the CoE from new evidence in addition to the strength of recommendation (SoR) from existing guidelines. The RS were subsequently refined and finalized by the SC. There are four OPs and a total of 18 statements including 13 for Still's disease without MAS and 5 for that with MAS.

A modified Delphi process was used to develop consensus through one face- to-face meeting followed by one virtual meeting. Consensus for each recommendation statement was determined through anonymous dichotomous voting ("agree" or "disagree"). Consensus was predefined as agreement by at least 80% of the CP members. If consensus was not achieved, anonymized feedback and reasons for disagreement from the preceding round were reviewed, followed by discussion to refine the statement. The revised statement was then subjected to a further round of anonymous voting. After the final recommendation statements were agreed upon, members of the SC and CP independently rated their level of agreement (LoA) with each overarching principle and recommendation statement on a scale from 0 (completely disagree) to 10 (completely agree). The mean and standard deviation (SD) of the LoA scores were calculated and reported.

Three categories of SoR were defined. A strong recommendation (using the terms "recommend" or "should") indicated that the TF was confident that the desirable effects of an intervention outweighed its undesirable effects, or vice versa. A weak recommendation (using the terms "suggest", "may", or "might") indicated that the desirable effects were likely to outweigh the undesirable effects, or vice versa; hence each patient's circumstances, values, and preferences should be considered carefully. A shared decision- making discussion between the patient/caregiver and the treating practitioner is crucial for interventions with a weak SoR. Where evidence was insufficient to support either a recommendation for or against an intervention, no SoR was assigned.

2.4 | External Review

These consensus recommendations were presented in public forums at the 28th Asia-Pacific League of Associations for Rheumatology Congress (APLAR 2026, Seoul, Republic of Korea), the Taiwan Academy of Pediatric Allergy, Asthma, Immunology & Rheumatology, and the 2nd Pediatric Rheumatology School in Taipei, Taiwan for external review; the first draft was also sent to two pediatric rheumatology experts (see Acknowledgement) for external review.

2.5 | Update

Acknowledging the fast- paced advancements in Still's disease research and management, the TF will undertake a revision of these consensus recommendations 5 years after their publication.

3 | Results

There are four overarching principles (OP) and thirteen statements for Still's disease without MAS and five statements for Still's disease with MAS. Nonetheless, the majority of statements constitute weak recommendations due to the low to very low certainty of supporting data, accounting for 94%.

3.1 | Overarching Principles (Table 1)

Early recognition and prompt initiation of therapy constitute the cornerstone of JIA management (OP1). This paradigm is particularly salient in Still's disease, where timely intervention has consistently demonstrated superior efficacy and improves long-term outcomes, most notably with biologic agents, such as IL- 1i and IL- 6i (window of opportunity) [20–22]. Accurately diagnosing Still's disease can be challenging, particularly in the absence of arthritis. Malignancies, infectious diseases, autoinflammatory and other immune- mediated inflammatory diseases must be carefully excluded, providing that the infectious diseases are more prevalent in the region and the use of high- dose glucocorticoids can mask other immune- mediated or inflammatory disease manifestations [23]. The diagnostic approach to Still's disease is not addressed in this recommendation; practitioners are advised to consult existing guidelines [13, 23].

Management of patients with Still's disease needs to be adapted to the individual, taking into account the different circumstances (cost, drug availability and insurance practices), values and preferences, health beliefs, and cultural and ethnic background of each patient. This approach increases adherence and therefore improves outcomes. Treatment target and treatment strategy should be discussed through shared decision- making between the patient, caregiver, and treatment team (OP2).

TABLE 1 | APLAR consensus statements on systemic JIA/Still's disease management.

Recommendation statements	SoR	CoE	%A	LoA
Overarching principles				
1. Prompt diagnosis and treatment initiation of Still's disease is essential to achieve better outcomes while preventing long-term damage.	NA	NA	100.0%	10.0 (0)
2. Treatment target and treatment plan are based on a share- decision between the patient/caregiver and the treatment team.	NA	NA	100.0%	9.9 (0.3)
3. A treat- to-target approach is essential, with regular assessment of disease activity and timely modification of therapy, aiming for drug- free remission, when possible, or clinically inactive disease with minimal therapy.	NA	NA	100.0%	10.0 (0)
4. Long- term systemic glucocorticoid therapy is discouraged due to its long-term adverse events.	NA	NA	100.0%	10.0 (0)
The following recommendation statements apply to children and young adults with treatment naïve, newly diagnosed active Still's disease without MAS				
1. We suggest against NSAIDs as initial monotherapy	2	D	93.8%	9.5 (0.7)
2. We suggest against systemic glucocorticoids as monotherapy. However, systemic glucocorticoids may be used as a bridging therapy.	2	D	100.0%	9.2 (0.9)
3. If possible, we suggest initiating treatment with IL- 6 inhibitors or IL-1 inhibitors. However, conventional synthetic DMARDs may be considered if IL- 6 inhibitors or IL-1 inhibitors are not available.	2	B	93.8%	9.6 (0.6)
4. When conventional synthetic DMARDs are being considered as initial therapy, methotrexate may be considered for patients with predominant articular disease, while cyclosporin may be considered for those with predominant systemic disease. In some cases, a combination of the two may also be considered. This strategy requires careful toxicity monitoring.	2	C (MTX) D (CsA)	100.0%	9.6 (0.8)
5. We recommend against using TNF inhibitors as initial therapy.	1	D	93.8%	9.7 (0.6)
The following recommendation statements apply to children and young adults with Still's disease without MAS who do not respond to initial therapy				
6. In patients initiated on an IL- 6 inhibitor or IL-1 inhibitor at their respective therapeutic dose (or after dose optimization), switching to either IL- 1 inhibitor or IL-6 inhibitor is recommended, if feasible.	1	C	100.0%	10.0 (0.2)
7. In patients initiated with a conventional synthetic DMARD at a therapeutic dose (or after dose optimization), if possible, switching to or adding either IL- 6 inhibitor or IL-1 inhibitor is recommended.	1	C	100.0%	9.9 (0.5)
8. In patients with no biologic DMARD access OR resistant to both IL- 1 inhibitors and IL-6 inhibitors, addition of or switching to any of the following medications may be considered. • Calcineurin inhibitors (cyclosporin A or tacrolimus), particularly in patients with systemic features • Methotrexate, particularly in patients with predominant articular manifestation • Thalidomide • Cyclophosphamide • JAK inhibitors All these strategies require great caution on toxicity monitoring.	2	D (CsA) C (MTX) D (THA, CTX, JAKi)	100.0%	9.6 (0.7)

(Continues)

TABLE 1 | (Continued)

Recommendation statements		SoR	CoE	%A	LoA
9.	There is insufficient evidence to recommend for or against IVIG, colchicine, or etoposide.	—	D (IVIG) N (Col, Eto)	100.0%	9.8 (0.5)
The following recommendation statements apply to children and young adults with Still's disease without MAS who are in clinically inactive disease					
10.	We suggest tapering off systemic glucocorticoids as soon as inactive disease is achieved, with the goal of discontinuation ideally within 6 months of glucocorticoid initiation.	2	C	100.0%	10.0 (0)
11.	We suggest maintaining a sustained inactive disease for at least 6 months after systemic glucocorticoid discontinuation, prior to tapering DMARDs, regardless of DMARD classes initiated.	2	D	87.5%	9.2 (1.1)
12.	We suggest extending the dosing interval of biologic DMARDs rather than decreasing the dosage.	2	D	100.0%	9.5 (0.9)
13.	In patients with the combination of conventional synthetic DMARD and biologic DMARD, tapering either of the medications first may be considered. There is no preferred order of tapering.	2	D	100.0%	9.4 (0.8)
The following recommendation statements apply to children and young adults with Macrophage Activation Syndrome associated with active Still's disease					
14.	If there is no contraindication, we recommend pulse methylprednisolone as initial therapy as soon as possible.	1	B	100.0%	10.0 (0.2)
15.	In patients with inadequate response to pulse methylprednisolone within the first 48–72 h, we suggest the addition of cyclosporin A or anakinra or IFN- γ inhibitors (i.e., emapalumab). IL-6 inhibitors may be considered with caution.	2	C (CsA, ANK, IFN-γi) D (TCZ)	100.0%	9.6 (0.7)
16.	Plasma exchange or etoposide might be considered in patients who are refractory to biologic DMARDs and cyclosporin A.	2	D (PLEX) C (Eto)	93.8%	9.2 (0.9)
17.	JAK inhibitors might be considered in patients who are refractory to biologic DMARDs and conventional synthetic DMARDs.	2	D (BAR, RUX, TOF)	100.0%	9.4 (0.9)
18.	In order to achieve the ultimate goal (drug- free remission), the following intermediate targets are suggested: • At day 7, resolution of fever and reduction of CRP by > 50%, • At week 4, no fever, reduction of active (or swollen) joint count by > 50%, normal CRP and physician and patient/parent global assessment less than 20 on a 0–100 VAS, • At month 3, CID with glucocorticoids less than 0.2 mg/kg/day, • At month 6, CID without glucocorticoids.	2	D	100.0%	9.6 (0.7)

Note: SoR (Strength of Recommendation): 0 = absence of SoR, 1 = Strong Recommendation, 2 = Weak Recommendation; CoE (Certainty of Evidence): A = High, B = Moderate, C = Low and D = Very low; %A (Agreement): Percentage of consensus panel member agreeing with the recommendation statement.

Abbreviations: ANK, anakinra; BAR, baricitinib; Col, colchicine; CsA, cyclosporin A; DMARD, disease modifying anti- rheumatic drug; Eto, etoposide; IFN-γi, interferon γ inhibitor; IVIG, intravenous immunoglobulin; LoA, Level of Agreement (0 totally disagree –10 totally agree; mean [SD, standard deviation]); MAS, macrophage activation syndrome; N, no evidence; PLEX, plasma exchange; RTX, rituximab; RUX, ruxolitinib; TCZ, tocilizumab; TNFi, tumor necrosis factor inhibitor; TOF, Tofacitinib.

Treat- to-target refers to a target-oriented strategy for the management of chronic diseases, as opposed to the traditional symptom- focused methodology. This involves frequent treatment adjustment, guided by quantitative indices and has been shown to improve outcomes in adult rheumatoid arthritis irrespective of the therapeutic agent used [24–26]. A similar effect was seen in a recent German JIA multicenter cohort study (BiKER cohort) [27]. The targets are divided into short- , medium- and long- term targets (see below) due to the urgent need to control the inflammation in order to avoid life- threatening complications, including MAS and the window of opportunity for the treatment of Still's disease (OP3) [20–22].

While the sequelae of glucocorticoid administration are well-characterized, certain pediatric complications—including cataracts, dermal striae, and avascular necrosis—represent significant, permanent morbidities. In resource- limited regions such as the Asia Pacific, access to biologic disease modifying anti- rheumatic drugs (bDMARDs)—particularly IL-6i, and even more so, IL- 1i—is constrained, whether due to prohibitive costs or outright unavailability [11]. Consequently, glucocorticoids remain indispensable for achieving rapid control of systemic inflammation in Still's disease. Moreover, in MAS complicating Still's disease, high- dose pulse methylprednisolone continues to represent the first- line therapeutic choice in our region. Nonetheless, the TF advocates systemic glucocorticoids be administered at the lowest effective dose for the shortest possible duration (OP4).

3.2 | Consensus Statements on Still's Disease Management (Table 1)

3.2.1 | Induction Therapy

Treatment of naïve, newly diagnosed active Still's disease without MAS (Figure 2)

1. *We suggest against NSAIDs as initial monotherapy (PICO 1)*

Non- steroidal anti-inflammatory drugs (NSAIDs) are weakly recommended as initial monotherapy in the American College of Rheumatology (ACR) recommendations [12]. However, they are mentioned in the newer European Alliance of Associations for Rheumatology (EULAR)/Pediatric Rheumatology European Society (PReS) recommendations to use for symptomatic treatment of fever and pain during the diagnostic workup period [13]. Additional observational studies, including one from the region, demonstrated that the majority of patients initiated on NSAIDs monotherapy required additional glucocorticoids or other disease modifying anti- rheumatic drugs (DMARDs) [28, 29]. Given the limited availability of healthcare facilities, the scarcity of pediatric rheumatologists, and the low disease awareness across many areas in the region, the TF advises against the use of NSAIDs as first- line monotherapy in order to avoid further delays in initiating appropriate treatment. This RS is a weak recommendation based on very low CoE.

2. *We suggest against systemic glucocorticoids as monotherapy. However, systemic glucocorticoids may be used as a bridging therapy. (PICO 1)*

According to the ACR recommendations, oral glucocorticoids are weakly recommended against as initial monotherapy [12]. They are instead primarily indicated as interim therapy while awaiting access to bDMARDs or as bridging treatment until conventional synthetic DMARDs (csDMARDs) achieve therapeutic effect, particularly within the first 3 months. Glucocorticoids as bridging therapy were defined as a short course of oral form ($\leq$ 2 mg/kg/day with a maximum of 60 mg/day). The current recommendations are consistent with those of the ACR. However, the SoR is weak, reflecting the very low CoE. Observational studies have indicated that early initiation of glucocorticoids may accelerate remission, though they do not alter the overall disease trajectory [30–33]. Nonetheless, the TF recognizes that in circumstances where access to biologics is not feasible, the combination of glucocorticoids with csDMARDs is necessary to achieve control of systemic and/or articular disease. As outlined in OP4, glucocorticoids should be administered at the lowest effective dose for the shortest required duration to minimize long- term adverse complications.

3. *If possible, we suggest initiating treatment with IL- 6 inhibitors or IL- 1 inhibitors. However, conventional synthetic DMARDs may be considered if IL- 6 inhibitors or IL-1 inhibitors are not available. (PICO 2, 3)*

Both Western guidelines recommend initiating bDMARDs targeting IL- 1 or IL-6 as monotherapy and emphasize that treatment should commence as soon as possible [12, 13]. In the absence of controlled studies, no preferred agent was endorsed. Recent meta- analyzes demonstrate that both IL-1 and IL-6 inhibitors are efficacious, significantly improving ACR50 response rates at week 4 compared to baseline. Pooled analysis of four randomized controlled trials (RCTs) evaluating three IL- 1i (rilonacept, anakinra, canakinumab) demonstrated a significant association with treatment response (OR = 6.92, 95% CI 2.24–21.36) [34]. Similarly, tocilizumab treatment was associated with an increased likelihood of response compared with placebo (OR = 8.08, 95% CI: 1.89–34.57) [34]. Real-world longitudinal studies also demonstrated that early initiation of IL- 1i or IL-6i was associated with high rates of clinically inactive disease [34]. In addition, five recent observation studies, comparing first- line or early bDMARDs with those who did not, reported that early initiation of biologics, either IL- 1i or IL-6i, was linked to higher rates of event- free remission and successful glucocorticoid withdrawal compared with csDMARDs [35–39]. Recent evidence supports the efficacy and safety of biologic therapy in Still's disease. Tocilizumab (TCZ), evaluated in two open- label and eight observational studies, demonstrated high efficacy with minimal serious adverse events [40–49]. Anakinra (ANK), across one RCT and two observational studies, showed consistently high response rate [50–52]. Similarly, canakinumab (CAM), assessed in two open- label and five real-world observational studies, reinforces its role not only in refractory but also earlier disease, with substantial proportions achieving early response. Efficacy was sustained during long- term follow-up without significant adverse events [53–60]. Furthermore, a recent network meta-analysis of RCTs on the efficacy and safety of bDMARDs in Still's disease. Nine trials with 430 patients were included. All bDMARDs were associated with greater odds of ACR50 response compared with placebo. There was no statistically significant

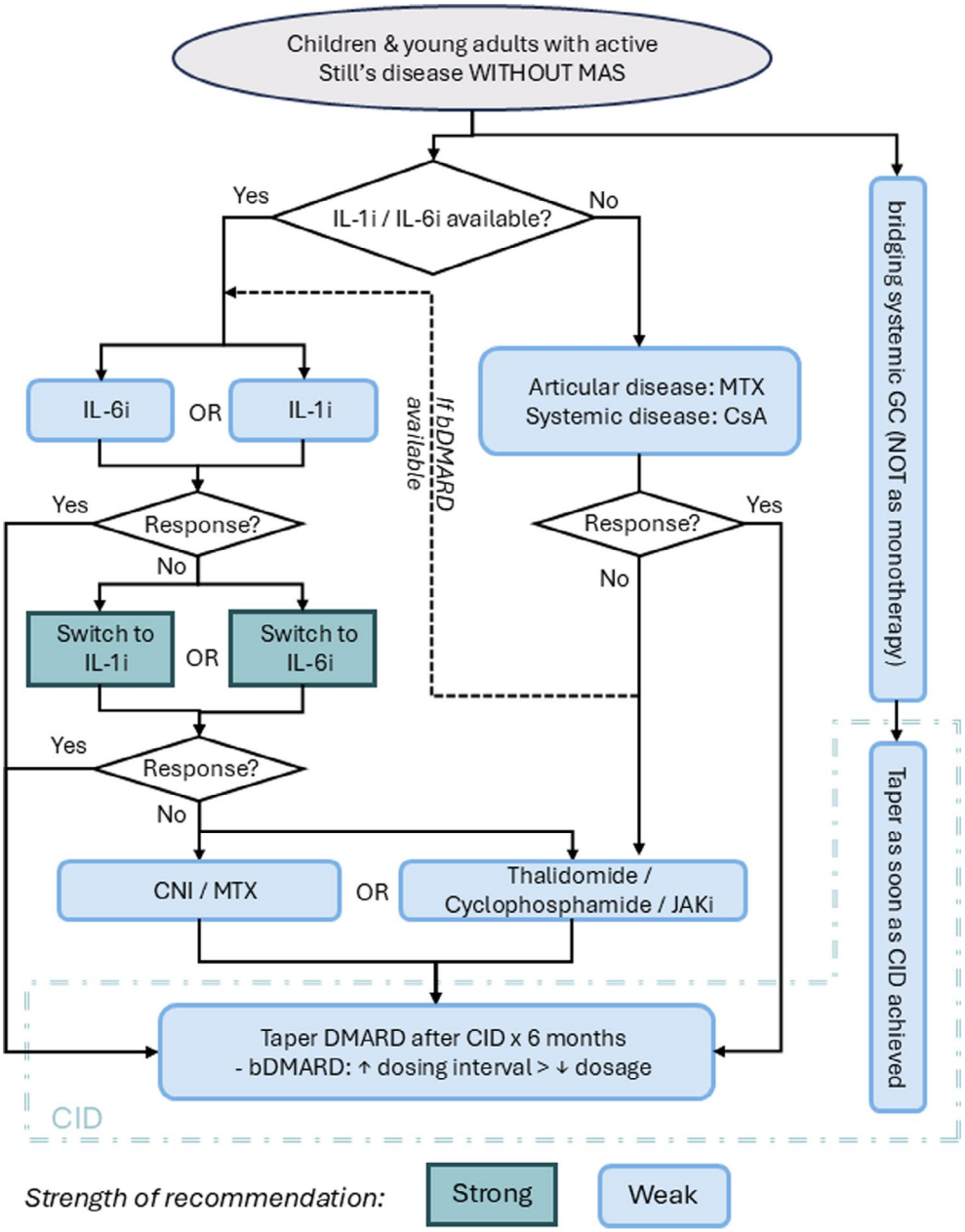

FIGURE 2 | Treatment algorithm for Still's disease without macrophage activation syndrome. Treatment algorithms should always be interpreted in conjunction with individual clinical judgment, taking into account each patient's unique circumstances. bDMARD, biological disease-modifying anti- rheumatic drug; CID, clinically inactive disease; CNI, calcineurin inhibitors; CsA, cyclosporin A; GC, glucocorticoid; IL-1i, interleukin-1 inhibitors; IL- 6i, interleukin-6 inhibitors; JAKi, Jak kinase inhibitors; MAS, macrophage activation syndrome; MTX, methotrexate.

association between biologic drugs and serious adverse events. The multivariate meta- analysis found no difference between bDMARDs (ANK, CAM, TCZ and rilonacept) [61]. Accordingly, no sequence for bDMARD initiation is currently recommended.

Findings from our recent regional survey indicated that while TCZ is available in most of the participating countries, access to ANK or CAM is considerably more limited, with fewer than half of the countries reporting availability compared to the widespread accessibility of csDMARDs. Despite some degree of access to these bDMARDs, their high cost continues to represent a major barrier to utilization [11]. Consequently, csDMARDs remain the predominant therapeutic option across much of the Asia-Pacific region. To prevent therapy delay, the TF suggests initiation of csDMARDs in combination with a bridging glucocorticoids regimen to control active disease when bDMARDs are unavailable/feasible. This constitutes a weak recommendation despite the moderate overall CoE for early bDMARD, reflecting limitations in regional resource availability.

4. *When conventional synthetic DMARDs are being considered as initial therapy, methotrexate may be considered for patients with predominant articular disease, while cyclosporin may be considered for those with predominant systemic disease. In some cases, a combination of the two may also be considered. This strategy requires careful toxicity monitoring. (PICO 2)*

The 2021 ACR guideline strongly recommends against csDMARDs as initial monotherapy despite very low CoE, whereas the 2024 EULAR/PReS recommendation does not address csDMARDs as first- line therapy [2, 13]. Both Western guidelines, however, acknowledged the role of csDMARDs in circumstances where IL- 1i or IL-6i are inaccessible or unavailable. As outlined above, both bDMARD classes demonstrate superior efficacy compared with csDMARDs monotherapy, achieving a higher rate of inactive disease, sustaining effect during long- term follow-up, and reducing glucocorticoids exposure to discontinuation, all with minimal serious adverse events including MAS observed [34, 61].

Recent systematic literature review (SLR) indicates that csDMARDs are employed either following inadequate glucocorticoid response or in combination with bDMARDs [34]. Evidence regarding methotrexate (MTX) in Still's disease is limited by heterogeneity in dosage and administration routes, complicating cross- study comparisons [62–66]. Nonetheless, MTX has demonstrated efficacy, achieving inactive disease in up to 70% of patients, and significantly reducing glucocorticoid dose, irrespective of concomitant arthritis [63–65, 67].

Observational studies of calcineurin inhibitors (CNI), including cyclosporine (CsA) and tacrolimus, have demonstrated efficacy in Still's disease, with longer event- free survival, higher rates of clinical remission, and reduced glucocorticoid requirements compared to patients without CNI [68–70]. MTX was also combined with CsA in one prospective and two retrospective studies [71–73], yielding clinically inactive disease (CID) rates ranging from 73% to 86%.

Thalidomide has been investigated in two observational studies—one prospective and one retrospective, demonstrating disease control rates of 73%–85%, with improvements in both arthritis and fever [74, 75]. Reported adverse events included transient paresthesia, reversible aminotransferase elevations, and somnolence. Owing to its teratogenic potential and the likelihood that these recommendations will be applied by non-rheumatologists, the TF advises caution and does not advocate thalidomide use except under exceptional circumstances.

The TF recommends MTX for predominantly articular disease and CsA for predominantly systemic disease, with the option of MTX + CsA combination therapy in severe or refractory cases. Given the potential for increased adverse events, this approach should be applied with caution. This recommendation is based on regional expert consensus and supported by evidence of low to very low certainty, resulting in a weak SoR.

5. *We recommend against using TNF inhibitors as initial therapy. (PICO 3)*

No trials of TNF inhibitors (TNFi) have been conducted in Still's disease. However, a recent SLR and meta- analysis reported pooled efficacy rates (ACR70 or CID) of 23% (95% CI 13%–34%) in AOSD and 28% (95% CI 15%–43%) in sJIA [34]. The TF upgrades the strength of this recommendation despite very low CoE, reflecting both the comparatively limited efficacy of Tumor necrosis factor- inhibitors (TNFi) relative to other DMARDs and the consensus panel's prioritization of avoiding harm from treatment delays. Consequently, the SoR is high.

The following recommendation statements apply to children and young adults with Still's disease without MAS who do not respond to initial therapy.

6. *In patients initiated on an IL- 6 inhibitor or IL-1 inhibitor at their respective therapeutic dose (or after dose optimization), switching to either IL- 1 inhibitor or IL-6 inhibitor is recommended, if feasible. (PICO 5)*

Evidence from randomized trials and observational studies demonstrates that IL- 1i or IL-6i provide higher efficacy, faster remission, and lower toxicity compared with csDMARDs. Recent data further support their role in facilitating glucocorticoid discontinuation, in contrast to conventional agents, and underscore the risks associated with long- term glucocorticoid exposure [34]. Switching between IL- 1i and IL-6i has been shown to yield comparable outcomes, consistent with the 2021 ACR guideline [12, 53, 58, 76, 77]. On this basis, the TF upgraded the recommendation. Accordingly, the SoR is strong despite the low CoE, reflecting the clinical benefits of bDMARDs and the emphasis on minimizing glucocorticoid use. This also highlights that patients may respond differently to IL- 1i and IL-6i, allowing treatment flexibility and avoiding therapeutic delay.

7. *In patients initiated with a conventional synthetic DMARD at a therapeutic dose (or after dose optimization), if possible, switching to or adding either IL- 6 inhibitor or IL-1 inhibitor is recommended. (PICO 5)*

Randomized controlled trials and observational studies consistently demonstrate the superior efficacy and safety of IL- 1 and IL- 6 inhibitors in Still's disease [34]. The initiation of csDMARDs should be reserved for circumstances in which both bDMARD classes are inaccessible or infeasible. Two scenarios warrant specific consideration: (1) if either IL- 1i or IL-6i subsequently become available, switching to or adding one of these agents is recommended, as *current evidence does not indicate superiority of one over the other*; and (2) if both biologic options remain unavailable, a different class of csDMARD—with or without bridging glucocorticoids—should be initiated promptly to control active disease. This recommendation is strengthened despite low CoE, reflecting the clear therapeutic benefit of IL- 1 and IL-6 inhibitors and the imperative to avoid delays in disease control.

8. *In patients with no biologic DMARD access or resistant to both IL- 1 inhibitors and IL-6 inhibitors, addition of or switching to any of the following medications may be considered.*

   - *Calcineurin inhibitors (cyclosporin A or tacrolimus), particularly in patients with systemic features.*
   - *Methotrexate, particularly in patients with predominant articular manifestation.*
   - *Thalidomide*
   - *Cyclophosphamide*
   - *JAK inhibitors*

All these strategies require great caution on toxicity monitoring. (PICO 2, 4).

Supporting evidence for the use of CNI, MTX, and thalidomide has been discussed above. Regarding cyclophosphamide, two

observational studies have evaluated its use in sJIA. The first involved 18 patients treated with a regimen of three consecutive days of pulse methylprednisolone, low- dose intravenous cyclophosphamide (400 mg/m^2) on the third day, and oral MTX (10 mg/m^2/week), repeated every 3 months [67]. The second study included four patients who received 6–10 monthly intravenous pulse methylprednisolone (30 mg/kg, max 1 g) in combination with cyclophosphamide (500–1000 mg/m^2) [78]. Both reports suggested some therapeutic efficacy; however, the CoE remains very low.

For JAK inhibitors (JAKi), five small case series involving 7–14 patients with sJIA or AOSD, together with one meta- analysis, have reported outcomes with tofacitinib, baricitinib, ruxolitinib, or upadacitinib [79–84]. Complete remission was achieved in up to 71% of patients, while partial remission was observed in up to 43%. The role of JAKi in Still's disease without MAS remains confined to refractory cases that have failed IL- 1 and IL-6 inhibitors, with or without concomitant csDMARDs.

This statement is a weak recommendation based on low to very low certainty of supporting evidence.

9. *There is insufficient evidence to recommend for or against intravenous immunoglobulin (IVIG), colchicine, or etoposide. (PICO 4, 6)*

Evidence from one small RCT and four observational studies with variable follow-up did not demonstrate a significant effect of IVIG on prognosis or overall outcomes in Still's disease, although steroid- sparing benefits were noted [34, 85, 86]. Colchicine was primarily used for Still's disease–associated serositis in a single study [34], while no evidence supports the use of etoposide outside of MAS. Accordingly, the TF considers the available data insufficient to recommend these agents.

3.2.2 | Maintenance Therapy

The following recommendation statements apply to children and young adults with Still's disease without MAS who are in clinically inactive disease.

10. *We suggest tapering off systemic glucocorticoids as soon as inactive disease is achieved, with the goal of discontinuation ideally within 6 months of glucocorticoid initiation. (PICO 7)*

The TF acknowledges the necessity of high- dose glucocorticoids (> 1 mg/kg/day) to control initial disease activity as bridging therapy, particularly in settings where bDMARDs are unavailable. The long- term adverse effects of glucocorticoids are well established, notably growth impairment in children [87]. In such circumstances, a treatment duration of at least 2–3 months is often unavoidable; however, once therapeutic targets are achieved, tapering should be undertaken progressively, with discontinuation ideally within 6 months—a goal the TF considers attainable. The TF recognizes that a proportion of patients may require ongoing glucocorticoid therapy at variable doses, often in combination with a csDMARD or combined- csDMARDs to achieve steroid-sparing effects. Nevertheless, every effort should be directed toward minimizing glucocorticoid exposure by using the lowest effective dose for the shortest possible duration.

11. *We suggest maintaining a sustained inactive disease for at least 6 months after systemic glucocorticoid discontinuation, prior to tapering DMARDs, regardless of DMARD classes initiated. (PICO 7, 8)*

Data guiding the management of DMARDs following achievement of CID remain limited. The TF recommends discontinuation of glucocorticoids as the initial mandatory step. Although no definitive trial has established the optimal timing for DMARD tapering, the PRINTO MTX withdrawal study demonstrated that maintaining CID for 6 or 12 months did not alter flare rates; notably, sJIA was included despite small numbers [88, 89]. Regression analyzes adjusted for ILAR subtype revealed no differential risk of flare across subtypes. Based on these findings, the TF advises maintaining CID for at least 6 months off glucocorticoids before initiating DMARD tapering. A stepwise reduction every 3–6 months is recommended, consistent with prior Western guidelines, allowing re- escalation if flare occurs [13]. This approach aims to mitigate severe relapse risk and harmonize DMARD withdrawal strategies across JIA subtypes.

12. *We suggest extending the dosing interval of biologic DMARDs rather than decreasing the dosage. (PICO 8)*

Evidence is lacking to guide DMARD tapering following achievement of CID. The TF suggests extending the dosing interval of bDMARDs to minimize drug wastage, align with patient values and preferences, and enhance adherence by reducing injection frequency.

13. *In patients with the combination of conventional synthetic DMARD and biologic DMARD, tapering either of the medications first may be considered. There is no preferred order of tapering. (PICO 8)*

This is a weak recommendation, supported by very low- CoE from a single study. A recent observational analysis found no significant difference in flare rates whether MTX or bDMARDs were withdrawn first [90].

3.2.3 | Macrophage Activation Syndrome (Figure 3)

The following recommendation statements apply to children and young adults with Macrophage Activation Syndrome associated with active Still's disease.

14. *If there is no contraindication, we recommend pulse methylprednisolone as initial therapy as soon as possible. (PICO 9)*

Macrophage activation syndrome (MAS) is a life- threatening complication of Still's disease. Identification of triggering factors is essential, with infection being more common in the Asia-Pacific region; this necessitates prompt and thorough investigation, followed by targeted antimicrobial therapy. Such measures facilitate the management of both MAS and the underlying Still's disease. High- dose glucocorticoids remain the

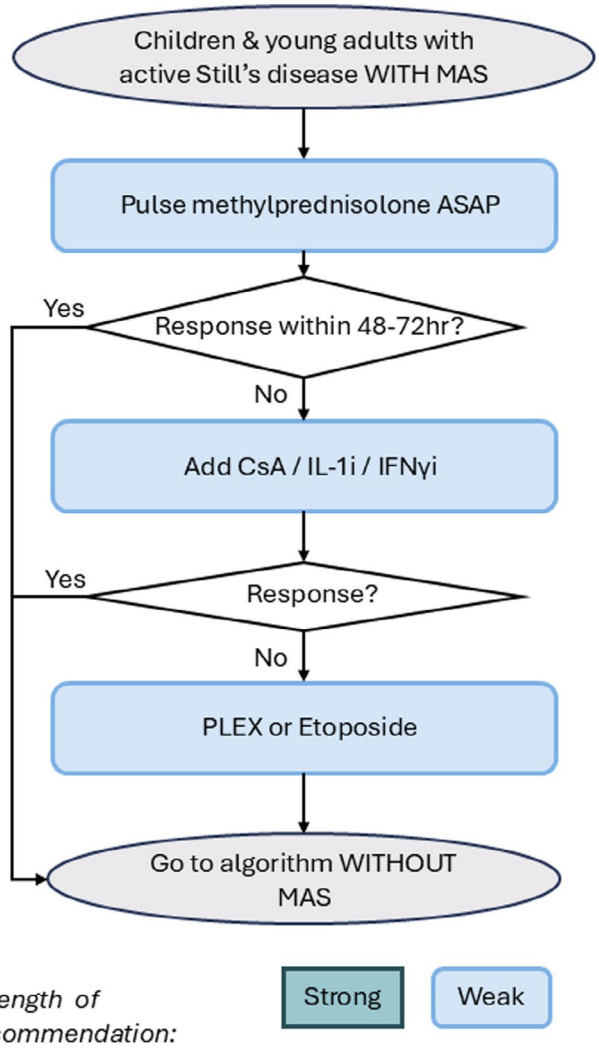

FIGURE 3 | Treatment algorithm for Still's disease with macrophage activation syndrome. Treatment algorithms should always be interpreted in conjunction with individual clinical judgment, taking into account each patient's unique circumstances. ASAP, as soon as possible; CsA, cyclosporin A; IFNγi, interferon- γ inhibitor; IL-1i, interleukin- 1 inhibitors; MAS, macrophage activation syndrome; PLEX, plasma exchange.

mainstay therapy and should be initiated without delay, typically with pulse methylprednisolone at 15–30 mg/kg/day (maximum 1 g/dose) for 3–5 days. Dexamethasone may be considered as an alternative, particularly in cases with central nervous system involvement, given its superior penetration of the blood–brain barrier.

15. *In patients with inadequate response to pulse methylprednisolone within the first 48–72 h, we suggest the addition of cyclosporin A or IL- 1 inhibitors or IFN-γ inhibitors (i.e., emapalumab). IL- 6 inhibitors may be considered with caution. (PICO 9)*

High- dose glucocorticoid therapy has demonstrated satisfactory efficacy in a substantial subset of patients with MAS associated with Still's disease, particularly when commenced early in the disease course. In severe or refractory MAS, where high- dose glucocorticoids prove inadequate, timely initiation of additional therapy within 48–72 h is recommended. To date, no RCTs have addressed MAS in Still's disease, apart from a recent small open- label study of emapalumab [91]. The TF highlights substantial and favorable evidence supporting CNIs, particularly CsA (oral or intravenous), in combination with glucocorticoids for disease control and steroid- sparing effects, as demonstrated in a SLR and a recent observational report [34, 68]. In resource- limited regions such as the Asia Pacific, the TF suggests the addition of a CNI following failure of high- dose glucocorticoid therapy, given their wider availability and accessibility compared with IL- 1i or emapalumab [11].

A recent meta- analysis demonstrated that MAS could occur during treatment with IL- 1 or IL-6 inhibitors, with pooled incidence rates of 2.7/100 patient- years (95% CI 1.8–3.9) for TCZ and 2.2/100 patient- years (95% CI 2.4–3.5) for ANK. Practitioners should exercise caution when MAS develops in patients with Still's disease receiving these agents, as key parameters—including fever and inflammatory markers—may be attenuated [92]. Observational studies of ANK in sJIA- associated MAS ($n = 44$) reported a complete response in 73% of patients, though concomitant CsA use was not clearly defined [34]. Emapalumab, the first monoclonal antibody targeting IFN- γ, demonstrated efficacy and safety in an open- label trial of patients with MAS refractory to high- dose glucocorticoids. Administered at an initial dose of 6 mg/kg followed by 3 mg/kg every 3 days until day 15, then twice weekly until day 28, in combination with high-dose methylprednisolone, emapalumab induced remission in 93% of patients by week 8, with the earliest response at day 9 [91]. However, its high cost and limited availability preclude widespread use in the Asia-Pacific region. By contrast, TCZ is more accessible, and recent regional observational studies have reported favorable efficacy and safety in MAS [93–95]. Given its higher incidence of MAS and greater masking of fever and inflammatory markers, particularly CRP, IL- 6 inhibition is generally reserved as second- line therapy after failure of other agents. In resource- limited settings, the TF anticipates CsA as first-line therapy following glucocorticoid failure, with TCZ positioned subsequently in the treatment sequence.

16. *Plasma exchange or etoposide might be considered in patients who are refractory to biologic DMARDs and cyclosporin A. (PICO 10)*

Plasma exchange, typically employed in combination with other therapies, has been reported in treatment- refractory Still's disease–associated MAS, predominantly in small observational studies, with favorable outcomes when therapeutic options are otherwise exhausted [34, 96, 97]. Similarly, low- dose etoposide has demonstrated efficacy across several observational cohorts, generally administered alongside other agents, with evidence of improved survival [34, 98].

This weak recommendation reflects the very low CoE supporting plasma exchange and low- dose etoposide in refractory MAS associated with Still's disease. The available data are limited to small observational studies, with inherent methodological constraints and risk of bias. Nevertheless, the TF acknowledges that in many parts of the region, therapeutic options are restricted, and patients with multiple drug failures face high mortality

risk. In such contexts, consideration of these modalities may be warranted, as their use has been associated with improved survival in observational cohorts. The balance of potential benefit against the uncertainty of evidence underpins the conditional nature of this recommendation.

17. *JAK inhibitors might be considered in patients who are refractory to biologic DMARDs and conventional synthetic DMARDs. (PICO 9)*

Although evidence is limited to small observational studies, JAK inhibitors—particularly the JAK1/JAK2 agents ruxolitinib and baricitinib—have shown efficacy in controlling MAS complicating Still's disease and in improving survival [34, 80–82, 84, 99, 100]. Reported outcomes indicate clinically meaningful benefit in patients with otherwise treatment- refractory MAS. In view of the paucity of effective alternatives and the high associated mortality, the TF considers JAKi a potential therapeutic option while underscoring the conditional/weak nature of this recommendation given the very low CoE.

18. *In order to achieve the ultimate goal (drug- free remission), the following intermediate targets are suggested:*

- At day 7, resolution of fever and reduction of CRP by > 50%,
- At week 4, no fever, reduction of active (or swollen) joint count by > 50%, normal CRP and physician and patient/parent global assessment less than 20 on a 0–100 VAS,
- At Month 3, CID with glucocorticoids less than 0.2 mg/kg/day,
- At Month 6, CID without glucocorticoids.

In line with the overarching principle of treat- to-target (T2T), where treatment is modified according to the disease activity. This approach is particularly encouraged in Still's disease management, where delayed treatment may result in severe or life-threatening complications. Consistent with the immediate targets (within 6 months) outlined in the EULAR/PReS recommendations, the TF adopts these targets, recognizing their simplicity and feasibility in guiding treatment across the region [34]. These targets are intended as guidance; however, in severely resource-limited settings, complete glucocorticoid discontinuation may not be achievable. In such circumstances, glucocorticoids may be maintained at the lowest effective dose for the shortest possible duration, while considering change or escalation of DMARD.

4 | Discussion

These consensus recommendations represent the second part of the APLAR JIA Guidelines Task Force initiative, following the regional consensus on polyarticular course JIA (pJIA), temporomandibular joint arthritis, and non- pharmacological management of JIA (in press). In contrast to pJIA, Still's disease is a clinically distinct autoinflammatory syndrome driven predominantly by IL- 1 and IL-6 pathways [13]. The therapeutic landscape for Still's disease has evolved rapidly over the past decade; however, access to biologic agents and implementation of treat- to-target strategies remain highly variable across the Asia-Pacific region. This variability is compounded by a critical shortage of pediatric rheumatologists, with most patients managed by non- pediatric rheumatologist practitioners, underscoring the need for a tailored regional approach. While conceptually aligned with recent international guidelines, including the 2021 ACR recommendations [12] and the 2024 EULAR/PReS consensus recommendations [13], the APLAR statements introduce region- specific modifications. These adaptations reflect both emerging evidence and pragmatic considerations unique to the Asia-Pacific context (Table 2).

4.1 | Early Therapeutic Strategy and Discouragement of NSAID or Glucocorticoid Monotherapy

A major distinction of the APLAR consensus recommendations is the explicit discouragement of NSAIDs and systemic glucocorticoids as initial monotherapy for treatment- naïve, active Still's disease without MAS, with preference given to early initiation of IL- 1 or IL-6 inhibitors where feasible. This contrasts with the 2021 ACR guideline, which conditionally recommended NSAIDs as possible first- line therapy in selected patients [12] but aligns with the 2024 EULAR/PReS recommendations emphasizing early biologic intervention [13]. Two considerations underpin this divergence. First, the APLAR task force recognizes Still's disease as a cytokine- driven autoinflammatory condition mediated primarily by IL- 1 and IL-6. Observational data demonstrate that NSAID monotherapy rarely achieves sustained remission and may delay timely escalation; more than half of patients initiated on NSAIDs ultimately require glucocorticoids or DMARDs [28, 29]. Second, glucocorticoid exposure remains a major determinant of long- term morbidity, particularly growth impairment in children [87]. Accordingly, the TF chose to include a steroid- sparing strategy as one of the explicit therapeutic objectives.

This recommendation reflects a more aggressive "window-of- opportunity" approach to Still's disease, aiming to prevent chronic joint damage and reduce steroid dependency through early cytokine blockade. Nevertheless, access to biologic agents remains highly variable across the Asia-Pacific region. To accommodate differences in regulatory approval and drug availability, the consensus statements deliberately frame biologic therapy as "if possible," ensuring flexibility while emphasizing the importance of early intervention where feasible.

4.2 | Role of Conventional Synthetic DMARDs

In contrast to the 2021 ACR guidelines, which did not endorse csDMARD monotherapy for Still's disease [12], the current APLAR consensus adopts a more pragmatic stance. Although IL- 1 or IL-6 inhibitors remain the preferred first-line therapy, MTX or CNI may be considered when biologics are unavailable. A greater consideration is given to csDMARD in this guideline to reflect real- world practice in many lower- and middle- income countries across the AsiaPacific region. The recommendations are further stratified by phenotype: MTX may provide benefit in patients with predominant articular involvement, whereas CNI may be more suitable for

TABLE 2 | Differences and similarities of the current APLAR recommendation with the existing Western guidelines.

Domain	APLAR guideline	2024 EULAR/PReS	2021 ACR
Treat to target (T2T)	Strongly frames management as T2T with the ultimate goal of drug-free remission; defines targets at day 7, week 4, month 3, month 6		Does not specify a time- anchored T2T target
Treatment naïve Still's disease without MAS: NSAIDs	Suggests against NSAIDs as initial monotherapy	The algorithm prioritizes early IL-1/IL-6 inhibitors without mentioning NSAIDs as a core initial monotherapy strategy	Conditionally recommends NSAIDs as initial monotherapy
Treatment naïve Still's disease without MAS: glucocorticoids	Suggests against systemic GCs as monotherapy, but allow bridging	Uses GCs by severity; emphasizes tapering as soon as possible with the explicit target of CID low dose at 3 months and CID off GCs at 6 months.	Conditionally recommends against oral GCs as initial monotherapy, but acknowledges use when biologics are not available; in the lowest dose/shortest duration possible
Treatment naïve Still's disease without MAS: IL1/IL- 6 inhibitors	Suggests initiating IL- 1/IL-6 inhibitors if possible; however, allows fallback to csDMARDs if biologics are not available.	Emphasizes early initiation with IL- 1/IL-6 inhibitors once the diagnosis is established	Conditionally recommends IL- 1/IL-6 inhibitors as initial monotherapy without preferred agent
Treatment naïve Still's disease without MAS: csDMARDs	Considers the use of MTX for predominant arthritis; CsA for systemic disease when biologics not available	Not positioned as first- line. The management algorithm is a biologic- first approach	Strongly recommends against csDMARDs as initial monotherapy in Still's disease without MAS
Treatment naïve Still's disease without MAS: TNFi	Specifically recommends against TNFi as initial therapy.	Did not explicitly recommend against the use of TNFi as initial therapy, but emphasizes the use of IL- 1/6 inhibitors	TNFi not recommended as initial Still's disease therapy
Still's disease without MAS with inadequate response to initial IL-1/IL-6 inhibitor strategy	Supports switching to another class when initial therapy fails.		
Refractory disease/no biologic access/resistant to IL-1 + IL-6 inhibitors	Lists options of CNI, MTX, thalidomide, cyclophosphamide and JAK inhibitors	Does not mention alternatives to IL- 1/IL-6 inhibitors if not available	Notes options for residual arthritis after IL- 1/IL-6 inhibitors (e.g., add MTX, switch to abatacept or TNFi)
Inactive disease: glucocorticoids	All support tapering off GCs but APLAR and EULAR/PReS gives specific target timepoints for GC discontinuation		
Inactive disease: tapering biologics	Maintains sustained inactive disease ≥ 6 months after GC discontinuation before tapering DMARDs	Maintenance of CID 3–6 months off GCs before bDMARD tapering; and ≥ 6 months CID off GCs before tapering is reiterated in the algorithm narrative	Conditionally recommends tapering/discontinuing bDMARDs after inactive disease, but timing/method unclear due to limited evidence
Inactive disease: tapering strategy	Prefers extend dosing interval rather than decrease dosage	Not specifically mentioned	Not specifically mentioned

(Continues)

TABLE 2 | (Continued)

Domain	APLAR guideline	2024 EULAR/PReS	2021 ACR
MAS: initial therapy	Recommends pulse methylprednisolone ASAP if no contraindication.		Conditionally recommends GCs as part of initial treatment for Still's disease with MAS
MAS: escalation of therapy	If inadequate response within 48–72 h, suggests adding CsA or IL- 1 inhibitors or IFN- γ inhibitors. Mentions the use of IL- 6 inhibitors with caution	Only considers IL1 inhibitors instead of IL- 6 inhibitors. Also mentions cyclosporin A and/or IFN- γ inhibitor as part of initial therapy	Conditionally recommends IL- 1/IL-6 inhibitors over CNI alone to achieve inactive disease and MAS resolution; acknowledges combination therapy may be needed in severe MAS.
MAS: refractory disease	PLEX or etoposide may be considered if refractory. JAK inhibitors may be considered	Not specifically mentioned	Formal recommendations deferred
Complications	Not present in the excerpted APLAR statements.	Has explicit statements: active screening (symptoms + PFTs; HRCT if symptomatic)	Discusses concern about Still's disease–associated lung disease

Abbreviations: CID, clinically inactive disease; CNI, calcineurin inhibitor; CsA, cyclosporin A; csDMARD, conventional synthetic disease modifying anti- rheumatic drug; GCs, glucocorticoids; HRCT, high resolution computed tomography; JAK, janus kinase; MAS, macrophage activation syndrome; PFT, pulmonary function test; PLEX, plasma exchange; RTX, rituximab; RUX, ruxolitinib; TCZ, tocilizumab; TNFi, tumor necrosis factor inhibitor; TOF, Tofacitinib.

predominant systemic inflammation and MAS phenotypes, despite the low CoE. Inclusion of csDMARDs thus balances limited evidence with the practical realities of regional healthcare systems and aligns with global efforts to expand access to essential therapies for childhood rheumatic diseases. Notably, MTX is listed in the pediatric section of the WHO Model List of Essential Medicines [101], underscoring its importance as a core treatment option in settings where biologics are inaccessible.

4.3 | Explicit Rejection of TNF Inhibitors as Initial Therapy

The APLAR consensus explicitly recommends against the use of TNFi as initial therapy for Still's disease. While prior guidelines did not endorse TNFi as first- line biologics, the current statement provides clearer guidance, supported by mechanistic and clinical evidence indicating that TNF blockade fails to adequately control systemic inflammation in this condition [34]. Although TNFi may be more accessible in the Asia-Pacific region due to biosimilars and lower cost, their use risks delaying initiation of IL- 1 or IL-6 inhibitors, or other potentially more effective csDMARDs. This explicit recommendation is intended to prevent inappropriate therapeutic sequencing and to optimize timely initiation of effective therapy.

4.4 | Treat- To-Target Approach and Drug Tapering

Similar to the 2024 EULAR/PReS guideline, the APLAR consensus statement outlines treatment targets at Day 7, Week 4, Month 3, and Month 6, culminating in glucocorticoid- free inactive disease by month 6 [13]. These milestones were not defined in the 2021 ACR guideline [12]. This reflects the adoption of a treat- to-target approach, consistent with paradigms established in other inflammatory rheumatic diseases [102]. The consensus statement specifies quantitative targets—including fever resolution, reduction in inflammatory markers, improvement in joint counts, and, critically, defined glucocorticoid thresholds—to reduce therapeutic inertia and encourage timely escalation. This framework also aims to harmonize outcome monitoring across diverse healthcare systems. However, these targets have not yet been validated in the Asia-Pacific setting, where access to bDMARDs, particularly IL- 1 and in some contexts IL-6 inhibitors, remains limited. Whether such targets can be consistently achieved under current resource constraints is therefore uncertain and constitutes a priority for future research. Modifications toward more attainable targets may be required following regional practice experience and data collection.

Another distinct point in this consensus statement is the tapering strategy for patients who achieve CID on biologic therapy. This has not been explored in previous guidelines. Our consensus statement suggests extending the dosing interval rather than reducing the individual dose. The evidence base for drug tapering in Still's disease is sparse. With improved outcomes and more patients achieving CID, practical tapering strategies are necessary. While interval extension is a more feasible approach, this recommendation should be interpreted cautiously, as it is

based on very low CoE and requires validation in prospective studies.

4.5 | Expanded Framework for Refractory Disease

For patients refractory to both IL- 1 and IL-6 inhibitors, or without access to biologics, the APLAR consensus provides a broader list of potential agents, including CNI, thalidomide, cyclophosphamide, and JAKi. Evidence supporting these therapies remains of very low certainty, yet their inclusion reflects real- world practice in parts of Asia. The conditional consideration of thalidomide is particularly contextual: although not widely endorsed in Western guidelines due to toxicity concerns, structured monitoring systems in some centers have enabled its use with demonstrable efficacy [74, 75]. Rather than omitting such options, the task force elected to provide explicit cautionary guidance to ensure clarity and safe application in resource-constrained environments. The monitoring should include electrophysical evaluation with electromyography (EMG) and nerve conduction velocity (NCV) tests, if feasible.

4.6 | Macrophage Activation Syndrome Management and Escalation Pathways

In cases of MAS complicating Still's disease, the APLAR consensus recommends pulse methylprednisolone as initial therapy, with subsequent addition of CsA, IL- 1i, particularly ANK, or emapalumab (IFN- γ inhibitor) in the event of inadequate response. This approach is consistent with prior guidelines, which recognized IL- 1 blockade and IFN-γ inhibition as effective strategies for MAS, though access to these agents remains limited across most of the Asia-Pacific countries [12, 13]. For refractory disease, the consensus panel also acknowledges potential roles for etoposide, JAKi, and plasma exchange. Inclusion of these options underscores both the severity of refractory MAS and the need for flexible escalation pathways, particularly in settings where availability of targeted therapies is constrained.

4.7 | Addressing Gaps in Evidence

Despite advances in understanding the pathophysiology of Still's disease, high- quality evidence remains scarce across most PICO domains. Observational studies are frequently underpowered due to small sample sizes, and outcome definitions remain heterogeneous. There is a pressing need for adequately designed RCTs evaluating therapies beyond IL- 1 and IL-6 inhibitors. Although biologics are increasingly regarded as first- line therapy in Still's disease without MAS, the relative efficacy of IL- 1 versus IL- 6 blockade remains uncertain. With improving outcomes and growing numbers of children achieving sustained remission, further data are required to define the optimal duration of biologic therapy prior to tapering. In addition, as experience with targeted synthetic DMARDs expands, cost- effectiveness analyzes will become increasingly important, particularly in low- and middle- income countries in the Asia-Pacific region. Addressing these knowledge gaps will necessitate regional collaboration and the establishment of patient registries.

Recent policy developments have significantly advanced global access to therapies for childhood rheumatic diseases. The WHO Model List of Essential Medicines (EML) has been updated to include agents for pediatric inflammatory joint disease; however, the current list is limited to MTX, intra- articular glucocorticoids, and selected TNFi [101]. While the EML establishes a minimum standard for medicines that should be available within national health systems, key cytokine- targeted therapies for Still's disease, such as IL- 1 and IL-6 inhibitors, remain excluded. This disparity highlights the gap between emerging evidence and evolving global policy frameworks. Sustained advocacy will be required to ensure that essential medicine lists keep pace with emerging evidence in pediatric rheumatology and reflect the therapeutic needs of children worldwide.

Several limitations must be acknowledged in applying this consensus statement to clinical practice. First, although the GRADE methodology was systematically applied, many recommendations rely on indirect evidence, particularly regarding tapering strategies and management of refractory MAS. Second, heterogeneity in study populations—including variability in inclusion of childhood- versus adult-onset Still's disease—and inconsistency in defining "inactive disease" limit comparability and reduce applicability to specific patient groups. Third, economic considerations are highly relevant in Asia-Pacific settings, yet formal studies remain scarce. While pragmatic and real- world factors influenced the development of these recommendations, they were not formally incorporated into the evidence synthesis. Finally, restricting the literature search to English- language publications introduces the potential for language and publication bias and may have resulted in the omission of relevant studies from the Asia-Pacific region. This is particularly pertinent given that clinical experience and real- world evidence on Still's disease may be reported in local languages, including Japanese and Chinese, potentially limiting the representation of regional practice patterns, uncommon complications such as macrophage activation syndrome, and treatment strategies influenced by local healthcare systems and resource availability. However, the English- language restriction was adopted to ensure a feasible, consistent, and methodologically rigorous review process across participating review teams. To mitigate this limitation, the TF comprises experts from across the Asia-Pacific region who interpreted the evidence in the context of regional clinical practice and healthcare constraints. Furthermore, the review deliberately included csDMARDs alongside biologic therapies to reflect treatments that remain widely available in many countries within the region. These measures were intended to enhance the applicability of the recommendations despite the limitations of the published evidence.

5 | Conclusion

This APLAR consensus represents a distinct and necessary evolution of existing guidance for Still's disease by explicitly addressing the realities of healthcare delivery across the Asia-Pacific region. While international recommendations have advanced a biologic- driven, treat-to-target paradigm supported by biologics, these strategies assume timely access to specialist care and high- cost therapies. In contrast, the APLAR recommendations are deliberately contextualized to heterogeneous health systems in which access to biologic agents remains variable. Importantly,

this work does not merely adapt existing recommendations but reframes them through a resource- stratified lens, integrating evidence with real- world feasibility and health system constraints. Persistent gaps in high- quality evidence highlight the need for collaborative research and regional registries to refine management strategies for this rare but potentially severe disease.

Author Contributions

T.A., S.F., L.F.D. and K.L.T. conceptualized the study. All authors contributed to drafting, discussing, modifying, and finalizing the consensus statements. T.A. and K.L.T. drafted the manuscript. K.L.T., C.M.A., K.A., P.C., M.C.M.C.- C., N.G.U.C., M.T.C., C.H.J., S.C.L., M.J.H.L., B.L., W.S. and M.B.V. did the systematic literature search and review. All authors edited and approved the final manuscript.

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The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

R.A.J. reports honoraria from Pfizer. All other authors declare no competing interests. This work was funded by the APLAR secretariat to support the online voting platform and other secretary supports. There are no commercial conflicts of interest to be declared.

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. **Appendix S1:** Task force members' list and their responsibility. **Appendix S2:** Evidence report and Summary of Finding (SoF) tables. **Appendix S3:** Search Inclusion Criteria. **Appendix S4:** Search Strategies. **Figure S1:** Preferred Reporting Items for Systematic reviews and Meta- Analyzes (PRISMA) Flowchart.

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LETTER TO THE EDITOR

Association of Neutrophil Percentage-to-Albumin Ratio With Urinary Albumin Excretion in U.S. Adults: Implications for Rheumatic Disease Research

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Dear Editor,

Rheumatic diseases affect more than 1 billion individuals globally [1] and are the leading cause of chronic musculoskeletal disability. Renal injury is a frequent and severe complication of many rheumatic diseases [2], yet early asymptomatic albuminuria remains largely difficult to detect because of suboptimal patient adherence to urine albumin-to-creatinine ratio (UACR) testing, especially in patients with oliguria or anuria, or those with critical illness who are unable to provide spontaneous urine samples [3]. The neutrophil percentage-to-albumin ratio (NPAR), a readily accessible composite biomarker reflecting systemic inflammation and nutritional status, may be associated with early renal function changes. Using data from the National Health and Nutrition Examination Survey (NHANES), this study examined the association between NPAR and log-transformed UACR (ln-UACR) in the general U.S. adult population, aiming to provide epidemiological evidence that may inform future investigations in rheumatic disease cohorts.

We conducted a cross-sectional study using five consecutive rounds of data from the NHANES database (2009–2018). Administered by the National Center for Health Statistics (NCHS), the NHANES is a cross-sectional database that supplies real-world data on health conditions and nutritional profiles of the U.S. general population. After rigorously screening the initial 47 715 participants against the exclusion criteria, a total of 26 818 adults (all aged 18 years or older) were ultimately included in the analysis (Figure 1). The NPAR was calculated using the formula $\text{NPAR} = \text{Neutrophil Percentage}\,(\%) \times 100 / \text{Albumin Concentration}\,(\text{g/dL})$ [4]. Urinary albumin-to-creatinine ratio (UACR, mg/g), the current reference standard for albuminuria assessment, was calculated from urinary albumin and urinary creatinine concentrations using standard unit conversion. Given its highly right-skewed distribution, UACR was natural log-transformed and used as the primary dependent variable in the main analyses. Estimated glomerular filtration rate (eGFR) was calculated using the 2021 CKD-EPI creatinine equation based on serum creatinine, age, and sex, and was included as a covariate in the fully adjusted model and for stratified sensitivity analyses.

All analyses incorporated sample weights (WTMEC2YR), stratification (SDMVSTRA), and clustering (SDMVPSU) per NCHS guidelines. A 10-year combined weight was derived by dividing WTMEC2YR by five across cycles. For analyses involving fasting subsample covariates, the fasting weight (WTSAF2YR) was applied instead. *Model 1* was unadjusted; *Model 2* adjusted for age, sex, and race; *Model 3* further adjusted for BMI, fasting blood glucose, lipid profile, and other covariates (Table S1) [5]. Additionally, we performed baseline analyses, RCS modeling, and subgroup analyses. To achieve methodological rigidity and accuracy in computing, all the statistical tests were conducted with DecisionLinnc (v1.0), Python (v3.10.6), and R (v4.2.3).

Abbreviations: BMI, body mass index; CDC, centers for disease control and prevention; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MPO, myeloperoxidase; NCHS, national center for health statistics; NHANES, national health and nutrition examination survey; NPAR, neutrophil percentage-to-albumin ratio; PIR, poverty-income ratio; RCS, restricted cubic spline; UACR, urine albumin-to-creatinine ratio.

Wenyu Bao and Huan Zhang contributed equally to this work.

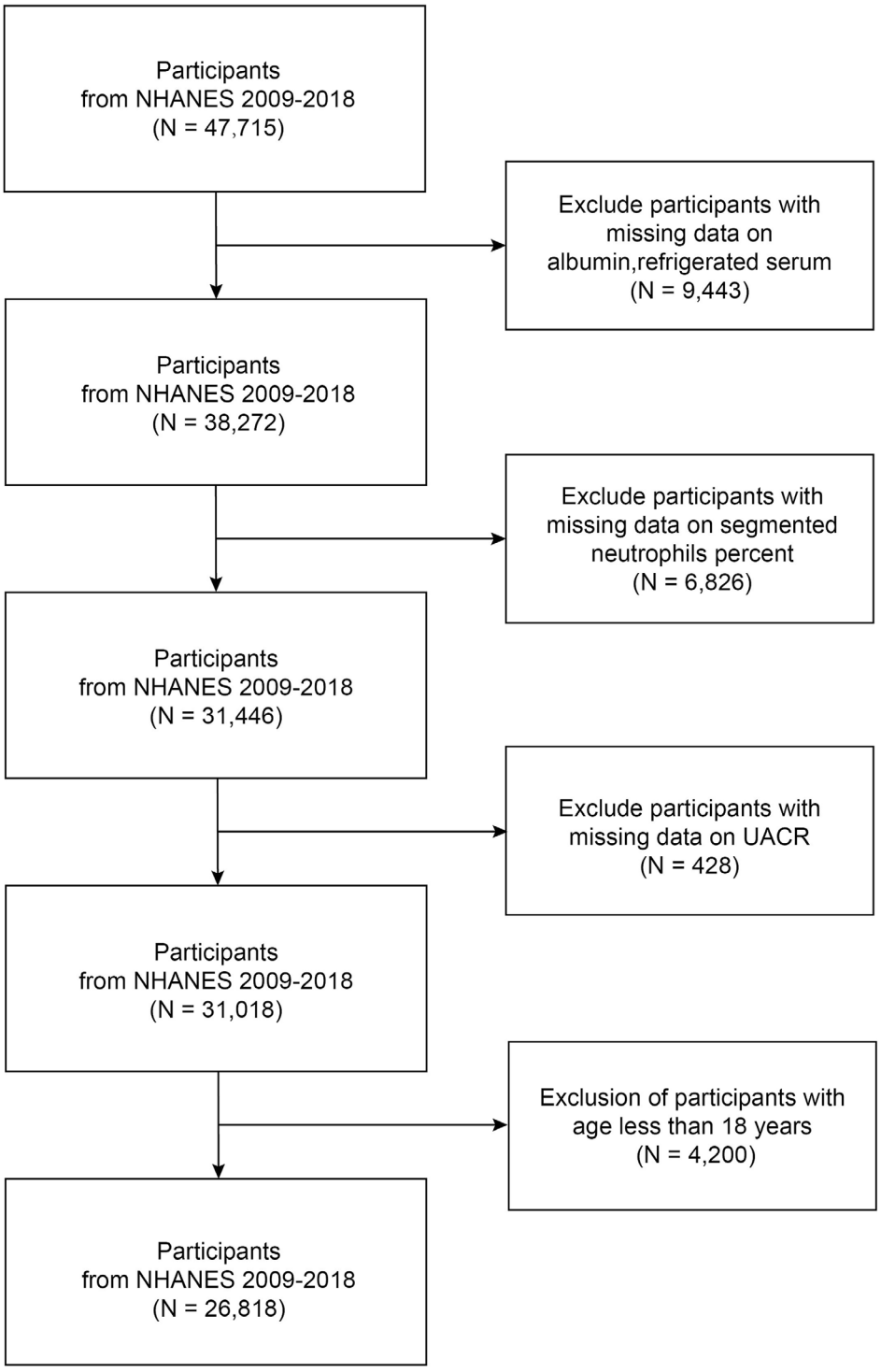

FIGURE 1 | Schematic depiction of participant selection and exclusion criteria.

A four- stratified baseline analysis of the population based on the NPAR (Table S2) revealed marked disparities among the quartiles for most of the demographic and clinical characteristics ($p < 0.05$). ln- UACR levels consistently increased across the as cending NPAR quartiles ($p < 0.001$). Variables such as sex, age, race, hypertension status, diabetes status, physical activity, marital status, education level, BMI, fasting blood glucose level, systolic and diastolic blood pressure, family poverty- income ratio (PIR), urine creatinine level, and smoking status significantly differed across the four NPAR quartiles (all $p < 0.05$). However, no significant variation in alcohol use status was observed among the quartiles ($p > 0.05$). Additionally, a baseline analysis of the population stratified into three groups on the basis of ln-UACR levels (Table S3) revealed that marked disparities were seen in all assessed demographic and clinical characteristics across ln- UACR tertiles, except total cholesterol.

We implemented the RCS to model the association between the NPAR and ln- UACR in U.S. adults. As depicted in Figure2a–c, NPAR demonstrated a significant non- linear association with

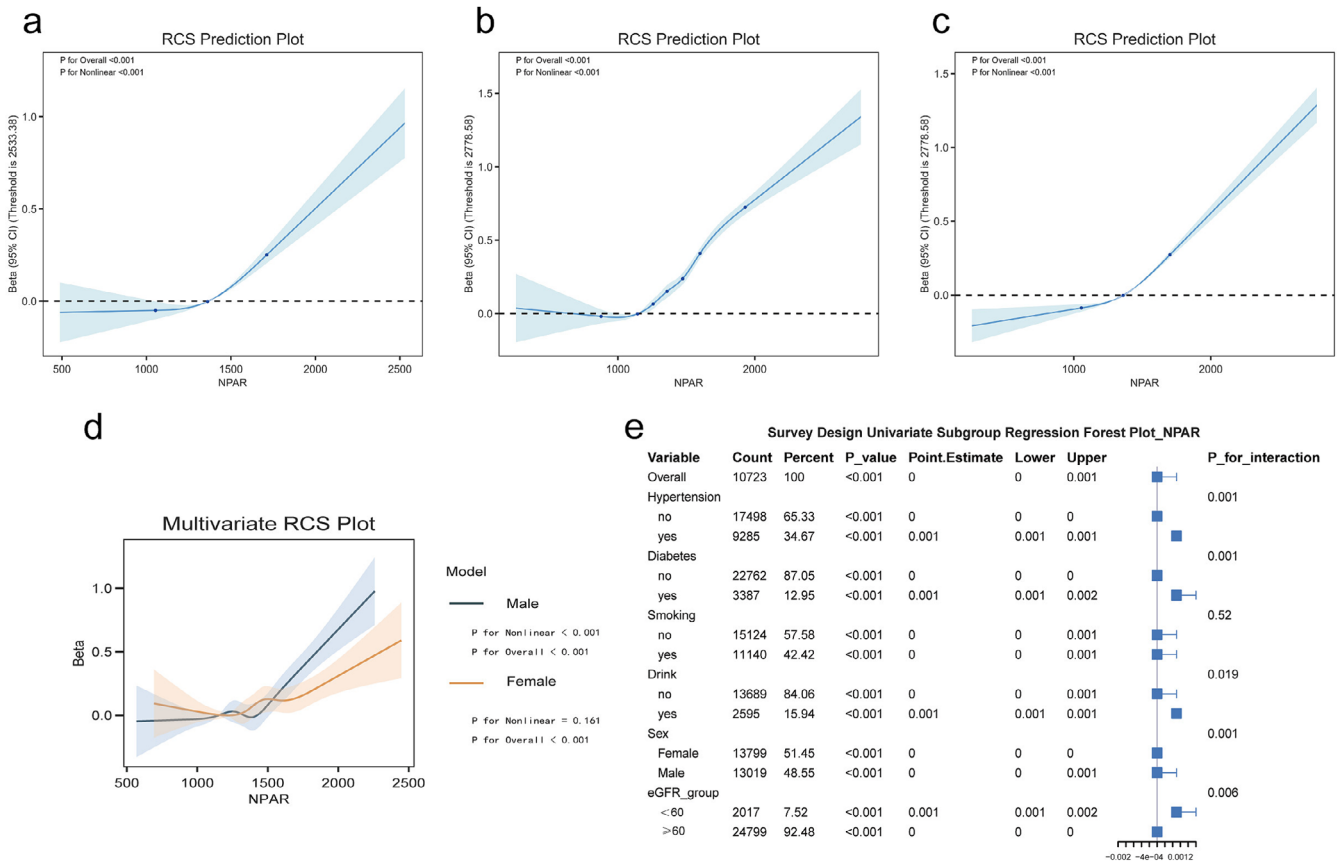

FIGURE 2 | RCS analysis and subgroup analysis between the NPAR and ln- UACR. (a–c) Nonlinear relationship between the NPAR and ln-UACR illustrated by a smooth curve fit. The solid blue line represents a smooth curve fit between the variables. The light blue band represents the 95% confidence interval of the fitted values. The fully adjusted model presented in (a) incorporates variables such as hypertension, diabetes status, smoking status, alcohol consumption, vigorous work activity, fasting glucose level, direct high- density lipoprotein (HDL) cholesterol level, total cholesterol level, low- density lipoprotein (LDL) cholesterol level (Friedewald), BMI, sex, age, race, level of education and marital status. (b) The unadjusted model. The partially adjusted model, as depicted in (c), accounts solely for sex, age, and race. (d) Multivariate- restricted cubic splines of NPAR and ln- UACR. (e) Results from subgroup analyses.

ln- UACR across all models (*p* for overall < 0.001; *p* for non-linearity < 0.001). In the fully adjusted model (Figure 2a), the curve displayed a complex non- linear pattern with a threshold effect identified at an NPAR value of 2533.38. The relationship showed a J- shaped trend, where ln-UACR levels remained relatively stable or slightly decreased at lower NPAR values but increased sharply once the threshold was exceeded. In the unadjusted model (Figure 2b) and the model adjusted for age, sex, and race (Figure 2c), a similar positive trend was observed, with a threshold effect at 2778.58. In these models, ln- UACR levels rose consistently as NPAR levels increased, particularly beyond the threshold, indicating a strong positive correlation between elevated NPAR and renal impairment markers.

Stratified analysis by sex revealed distinct patterns in this association (Figure 2d). In males, NPAR showed a significant non- linear correlation with ln- UACR (*p* for overall < 0.001; *p* for non- linearity < 0.001), with beta values increasing as NPAR rose. Conversely, in females, although the overall association was significant (*p* for overall < 0.001), the non- linearity was not statistically significant (*p* for non- linearity= 0.161), and the curve exhibited a flatter, more linear positive trend compared to males. This suggests that the impact of NPAR on renal function markers may be more pronounced or exhibit a different dose–response pattern in men.

Furthermore, subgroup analyses and interaction tests were performed to identify potential effect modifiers (Figure 2e). Significant interactions were observed for hypertension (*p* for interaction = 0.001), diabetes (*p* for interaction = 0.001), alcohol consumption (*p* for interaction = 0.019), sex (*p* for interaction = 0.001), and eGFR group (*p* for interaction = 0.006). These results indicate that the association between NPAR and ln- UACR is modified by metabolic status, gender, and baseline renal function. Specifically, the positive association appeared stronger in participants with hypertension, diabetes, and those with eGFR < 60. No significant interaction was found for smoking status (*p* for interaction = 0.52), suggesting the association is relatively consistent across smoking habits.

In conclusion, this large, nationally representative population-based study demonstrated a significant J- shaped association between the NPAR and ln- UACR in U.S. adults. The association is stronger in men, participants with hypertension or diabetes, and those with reduced eGFR.

These findings indicate that NPAR is independently associated with urinary albumin excretion, as reflected by ln- UACR, in the general U.S. adult population. While UACR remains the reference standard for albuminuria assessment, its reliance on urine

collection presents practical limitations in certain clinical scenarios [6]. NPAR, in contrast, can be derived from routine complete blood counts and serum albumin tests already performed in standard laboratory panels, potentially offering a practical adjunct for identifying individuals who may warrant further UACR evaluation. The threshold effect observed at an NPAR value of 2533.38 in the fully adjusted model may aid in such risk stratification, although these findings require prospective validation [7].

The biological plausibility of this association is supported by established pathophysiological mechanisms. In immune-mediated inflammatory conditions [8], activated neutrophils release mediators such as myeloperoxidase (MPO) [9], which can disrupt the charge barrier of the glomerular basement membrane. Hypoalbuminemia may further compromise endothelial integrity and contribute to renal interstitial edema [10]. These mechanistic links provide a coherent biological rationale for the observed association between NPAR and ln- UACR [11, 12].

This study has several strengths, including its large, nationally representative sample, rigorous adjustment for confounding variables, and incorporation of ln- UACR as a clinically validated outcome measure. However, several limitations should be acknowledged. First, the cross- sectional design precludes causal inference and assessment of the long- term prognostic value of NPAR. Second, although eGFR was included as a covariate, single- time-point measurements do not allow evaluation of chronic kidney disease progression or confirm persistent albuminuria. Third, the NHANES database does not capture rheumatic disease- specific clinical data, disease subtypes, activity measures, or treatment regimens. Therefore, our findings should be interpreted as population- level epidemiological evidence, and cautious extrapolation to rheumatology populations is warranted. Future prospective studies and longitudinal evaluations in well- characterized rheumatic disease cohorts are needed to validate these observations and investigate the underlying mechanisms across different stages of renal impairment [13].

Author Contributions

Wenyu Bao: conception and design, data analysis, and writing – original draft. **Huan Zhang** and **Jintao Mo:** reviewing and revising the manuscript, guidance for manuscript preparation, and final approval of the manuscript. **Chunyan Hua** and **Sheng Gao:** reviewing the manuscript, funding acquisition, and final approval of the manuscript. All the authors contributed to the article and approved the submitted version.

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Ethics Statement

This project received ethical approval from both the National Center for Health Statistics (NCHS) and the Centers for Disease Control and Prevention (CDC) Research Ethics Review Board (ethical code available at https://www.cdc.gov/nchs/nhanes/about/erb.html). All study participants provided written informed consent. We confirm that all methods are carried out in accordance with the relevant guidelines and principles of the Declaration of Helsinki.

Consent

The patients/participants provided written informed consent to participate in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Survey information can be publicly accessed online for use by researchers and data professionals worldwide. For more information, please refer to the website https://www.cdc.gov/nchs/nhanes. The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** List of covariates included in the weighted multivariate linear regression models for the association between neutrophil percentage- to-albumin ratio (NPAR) and urinary albumin levels, NHANES 2009–2018. **Table S2:** Baseline characteristics of 26 818 U.S. adults stratified by neutrophil percentage- to-albumin ratio (NPAR) quartiles, NHANES 2009–2018. **Table S3:** Baseline characteristics of 26 818 U.S. adults stratified by urinary albumin concentration tertiles, NHANES 2009–2018.

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REVIEW

Systematic Review on Artificial Intelligence in Rheumatology Practice: From Implementation Concerns to Imaging and Clinical Application

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ABSTRACT

Background: The integration of artificial intelligence (AI) into healthcare has shown significant promise in addressing complex diagnostic and therapeutic challenges in rheumatology. This review examines the current state of AI applications across rheumatological practice.

Objective: To systematically evaluate AI applications in rheumatology, assess their clinical performance and identify future research directions.

Methods: We conducted a comprehensive literature review of AI applications in rheumatology, focusing on diagnostic imaging, clinical decision support and disease monitoring across major rheumatic conditions.

Results: AI demonstrates promising performance across multiple domains, with diagnostic accuracies frequently exceeding 80%–90% for imaging interpretation and disease classification. Applications span from automated radiographic scoring to real-time disease monitoring.

Conclusions: While AI shows significant potential in rheumatology, successful clinical implementation requires addressing challenges related to data quality, algorithm transparency and clinical integration.

1 | Introduction

Rheumatology comprises a heterogeneous group of autoimmune and inflammatory disorders that pose substantial diagnostic and therapeutic challenges. The variability in clinical presentation and disease course demands advanced methods for diagnosis, monitoring and treatment optimization. Artificial intelligence (AI), particularly machine learning (ML), offers powerful capabilities for integrating multimodal data, such as imaging, biomarkers and electronic health records, to improve diagnostic accuracy, enable earlier detection and support personalized therapeutic strategies in rheumatology [1, 2] The field is especially well-suited for AI applications due to the availability of rich multimodal datasets, the chronic nature of many conditions requiring longitudinal assessment and the standardized imaging protocols that provide clear targets for algorithm development.

2 | Scope and Objectives

This review provides a comprehensive assessment of current AI applications across rheumatology, spanning diagnostic imaging, laboratory analysis, clinical decision support and patient monitoring. It synthesizes evidence on AI use in the major rheumatic diseases, including rheumatoid arthritis (RA), vasculitis and connective tissue disorders. It highlights emerging applications in specialized domains such as capillaroscopy and

Practitioner Points

- AI demonstrates strong diagnostic performance across multiple rheumatologic applications, including automated radiographic scoring (AUC 0.71–0.97), ultrasound- based synovitis quantification, and MRI interpretation for inflammatory lesions, often matching or exceeding expert- level accuracy. However, most studies exhibit a moderate- to-high risk of bias and limited adherence to reporting standards, emphasizing the need for rigorous external validation before clinical adoption.
- Successful clinical integration requires addressing practical implementation challenges beyond algorithmic performance, including seamless integration into electronic health record systems, mitigation of alert fatigue, adequate clinician training and workflow compatibility. Current implementations remain in pilot phases, with substantial barriers related to interoperability, user acceptance and organizational change management.
- Evidence linking AI implementation to improved patient outcomes remains limited. While AI tools show promise for earlier disease detection, flare prediction and treatment optimization, prospective studies demonstrating measurable improvements in clinical endpoints, such as disease progression rates, functional outcomes, quality of life, or healthcare utilization are absent. Future research must prioritize patient- centered outcome measures alongside technical performance metrics.

ultrasound. The analysis addresses both technical considerations, such as accuracy metrics and validation strategies, and practical challenges related to clinical integration. The strengths and limitations of existing AI methodologies are evaluated, and unmet needs in the literature are identified, offering direction for future research and pathways toward effective clinical implementation.

3 | Methodological Approach

The review methodology adhered to the Preferred Reporting Items for Systematic Reviews and Meta- Analyses guidelines and followed established methodological standards for evidence synthesis in digital health and medical artificial intelligence research. The research question was formulated according to the Population–Intervention–Comparator–Outcome framework. The population comprised patients affected by inflammatory and systemic rheumatic diseases; the intervention corresponded to AI- based analytical approaches, including machine learning, deep learning, radiomics and natural language processing models; the comparator consisted of conventional clinical evaluation or expert interpretation; and the outcomes included diagnostic performance, predictive accuracy, risk stratification capability and clinical decision- support effectiveness. A comprehensive literature search was performed in PubMed, Scopus and Web of Science databases to identify relevant studies published between January 2020 and March 2025. The search strategy combined Medical Subject Headings and keywords related to artificial intelligence technologies and rheumatology. Search terms included 'artificial intelligence,' 'machine learning,' 'deep learning,' 'neural networks,' 'radiomics,' and 'natural language processing,' combined with disease- specific terminology such as 'rheumatoid arthritis,' 'vasculitis,' 'spondyloarthritis,' 'systemic sclerosis,' 'psoriatic arthritis,' 'connective tissue diseases,' and imaging- related terms including 'ultrasound,' 'magnetic resonance imaging,' 'computed tomography,' and 'capillaroscopy.' Study selection was conducted using a sequential screening process involving title review, abstract assessment and full- text evaluation. Two independent reviewers screened all records for eligibility. Discrepancies were resolved through discussion and consensus. When agreement could not be reached, a third reviewer adjudicated the decision. Studies were eligible for inclusion if they investigated the application of AI techniques within rheumatology and reported original quantitative data derived from clinical cohorts, imaging datasets, registries, or electronic health records. Eligible studies were required to report objective performance metrics, accuracy, sensitivity, specificity, precision and recall. Studies were excluded if they lacked complete methodological description or had insufficient methodological transparency, preventing quality appraisal. Two reviewers independently performed data extraction to ensure the accuracy and reproducibility of collected information. Figures 1 and 2.

4 | What's the Matter?

A variety of ML methodologies have been widely adopted in the medical domain.

4.1 | Linear Regression

Linear regression, one of the most fundamental ML models, approximates the relationship between one or more numerical inputs and a continuous output using a linear equation; in multivariate regression each input feature carries a coefficient reflecting its contribution to the output [2], and the discrepancy between predicted and actual values is captured by residuals.

4.2 | Logistic Regression

Despite its name, logistic regression is used primarily for classification, estimating the probability of a categorical outcome from one or more input features [2]. Rather than a linear function, it applies the sigmoid (logistic) function, mapping inputs onto an S- shaped curve between 0 and 1; this probabilistic output suits binary classification and can be extended to multiclass problems.

4.3 | Decision Trees and Random Forests

Decision trees are a supervised method used for classification but also for regression [2]. From a root node, the data are recursively partitioned by the feature that best separates the classes, producing a hierarchy of decision and terminal (leaf) nodes that maximizes within- subset homogeneity and assigns the final

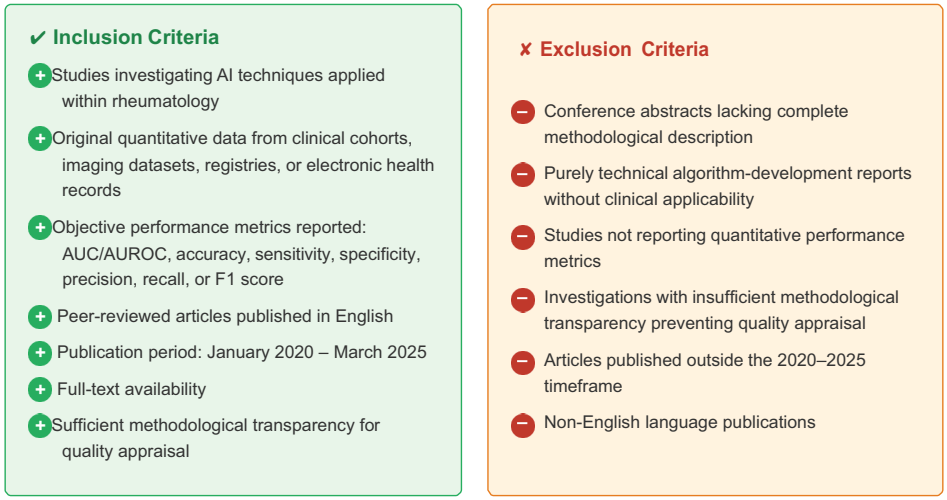

FIGURE 1 | Study selection process according to PRISMA 2020 guidelines. Records were identified through database searching (PubMed, Scopus, and Web of Science; $n = 2047$) and manual reference screening ($n = 47$). After duplicate removal, records were screened by title and abstract, and 75 studies were included in the qualitative synthesis.

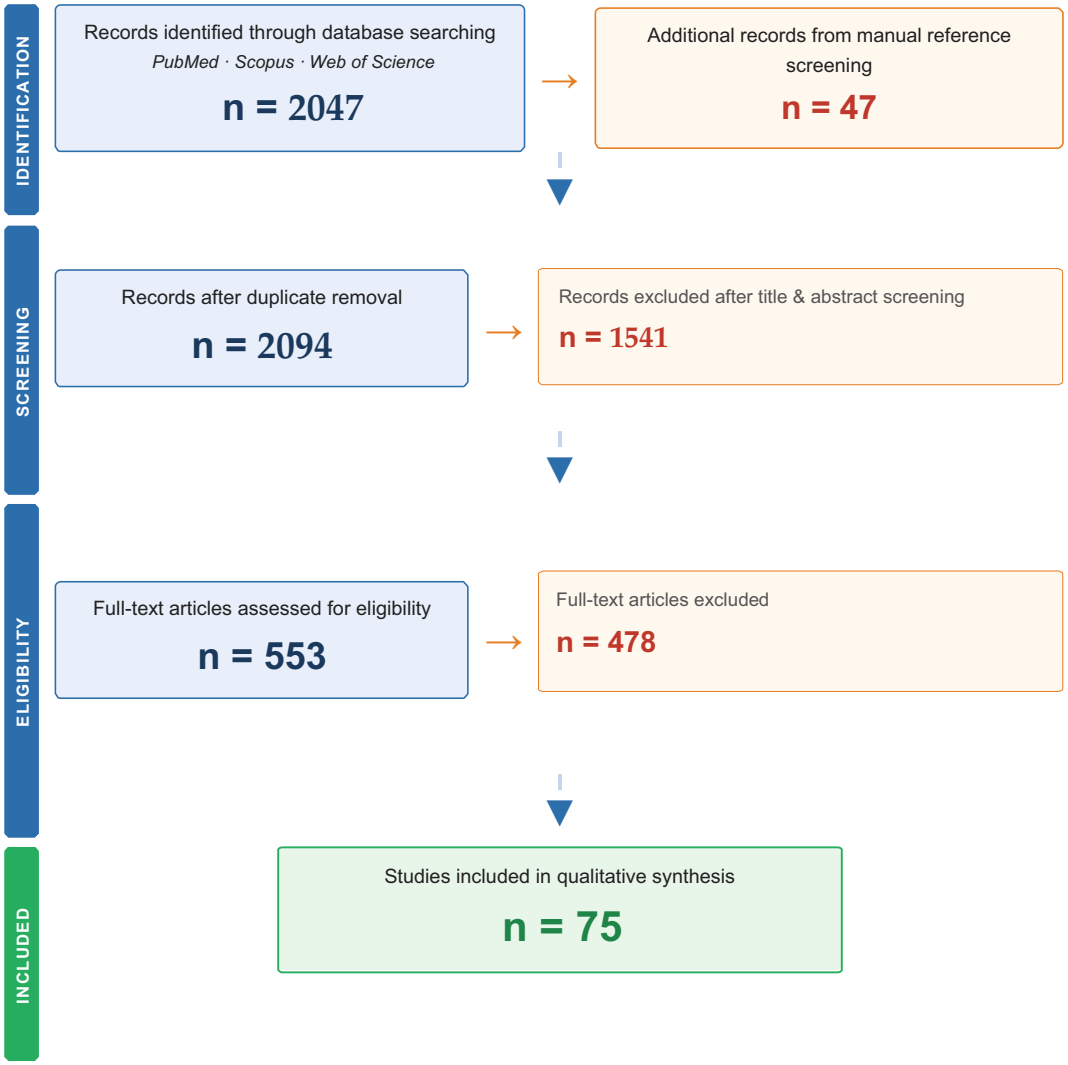

FIGURE 2 | Eligibility criteria for study selection. Inclusion criteria comprised peer- reviewed, English‐language studies (2020–2025) investigating artificial intelligence applications in rheumatology with original quantitative data and reported performance metrics. Studies lacking methodological transparency, clinical applicability, or quantitative outcomes were excluded. Discrepancies were resolved by consensus, and unresolved cases were adjudicated by a third reviewer.

label. Random forests extend this idea through ensemble learning: many trees built on different feature subsets and samples vote on the prediction, improving generalizability and reducing overfitting.

4.4 | Artificial Neural Networks (ANNs)

Artificial Neural Networks (ANNs) are inspired by the architecture of biological neural systems [3]. Interconnected nodes (analogous to neurons) transmit signals through weighted connections whose strengths are adjusted, following Hebbian principles, according to the correlation of node activations to improve predictive accuracy.

4.5 | Convolutional Neural Networks

For image data, Convolutional Neural Networks (CNNs) are a specialized class of ANNs that preserve spatial relationships between pixels [3]. Rather than treating each pixel independently, CNNs apply localized filters (convolutional kernels) across small patches of the image; each filter detects features such as edges or patterns through element- wise matrix multiplication, producing a feature map [4]. Successive convolutional layers extract increasingly abstract features, which are then passed to a feed-forward ANN for final classification [3]; this multi- layered process is a hallmark of DL [2].

5 | From Diagnosis to Treatment Optimization

5.1 | Arthritis

The diagnosis of RA continues to pose significant challenges, despite advancements in clinical methodologies. Symptoms such as arthralgia, morning stiffness and joint swelling frequently overlap with those of other musculoskeletal conditions, complicating differential diagnosis. Current diagnostic strategies typically combine clinical evaluation, serological assays and imaging modalities such as radiographs and ultrasonography. Nevertheless, these tools are not without limitations. Serological tests may yield false- negative results in the early disease phase, although imaging findings do not always show the state of inflammation [1, 5]. Furthermore, variability in clinical experience and the subjective interpretation of imaging contribute to inconsistencies in diagnostic accuracy. Despite updated diagnostic frameworks, delays and misdiagnoses remain prevalent, particularly in atypical or early- stage presentations. The management of RA necessitates continuous monitoring and dynamic treatment modifications in response to disease activity or adverse effects [6]. These persistent challenges have spurred growing interest in AI to improve the speed and accuracy of diagnostic and therapeutic decisions. Early diagnosis refers to the identification of a pathological condition at its initial phase before the manifestation of advanced symptoms or irreversible sequelae. In RA, early detection is critical for preventing structural joint damage and functional decline [7]. Research has shown that ML algorithms can synthesize clinical data, including patient- reported outcomes, serological tests and inflammatory biomarkers, to assess the likelihood of RA development in individuals with undifferentiated arthritis [8]. In RA, continuous monitoring is essential to optimize therapeutic efficacy and prevent complications. AI- enabled digital health platforms, including smartphone applications and wearable technologies, allow for real- time data acquisition on symptomatology, physical activity and inflammatory status [9]. For example, wearable devices equipped with biosensors can measure joint mobility and capture self- reported pain metrics. AI algorithms analyzed data streams to generate automated, individualized disease activity reports that can be transmitted to healthcare providers [10]. Moreover, digital tools such as chatbots and virtual health assistants can enhance patient self- management by supporting adherence to treatment regimens and facilitating symptom tracking [11, 12]. O'Neil et al. used ML to detect proteomic signatures linked to clinical remission and imminent flares in RA patients. Within the RETRO cohort of 130 individuals, 1307 serum proteins were quantified at baseline. Although clustering alone did not accurately predict flare, an XGBoost model trained on the baseline proteomic data achieved an AUC of 0.80 for predicting relapse 12 months post- medication withdrawal [13]. Meanwhile, deep learning (DL) algorithms applied to EHRs produced moderate performance (AUC = 0.7) in predicting flare severity, highlighting challenges posed by data inconsistencies across electronic records [14]. Selecting optimal RA treatments remains complex due to the extensive range of therapeutic options, including glucocorticoids, csDMARDs, tsDMARDs and bDMARDs, as well as heterogeneous patient responses and a lack of robust comparative data. AI tools hold promises for improving the prediction of both therapeutic efficacy and adverse event risks. Surendran et al. used ML to predict methotrexate- induced elevation of liver enzyme levels from EHR data, achieving high accuracy (F1 = 0.87), with baseline high- normal transaminase levels and elevated lymphocyte/neutrophil counts being major predictors [15]. A XGBoost classifier predicted 6- month non-remission in 222 DMARD- naïve RA patients initiating methotrexate, using a super learner approach combining elastic net, RnFr and SVM. This model reported AUCs of 0.75–0.76 and sensitivities of 0.77–0.81, with the Rheumatoid Arthritis Impact of Disease score identified as the strongest baseline predictor [16]. Kalweit et al. employed DL to stratify RA patients and predict their b/ts DMARD responses. Their analysis revealed that males, patients on ≥ 2 csDMARDs plus prednisone, and those with lower disease activity responded better to tocilizumab than adalimumab. Furthermore, seronegative females and seropositive females with high disease burden were less likely to respond to golimumab [17]. But a systematic review of 29 studies evaluating ML model performance and transparency in RA treatment response prediction found that adherence to TRIPOD reporting standards was moderate, with most models exhibiting moderate to elevated risk of bias. These findings stress the need for improved calibration, transparency and handling of missing data in RA- related ML research [18].

5.2 | Vasculitides

Vasculitides are a group of systemic, immune- mediated inflammatory disorders that affect blood vessels and present significant diagnostic complexities due to their heterogeneous clinical manifestations [19]. Early identification and therapeutic intervention are essential to optimize patient prognosis. In this context, AI has

emerged as a promising technology to support clinical decision-making in the management of vasculitides. In fact, prompt diagnosis is vital to minimize irreversible tissue injury and improve clinical outcomes [19]. AI methodologies, including both traditional ML algorithms, such as logistic regression, Random Forest and support vector machines (SVMs) and more advanced DL architectures, such as CNNs. They offer innovative opportunities to extract insights from complex biomedical data and support early detection efforts [20]. More recently, natural language processing (NLP) tools and large language models (LLMs) have demonstrated the capacity to interpret unstructured clinical narratives. AI- based tools can integrate and analyze multimodal data, including clinical documentation, laboratory parameters and imaging findings, thereby assisting with diagnosis, prognostication and treatment individualization [21]. Although AI has yielded encouraging results in other autoimmune contexts, its application to rare conditions like vasculitis remains underexplored. This underutilization is attributed to their low prevalence, which contributes to diagnostic delays and unfamiliarity among healthcare providers [22]. In diagnostic applications, 13 studies employed ML models to classify various forms of vasculitis. Some of these focused on Kawasaki's Disease (KD): Tsai et al. utilized XGBoost on 74 641 pediatric cases to distinguish KD from other febrile illnesses, achieving 92.5% sensitivity and 97.3% specificity using laboratory variables such as pyuria, urinary white cell count, alanine transaminase and C- reactive protein [23] and Li et al. employed LASSO regression and an SVM to differentiate KD from sepsis in 608 individuals, attaining an AUC of 0.878 with comparable clinical and biochemical features [24]. Other forms of vasculitides were similarly investigated. Vries et al. employed radiomics extracted from 18F- FDG PET scans to distinguish GCA from atherosclerosis, reaching 84% classification accuracy [25]. In Behçet disease, Güler et al. analyzed ophthalmic arterial Doppler signals from 106 participants, achieving 96.43% accuracy in healthy controls and 93.75% in patients using a multilayer perceptron [26]. Nie et al. applied XGBoost to distinguish abdominal Henoch–Schönlein purpura from acute appendicitis in 6965 pediatric cases, attaining 82.4% accuracy with 53 laboratory metrics [27]. Broader-spectrum studies were also conducted; Ryyppö et al. implemented ML models to identify vasculitis, myositis and glomerulonephritis across 114 897 individuals, including 2919 vasculitis cases, achieving a true positive rate of 76.7% and true negative rate of 98.4% via XGBoost [28]. In forecasting complications in GCA, Venerito et al. used Random Forest to identify flares after glucocorticoid tapering in 107 GCA patients, achieving 71.4% accuracy and an AUC of 0.76, surpassing logistic regression and decision trees [29]. For Behçet- related vision complications, Hammam et al. used XGBoost on 1094 patients, achieving an AUC of 0.85, 85% sensitivity and 86% specificity [30].

5.2.1 | AI in Dermatological Manifestations

5.2.1.1 | Psoriasis and Automated Assessment. Over the past two decades, research has focused on developing AI platforms to alleviate the clinical burden of psoriasis assessment. Significant advancements have occurred in integrating image segmentation with DL architecture. A 2019 investigation demonstrated that CNN, combined with automated lesion segmentation, can accurately distinguish psoriatic from non- psoriatic skin, achieving 94.8% accuracy even with confounding features such as hair or ill- defined lesion borders [31].

5.2.1.2 | Systemic Sclerosis Applications. In Systemic Sclerosis (SSc), AI has been deployed to enhance diagnostic stratification. Early applications involved neural network- based algorithms to quantify cutaneous thickening in morphea, improving diagnostic sensitivity [32]. AI- driven tools show promise for resource- constrained environments, with pilot projects training CNNs on smartphone- derived facial images to identify phenotypic features indicative of SSc, supporting early referral and triage by non- specialists [33].

5.2.2 | Radiological Applications

5.2.2.1 | Conventional Radiography. Conventional hand radiographs remain fundamental for identifying radiographic abnormalities in rheumatology. Artificial neural networks (ANNs) and DL approaches have shown promise in replicating expert- level scoring while reducing the use of manual interpretation. Izumi et al. designed a deep neural network model to detect wrist subluxation and ankylosis as part of an automated modified total Sharp score (mTSS) evaluation system, achieving a high AUC of 97% despite training on a limited dataset [34]. Radke et al. enhanced erosion detection by training RetinaNet models using adaptively adjusted Intersection over Union (IoU) thresholds, achieving 94% classification accuracy and mAP of 0.81, markedly outperforming static IoU models [35]. The RA2- DREAM Challenge facilitated the development of ML models capable of reliably quantifying joint damage based on radiographs, achieving high concordance with expert- assessed SvH scores (ranging from 0.71 to 0.82) [36]. Venäläinen et al. introduced and externally validated the AuRA (Automated RA Scoring Algorithm), developed during the same challenge. Trained on 367 radiographs and tested on an independent cohort of 205, AuRA yielded superior predictive accuracy compared to the two top RA2- DREAM models and demonstrated robust longitudinal correlation with expert scores [37].

5.2.2.2 | Computed Tomography. AI-driven classification and quantification methods in CT imaging are expected to outperform traditional visual assessment by improving reproducibility and enabling quantification of subtle lesions [38]. Recent years have witnessed increasing AI implementation in interstitial lung diseases (ILDs), with substantial literature addressing CT- based diagnosis [39, 40], quantification of parenchymal abnormalities [41] and prognostication via quantitative CT metrics [42]. These imaging biomarkers are pivotal for tracking disease progression [43]. Connective tissue diseases (CTDs) are systemic autoimmune disorders characterized by aberrant immune activation, leading to chronic inflammation and damage to the body's connective tissues. This group includes conditions such as RA, systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), idiopathic inflammatory myopathies, namely dermatomyositis (DM) and polymyositis (PM), SSc and mixed connective tissue disease (MCTD) [44]. Walsh et al. introduced SOFIA, a DL tool for identifying UIP patterns on CT. Using CT annotations from multiple radiologists as reference, they compared SOFIA's agreement with 91 radiologists, reporting

concordance rates of 73.3% for AI and 70.7% for human readers, suggesting AI parity with expert interpretation [45]. Given that ILD diagnosis is grounded in peripheral lung histopathology, attempts have been made to analyze corresponding CT regions (i.e., virtual wedge resections); Shaish et al. used HRCT images from 221 ILD cases, extracting approximately 500 virtual wedges per patient. Using a 16.5% threshold of wedges classified as UIP yielded 74% sensitivity and 58% specificity, highlighting potential diagnostic variability based on disease extent [46]. Some ILDs exhibit progressive fibrotic behavior and are referred to as progressive fibrosing ILD, progressive phenotype, or progressive pulmonary fibrosis [47]. The efficacy of antifibrotics has been demonstrated in trials that included fibrosis > 10% on CT and radiologic progression over time [48], necessitating accurate, reproducible fibrosis quantification. Given the limitations of visual HRCT assessment, such as inter- reader variability, various AI-based quantification tools using texture analysis, support vector machines and DL have emerged [41] Limitations inherent to AI include its task- specific nature-models cannot generalize beyond their training. Online AI systems may produce inaccurate results due to flawed input data. The 'black box' issue difficulty in interpreting AI decision logic, complicates error tracing and resolution. Interpretability thus remains a critical hurdle for AI integration into clinical workflows [1]. Presently, physicians retain ultimate responsibility for AI- assisted diagnoses, though this paradigm may evolve as technologies mature.

5.2.2.3 | Ultrasonography. Ultrasound (US), recognized as an adaptable and environmentally sustainable imaging modality, has become an essential diagnostic tool in clinical settings. Its distinctive benefits, including the absence of ionizing radiation, portability, affordability and the ability to acquire real- time images, have made it increasingly favored in routine practice. In particular, the continuous advancement of ultrasonographic technologies, such as high- frequency ultrasound, has significantly broadened their clinical applications. As a result, US is now widely adopted as one of the primary imaging modalities in rheumatology [49]. The enhanced soft- tissue resolution of US has led to its increasing utility in evaluating the musculoskeletal system. DL- based ultrasound approaches have been applied to a variety of tasks, including the diagnosis of myopathies [50] and the segmentation of muscular structures [51]. For instance, Burlina et al. introduced a CNN- based framework to classify inflammatory muscle disorders, yielding improved diagnostic performance for neuromuscular conditions [50]. When compared with traditional ML techniques, their CNN model demonstrated superior accuracy in classifying myositis subtypes: 76.2% ± 3.1% versus 72.3% ± 3.3% (normal vs. affected including inclusion body myositis, dermatomyositis and polymyositis); 86.6% ± 2.4% versus 84.3% ± 2.3% (normal vs. inclusion body myositis); and 74.8% ± 3.9% versus 68.9% ± 2.5% (inclusion body myositis vs. dermatomyositis/polymyositis) [50]. Temporal artery ultrasound (TAUS) has emerged as a promising technique for addressing this issue, with the European Alliance of Associations for Rheumatology recommending it as the preferred initial imaging modality for evaluating suspected GCA cases [52]. Although TAUS demonstrates reasonable diagnostic performance, its clinical utility is limited by only moderate interobserver reliability and the need for substantial operator training—factors that hinder its broader implementation [53]. In this context, DL methods offer a compelling opportunity to enhance diagnostic consistency and potentially reduce the learning curve associated with TAUS interpretation. Roncato et al. introduced a CNN approach specifically designed to detect the halo sign [54]. The initial phase of their model involved semantic segmentation of both transverse and longitudinal views from color Doppler and power Doppler ultrasound scans of temporal arteries. Regions of interest were delineated around arterial segments and adjacent soft tissues, with each pixel annotated as either halo- positive or halo-negative. To implement this segmentation task, the researchers employed the U- Net architecture, a CNN model specifically optimized for biomedical image segmentation tasks [54]. Final image classification (positive or negative for the halo sign) was determined based on the proportion of pixels within the predefined region labeled as halo positive. A higher density of such pixels correlated with an increased likelihood of a true halo sign being present [54]. The model's performance was evaluated on two datasets: the first consisting of images acquired by a single operator using a standardized imaging protocol, and the second comprising images collected by multiple operators employing diverse acquisition parameters. The CNN demonstrated superior accuracy on the standardized dataset (AUC 0.95) compared to the more heterogeneous dataset (AUC 0.82) [54]. These findings suggest that although training the model on a wider range of image qualities and acquisition techniques may improve its robustness, DL can also play a pivotal role in guiding real- time image acquisition. Such computer- assisted acquisition systems could help clinicians and sonographers obtain standardized and diagnostically valuable views during live ultrasound examinations. In power Doppler ultrasound imaging, the number of existing automated techniques for quantifying synovitis remains limited when compared to other imaging modalities. This is attributable to the inherent technical complexity of interpreting ultrasound images, primarily due to the high noise levels. A method combining traditional image processing approaches and ML has been proposed for the automated identification of anatomical structures such as skin, bone and synovial tissue, with the latter quantified based on region area [55]. More recently, DL models have been introduced to quantify synovitis across the entire ultrasound image. These models typically involve training CNNs using visual scoring systems as ground truth references [56].

5.3 | Magnetic Resonance

MRI has emerged as a crucial non- invasive imaging modality, offering unparalleled visualization of anatomical and pathological alterations associated with rheumatic diseases. It serves an essential function in the diagnosis and clinical management of conditions such as RA and spondyloarthritis (SpA), among others. MRI facilitates evaluation of joint and periarticular soft tissue involvement, inflammation and structural damage without the need for invasive procedures. However, interpreting MRI images remains labor- intensive, time-consuming and subject to variability both within and between observers. Furthermore, the high operational costs and limited access to MRI scanners impede their widespread use in routine rheumatological practice [57]. Advancements in AI present significant opportunities to revolutionize rheumatology by enhancing and

automating various aspects of MRI analysis [58]. SpA encompasses a spectrum of inflammatory disorders affecting the axial and peripheral skeleton. Early identification, classification and disease monitoring are imperative to prevent irreversible joint damage and functional decline. AI applications in SpA management show considerable promise, spanning early detection, disease characterization and longitudinal tracking [59]. Zheng et al. developed a DL model capable of detecting multifocal inflammatory lesions in the hips of SpA patients on MRI, achieving diagnostic performance on par with expert radiologists [60]. Bressem et al. validated a DL- based diagnostic tool in a multicenter retrospective cohort of 593 patients, achieving AUCs of 0.94 for inflammatory lesions, 0.88 for ASAS- defined inflammatory features, and 0.89 for structural changes. Sensitivity and specificity ranged from 71% to 88%, demonstrating generalizability across imaging protocols and scanner types [61]. Collectively, DL architectures have demonstrated robust utility in SpA management, frequently matching or exceeding the diagnostic accuracy of clinical experts. Schlereth et al. designed and validated an automated CNN- based scoring system to evaluate bone erosions, osteitis and synovitis in hand MRI scans of patients with RA and psoriatic arthritis (PsA) [62]. The model was trained on MRI data from 211 patients and externally validated on an independent cohort of 220 patients. It demonstrated robust diagnostic performance, achieving macro- level AUCs of 92% for erosions, 91% for osteitis and 85% for synovitis, along with Spearman correlation coefficients of 90%, 78% and 69%, respectively, when compared with expert annotations [62]. Folle et al. applied ResNet- based deep neural networks to distinguish inflammatory MRI patterns. among seropositive RA, seronegative RA and PsA, reporting area under the receiver operating characteristic curves (AUROCs) of 75%, 74% and 67%, respectively. The inclusion or exclusion of contrast- enhanced sequences and clinical metadata had minimal impact on classification performance. Interestingly, when this model was tested on psoriasis patients without clinical arthritis, it frequently classified them as PsA, implying the potential to detect early PsA-like MRI signatures before clinical manifestation [63].

5.4 | Capillaroscopy

Nailfold capillaroscopy (NFC) is a key diagnostic tool in SSc, enabling early detection of microvascular abnormalities characteristic of the capillaroscopic scleroderma pattern [61]. Structural changes, including capillary enlargement, giant capillaries, capillary loss and microhemorrhages, reflect progressive microangiopathy and determine the established early, active and late NFC stages [62]. NFC abnormalities correlate with disease activity, severity and prognosis, underscoring the importance of accurate image evaluation [63]. However, assessment remains limited by operator dependence, variability and time- consuming analysis [64]. To address these challenges, automated NFC image analysis has gained momentum. Systems such as AUTOCAPI enable fully automated evaluation within seconds, requiring no pre-processing [65], and supporting more consistent clinical and research assessments [64]. ML- based approaches have further advanced the field: the Manchester system uses a random forest model to extract quantitative vascular metrics within minutes [66], while state- of-the-art DL architectures such as Vision Transformers (ViT) [67] demonstrate excellent performance in detecting giant capillaries, microhemorrhages, capillary loss and capillary enlargement [68]. Notably, ViT models show performance comparable to human experts while offering far greater consistency and rapid processing (0.19 s per image) [68]. These tools hold promise for broad clinical deployment and may extend beyond SSc to other diseases with microvascular involvement [69].

5.5 | Human Factor

Clinicians, as the primary users of AI technologies, bring with them a range of personal experiences, cognitive styles, decision-making habits and varying levels of clinical expertise. These human elements introduce the potential for cognitive biases and reasoning errors, which must be considered during the development and deployment of AI- based diagnostic tools [64]. The dual process theory of cognition [28, 29] offers a useful framework for understanding how such biases arise [65]. This theory distinguishes between two modes of thinking: System 1, which is fast, automatic and intuitive, and System 2, which is deliberate, slower and analytical [66]. While each system serves specific cognitive functions, System 2 can, to a certain extent, oversee and regulate the output of System 1 [66]. Errors in clinical reasoning may occur during either type of cognitive processing [66]. However, it has been argued that many cognitive failures stem from excessive reliance on System 1 and an under- engaged or inactive System 2 [66]. Common biases linked to intuitive (System 1) reasoning include confirmation bias, anchoring, overconfidence, availability bias and framing effects [66]. To evaluate an individual's capacity to override intuitive but incorrect responses, the Cognitive Reflection Test (CRT) was developed [67]. This three- question assessment contrasts immediate, instinctive answers with correct responses that require analytical reasoning. Individuals who score highly on the CRT are believed to rely more on System 2 processing and to scrutinize intuitive judgments more critically [66]. Brennan et al. explored how physicians interacted with an ML model designed to assess preoperative risk and employed the CRT to characterize participants' cognitive styles [68]. The algorithm outperformed clinicians in accuracy, and physicians' decision quality improved following interaction with the model. Although not statistically significant, results suggested that those with higher CRT scores (indicating a more analytic style) were more likely to revise their decisions based on the algorithm's input than more intuitive clinicians [68]. Cognitive errors are considered the leading cause of diagnostic mistakes in medicine [69]. Many of these errors are attributed to flawed reasoning or biased judgment [69]. Over 30 cognitive biases and heuristics have been implicated in medical errors [48]. AI- based diagnostic systems may interact with human cognitive biases in many ways [70]. Some biases could be amplified; others diminished or remain unaffected depending on how the AI is employed and on the users' cognitive style. For instance, if an AI offers a diagnosis that differs from the clinician's own, it may prompt more analytical reflection [70]. Conversely, if the AI's suggestion precedes the clinician's assessment, it could result in anchoring or inhibit reconsideration of an erroneous diagnosis. If the AI confirms a pre- existing opinion, the clinician may fall victim to confirmation bias or premature diagnostic closure. Alternatively, some clinicians may disregard AI outputs altogether [70]. Nonetheless, AI introduces its own

TABLE 1 | Summary of key AI applications in rheumatology.

Clinical domain	Study	AI method	Application	Cohort size	Performance metrics
Rheumatoid arthritis					
Flare prediction	O'Neil et al. [10]	XGBoost	Predict relapse 12 months after medication withdrawal using proteomic signatures	130 patients	AUC 0.80
Treatment adverse events	Surendran et al. [12]	Machine learning	Predict methotrexate-induced liver enzyme elevation using EHR data	569 patients	F1 score 0.87
Treatment response	G. Li et al. [13]	Super learner (Elastic Net, Random Forest, SVM)	Predict 6-month non-remission in DMARD- naïve patients	222 patients	AUC 0.75–0.76; Sensitivity 0.77–0.81
Treatment stratification	Kalweit et al. [14]	Deep learning	Predict b/tsDMARD response and patient stratification	3516 patients	Stratification based on demographics and disease activity
Vasculitis					
Kawasaki disease	Tsai et al. [20]	XGBoost	Differentiate Kawasaki disease from febrile illnesses	74 641 pediatric cases	Sensitivity 92.5%; Specificity 97.3%
Kawasaki disease	Li et al. [21]	LASSO + SVM	Differentiate Kawasaki disease from sepsis	608 patients	AUC 0.878
Giant cell arteritis	Vries et al. [22]	PET radiomics	Distinguish GCA from atherosclerosis	238 patients	Accuracy 84%
Behçet disease	Güler et al. [23]	Multilayer perceptron	Analyze ophthalmic arterial Doppler signals	106 patients	Accuracy 96.43%
Henoch–Schönlein purpura	Nie et al. [24]	XGBoost	Differentiate abdominal HSP from acute appendicitis	6965 pediatric cases	Accuracy 82.4%
Multi-vasculitis classification	Ryyppö et al. [25]	XGBoost	Identify vasculitis, myositis and glomerulonephritis	114 897 patients (2919 vasculitis cases)	TPR 76.7%; TNR 98.4%
GCA complications	Venerito et al. [26]	Random forest	Predict flares after glucocorticoid tapering	107 GCA patients	Accuracy 71.4%; AUC 0.76

risks, notably automation bias, the undue reliance on automated systems, which may lead to acceptance of incorrect outputs and neglect of contradictory evidence [64].

6 | Limitations

ML in medicine faces several technical limitations that affect model performance, arising from algorithmic constraints to issues related to data quality and availability [3]. A major challenge is data scarcity, particularly in rare autoimmune rheumatic diseases, where low prevalence restricts the development of statistically robust models; multicenter collaborations and data- sharing platforms offer potential solutions to this limitation [3]. Another key concern is the lack of representativeness within training datasets, as models trained on non- diverse populations often fail to generalize across heterogeneous clinical settings. Ensuring demographic and clinical diversity is therefore essential, while institutional differences in clinical practice further compromise external validity [71]. Additionally, real- world data, unlike rigorously curated clinical trial datasets, are characterized by variability, structural heterogeneity, noise and missing values, all of which increase the risk of bias and reduce the reliability of ML outputs [72]. Crucially, the external validity and generalizability of most published models remain insufficiently established. The majority of algorithms are developed and tested within single- center cohorts, and their reported accuracy frequently deteriorates when applied to external populations because of distribution shift arising from differences in scanner hardware, acquisition and reconstruction protocols, patient case- mix, disease prevalence and local labeling conventions. Robust generalizability therefore cannot be inferred from internal cross- validation alone: it requires prospective, multicenter external validation on geographically and demographically distinct cohorts, ideally followed by prospective evaluation within the intended clinical workflow. Adherence to established reporting and appraisal frameworks, such as TRIPOD- AI for the reporting of prediction models and PROBAST for risk- of-bias assessment, would improve transparency, temper the optimistic performance estimates that characterize much of the current literature, and enable meaningful comparison across studies [73, 74].

7 | Expert Considerations

AI development can be likened to medical training: high- quality data and structured guidance are essential to achieve reliable performance, particularly in rare fields such as rheumatology. Establishing centralized, standardized data- sharing infrastructures would improve algorithm robustness, diagnostic accuracy, clinical management and trial design. However, beyond data availability, adequate development time is crucial; rapid creation of high- performing, bias-free AI systems is unrealistic (Table1).

Equally important is the recognition that AI outputs cannot be interpreted in isolation from clinical judgment. In rheumatology, diagnostic and classification criteria, definitions of disease activity and treatment recommendations are not entirely value- neutral or purely objective; they derive, at least in part, from expert consensus and collective clinical judgment [73, 75]. Consequently, the reference standards against which AI models are trained and evaluated inherit this interpretive, consensus-based character and their outputs require critical appraisal rather than uncritical acceptance. The clinician's role remains essential in contextualizing algorithmic predictions within the individual patient's history, comorbidities and preferences, in recognizing when a model is operating outside the data distribution on which it was trained, and in retaining ultimate accountability for the clinical decision. Rather than replacing expert reasoning, AI is therefore best positioned as a decision- support adjunct that augments, but does not supplant, human clinical judgment, thereby guarding against automation bias while preserving the interpretive flexibility that consensus- based rheumatologic practice demands [75].

8 | Conclusion

Artificial intelligence is progressively transforming rheumatology by enhancing diagnostic accuracy, supporting prognostic stratification and enabling personalized therapeutic strategies. Evidence demonstrates robust performance in imaging analysis and predictive modeling; however, translation into routine clinical practice remains limited. Robust external validation, standardized reporting, transparent model development and integration within clinical workflows are essential prerequisites for sustainable implementation. Future research should prioritize prospective validation studies and the evaluation of patient- centered clinical outcomes to establish the AI's impact in rheumatology. These tools should be deployed as decision-support adjuncts that assist rather than replace expert clinical judgment, which remains indispensable for the critical appraisal, contextualization and accountable use of AI- generated outputs [73, 75].

Author Contributions

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EDITORIAL

Biologics, Janus Kinase Inhibitors, and Cancer: The Comparator on Trial

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Whether Janus kinase (JAK) inhibitors raise the risk of malignancy has no single answer, because the estimate depends on the comparator against which they are judged. Concern that immunomodulatory therapy might promote malignancy has shadowed rheumatology for two decades, yet the accumulated evidence yields two different messages. For biologic disease-modifying antirheumatic drugs (bDMARDs), randomized trials and registries, including studies of patients with prior cancer, have been largely reassuring. Early lymphoma signals during tumor necrosis factor inhibitor (TNFi) therapy were difficult to separate from the disease itself, because rheumatoid arthritis (RA) raises lymphoma risk in proportion to inflammatory activity [1]. Once disease-driven confounding was addressed, pooled randomized trials showed no significant excess malignancy with bDMARDs versus conventional synthetic DMARDs (csDMARDs) or placebo [2]. Reassurance extended to patients with prior malignancy, in whom bDMARDs were not associated with new or recurrent cancer [3], and contemporary registry data in patients without prior cancer are concordant: the BIOBADASER III analysis found no increased cancer risk across non-TNFi bDMARDs and targeted synthetic DMARDs compared with TNFi [4].

JAK inhibitors reopened the question. In ORAL Surveillance, a regulator-mandated, open-label trial enrolling patients with RA aged 50 years or older with at least one cardiovascular risk factor, tofacitinib failed to meet noninferiority against a TNFi for malignancy, with adjudicated cancers excluding nonmelanoma skin cancer occurring in 4.2% versus 2.9% of patients [5, 6]. Regulatory boxed-warning updates for relevant JAK inhibitors followed [7]. This uncertainty, and the need to interpret cancer signals cautiously rather than as a fixed class label, has been noted previously [8]. Yet subsequent synthesis complicated the headline: a meta-analysis across 78 randomized trials and long-term extension studies spanning joint, skin, and bowel disease found no clear excess malignancy for JAK inhibitors compared with placebo or methotrexate, but a higher incidence when compared with a TNFi [9]. These are three distinct comparisons rather than a single verdict on the class; a non-significant difference against placebo or methotrexate is not proof of equivalence. A larger Bayesian network meta-analysis, spanning 305 randomized trials and long-term extension studies and more than 160 000 participants with rheumatoid arthritis, psoriatic disease, or inflammatory bowel disease, has since reported higher relative estimates for all malignancies, including nonmelanoma skin cancer, with JAK inhibitors versus both a TNFi and standard care, defined as placebo or methotrexate monotherapy; the modeled absolute excess versus a TNFi was negligible in standard-risk populations but clinically meaningful in higher-risk populations [10]. The comparator governs the relative estimate; the baseline risk of the patient governs what that estimate means. A simplified mechanistic contrast between JAK inhibition and TNF blockade is shown in Figure 1; the schematic is intended to orient readers to therapeutic level and signaling scope, not to explain comparative malignancy-risk estimates mechanistically. Real-world cohorts have been mixed [11], and evidence from inflammatory bowel disease has largely not reproduced

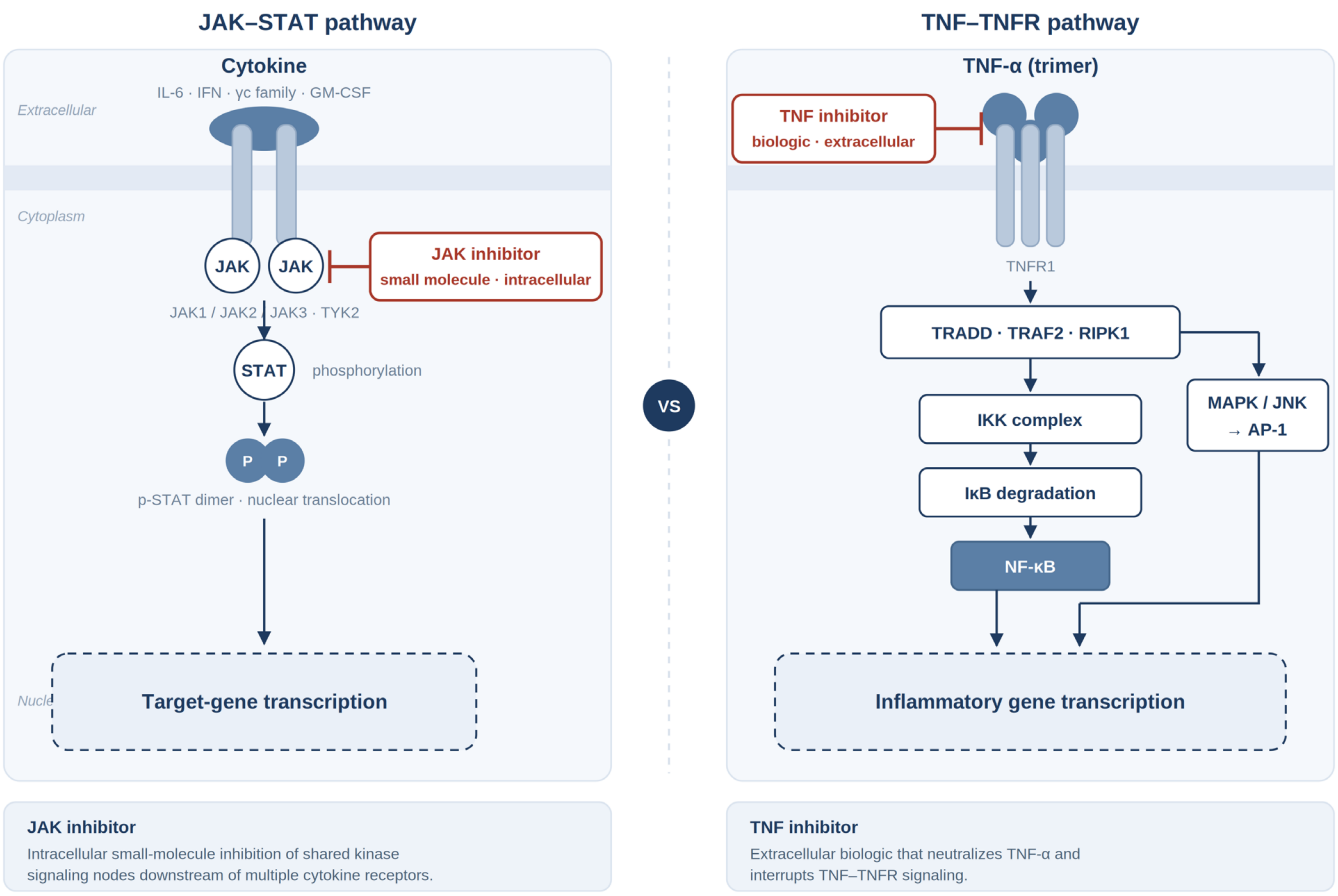

FIGURE 1 | Original, author- directed schematic contrasting JAK inhibitors and TNF inhibitors, based on the established JAK–STAT and TNF–TNFR signaling literature; the TNF arm depicts representative canonical TNFR1 signaling. JAK inhibitors are intracellular small molecules that block shared kinase signaling nodes (JAK1/2/3, TYK2) downstream of multiple cytokine receptors, attenuating STAT- dependent transcription. TNF inhibitors are extracellular biologics (monoclonal antibodies or a soluble receptor) that neutralize TNF- α, thereby interrupting representative TNFR1- dependent activation of NF-κB and MAPK/JNK–AP-1 signaling. This schematic contrasts the cellular level and signaling scope of the two therapeutic classes and does not imply that these mechanistic differences explain comparative malignancy- risk estimates. AP-1, activator protein 1; GM- CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IKK, IκB kinase; IL, interleukin; JAK, Janus kinase; JNK, c-Jun N- terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; p-STAT, phosphorylated STAT; RIPK1, receptor-interacting serine/threonine- protein kinase 1; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, TNFR1- associated death domain protein; TRAF2, TNF receptor-associated factor 2; TYK2, tyrosine kinase 2; γc, common gamma chain.

a consistent signal [12]. Even within RA, recent population-based work on hydroxychloroquine and malignancy illustrates that neutral safety findings require design- conscious interpretation, not just a drug- class label [13].

The central methodological lesson is that estimates which appear to conflict—no clear excess signal versus placebo or methotrexate in one synthesis, higher relative estimates versus a TNFi and versus standard care in another—are in large part statements about the comparator and the population in which they were measured. If TNFi carries a comparatively low malignancy rate, or if the enriched ORAL Surveillance population magnified a difference not visible in younger, lower- risk patients, then the inference that JAK inhibitors uniformly increase cancer risk is too blunt. The clinically relevant conclusion is not that one class is safe and the other unsafe, but that malignancy risk cannot be interpreted apart from the comparator, population, and clinical question.

Confounding by indication and channeling can manufacture or obscure safety signals in observational rheumatology when patients who differ in age, smoking, cardiovascular risk, prior malignancy, disease activity, or line of therapy are selectively directed toward particular agents, or when clinicians selectively avoid a TNFi or a JAK inhibitor after safety warnings [14]. Figure 2 sets out a comparator- conscious framework for designing and interpreting such studies, from definition of the target population through comparator selection to interpretation of the resulting estimate. The estimate reflects the comparator, enriched population, underlying disease, and confounding structure as much as the drug itself.

Guidelines have responded sensibly, moving toward individualized risk stratification rather than class- level prohibition. EULAR recommendations advise explicit appraisal of cardiovascular and malignancy risk before initiating a JAK inhibitor [15], and dedicated points to consider now address targeted therapy in patients

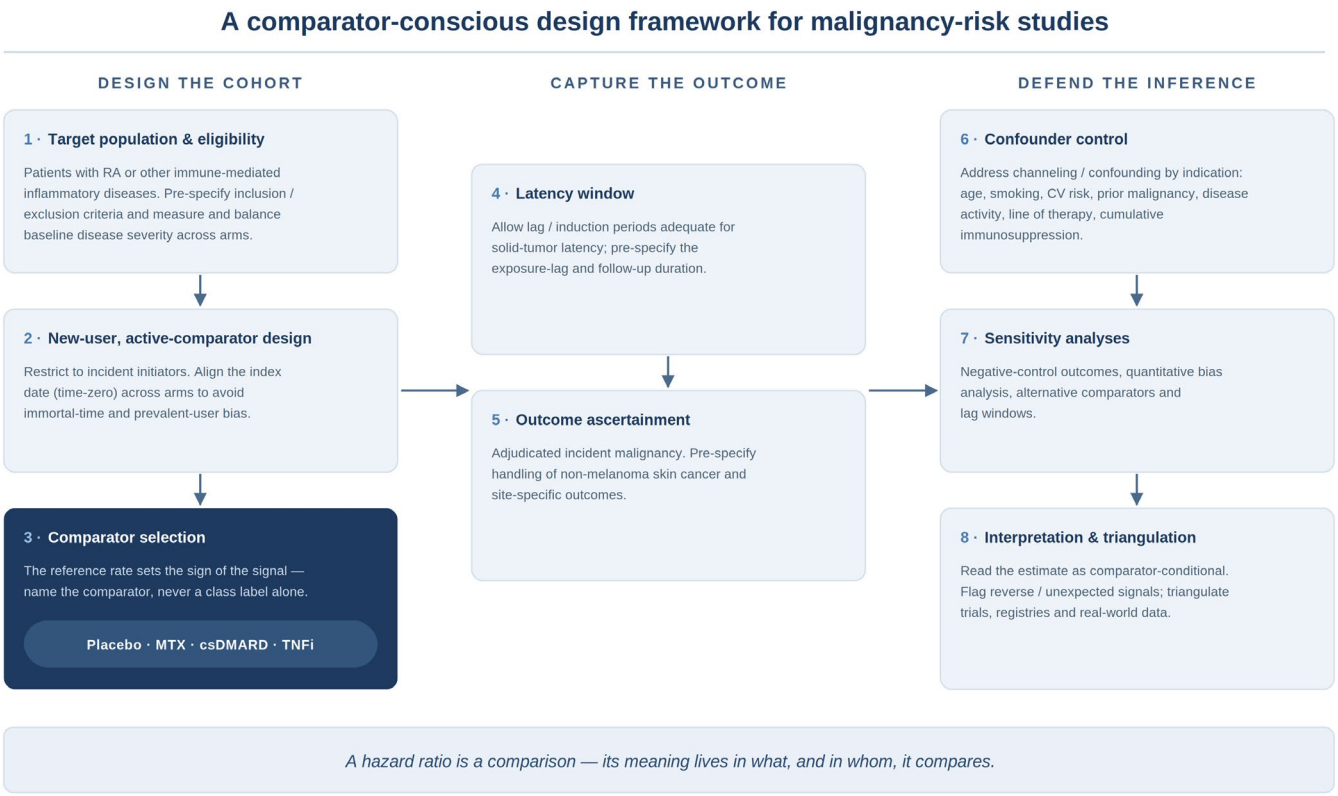

FIGURE 2 | Comparator- conscious framework for interpreting malignancy-risk estimates in targeted-therapy studies. Comparative safety questions are addressed in three stages: Designing the cohort (target population, eligibility, active- comparator new-user design, index-date alignment, and comparator selection), capturing the outcome (latency window and adjudicated malignancy ascertainment), and defending the inference (control of channeling and confounding by indication, sensitivity analyses, and interpretation of the estimate as comparator- conditional while triangulating randomized trials with registry and other real- world data). The comparator chosen—placebo, methotrexate, csDMARD, or TNFi—sets the reference rate and therefore the direction and magnitude of any observed signal. This original, author- directed framework was developed by the authors and draws on established active- comparator and new-user design principles. csDMARD, conventional synthetic DMARD; CV, cardiovascular; MTX, methotrexate; RA, rheumatoid arthritis; TNFi, tumor necrosis factor inhibitor.

with inflammatory arthritis and a history of cancer [16]. The clinically useful question is therefore not "does this class cause cancer?" but "in this patient, at this age, with this vascular and oncologic history, what is the incremental risk against the realistic alternative?" On current evidence, a pragmatic position is defensible: in patients older than 50 years with cardiovascular risk, a TNFi may remain a reasonable default when clinically appropriate, and JAK inhibitor use should involve explicit, documented risk discussion; in younger patients without these risk factors, ORAL Surveillance should not be extrapolated uncritically, although its safety signal should not be dismissed.

The unmet need is not another class- level pronouncement. We need head- to-head, adequately powered comparative data with sufficient latency for solid tumors, reported against a named comparator and population rather than as a class label. Two priorities follow. First, observational registries and databases should address the comparator question directly through active- comparator, new-user designs [17] and target- trial emulation, with transparent handling of confounding and channeling [14]. Large real- world platforms can contribute to this effort, but their role should be framed carefully. Analyses using federated electronic- record networks such as TriNetX and population- wide claims databases such as Taiwan's National Health Insurance Research Database can emulate head- to-head comparisons we are unlikely to test in trials, yet these data sources typically lack key confounders—smoking intensity, screening behavior, disease activity, line of therapy, and cumulative immunosuppression—and remain vulnerable to residual channeling. Such platforms are therefore best suited to signal detection, hypothesis generation, and triangulation with registry and trial data, rather than serving as standalone arbiters of causal cancer risk, and their structural limitations must be made explicit [18]. Recent Asia-Pacific work comparing JAK inhibitors with TNFi for uveitis risk in spondyloarthritis and psoriatic disease illustrates how target- trial emulation can sharpen comparative safety questions, provided such caveats are respected [19]. Second, the field should move from average class effects toward individual risk prediction, integrating age, smoking, vascular risk, prior malignancy, disease activity, and competing treatment alternatives. Until then, the honest reading of the bDMARDs- versus-JAK-inhibitor story is neither alarm nor reassurance but precision: a hazard ratio is a comparison, and its meaning lives in what, and in whom, it compares.

Author Contributions

Shih- Tsung Chang: conceptualization, literature review, visualization, original draft preparation, review and editing. Teng- Chieh Hsu:

conceptualization, supervision, critical revision for important intellectual content, review and editing. Both authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Ethics approval was not required because this article does not report original human- subject research.

Conflicts of Interest

The authors declare no conflicts of interest.

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No new data were generated or analyzed in support of this work.

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LETTER TO THE EDITOR

Herpes Zoster Vaccination and Dementia Occurrence-Correspondence

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To the Editor,

We read with interest the article by Pomirchy et al. reporting a quasi-experimental study that leverages a regression discontinuity (RD) design to demonstrate reduced incident dementia diagnoses following herpes zoster (HZ) vaccination eligibility in Australia, replicating prior findings from Wales [1]. While the RD approach strengthens causal inference, several limitations warrant consideration.

First, while RD designs rely on the assumption of local randomization at the cutoff, they may not fully eliminate selection bias. We argue that unobserved behavioral shifts or residual lifestyle differences near that cutoff may still occur. Individuals who seek vaccination often concurrently engage in cognitively protective behaviors, such as regular physical activity, balanced diets, and proactive healthcare utilization that are seldom captured in electronic health records yet independently associated with dementia risk [2, 3]. Although no abrupt discontinuities in preventive service use or comorbid diagnoses were observed at the eligibility cutoff, residual lifestyle differences may inflate the apparent protective effect of HZ vaccination.

Second, the analysis does not adjust for prior HZ infection history or underlying varicella-zoster virus (VZV) exposure. Viral reactivation, rather than vaccination status alone, may mediate neuroinflammation and vascular injury contributing to dementia pathogenesis [4]. In the absence of serologic or clinical infection data, it remains difficult to disentangle the vaccine's protective role from confounding by infection susceptibility.

Third, baseline cognitive status before vaccine eligibility was not assessed. Early cognitive decline may reduce vaccination uptake through impaired decisional capacity, limited healthcare access, or provider hesitancy [5]. If individuals with subtle, undiagnosed impairment were less likely to receive HZ vaccination and more likely to develop dementia, reverse causation or selection bias could influence the observed association.

In conclusion, to improve causal attribution and inform policy, future studies should integrate behavioral health proxies, infection history, and baseline cognitive assessments into RD analytic frameworks.

Author Contributions

Hao-Yun Chen: writing – original draft. Yan-Yu Liu: writing – original draft. Su-Boon Yong: writing – review and editing.

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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.

Abbreviations: HZ, herpes zoster; RD, regression discontinuity; VZV, varicella-zoster virus.

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ORIGINAL ARTICLE

Immunosuppressant Use Mediates Neuroprotection Against Alzheimer's Disease in Rheumatoid Arthritis—A Two-Step Mendelian Randomization and NHANES Study

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ABSTRACT

Objectives: To investigate whether immunosuppressant (IS) use contributes to the inverse association between rheumatoid arthritis (RA) and Alzheimer's disease (AD).

Methods: We analyzed NHANES 2011–2014 data, including 217 RA patients aged ≥60 years on prescription medications. Cognitive function was assessed using the Digit Symbol Substitution Test (DSST), CERAD, Animal Fluency Test (AFT), and a global cognition *z*-score (*Z*-score). Associations between IS use and cognition were evaluated using multivariable linear regression. Additionally, two-sample Mendelian randomization (TSMR) and multivariable MR (MVMR) analyses were performed with GWAS datasets for RA, AD, and IS to examine potential causal effects.

Results: We observed that patients with RA using IS performed better in *z.DDST* (β: 0.335, $p = 0.036$) and *Z*-score (β: 0.214, $p = 0.032$) after adjusting for covariates, with sex-specific differences in cognitive domains. TSMR indicated that genetically predicted RA was associated with lower AD risk (OR: 0.936, $p = 4.531\text{E}{-}04$), and this effect was largely mediated by IS use. MVMR further validated the independent neuroprotective effect of IS on AD (OR = 0.884, $p = 0.003$) after adjusting for glucocorticoid and NSAID use, while these other medications showed no significant association with AD risk.

Conclusions: These results suggest that the reduced risk of AD observed in RA patients may be partly related to IS use, highlighting a potential role of IS in improving cognitive function and modulating AD risk.

1 | Introduction

Alzheimer's disease (AD) is the most common cause of dementia, affecting nearly 7 million Americans aged 65 and older and posing a considerable societal and economic burden [1]. Chronic inflammation in the brain has been implicated in neuronal damage, where oxidative stress and apoptosis contribute to the cognitive decline that characterizes AD [2]. Autoimmune diseases may influence the risk of developing AD, but studies so far have reported conflicting findings [3, 4].

Abbreviations: AD, Alzheimer's disease; AFT, the Animal Fluency Test; Aβ, amyloid β; CERAD, the Consortium to Establish a Registry for Alzheimer's Disease Word Acquisition Test; CI, confidence interval; DSST, the Digit Symbol Substitution Test; GC, glucocorticoids; GWAS, Genome-Wide Association Study; IS, immunosuppressants; IV, instrumental variable; IVW, inverse variance weighted; MR, Mendelian randomization; MVMR, multivariate Mendelian randomization; NCHS, the National Center for Health Statistics; NHANES, National Health and Nutrition Examination Survey; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio; RA, rheumatoid arthritis; RCTs, randomized controlled trials; TSMR, two-step Mendelian randomization; WM, weighted median; *Z*-score, global cognition *z*-score.

Rheumatoid arthritis (RA) is a systemic autoimmune disease marked by chronic joint inflammation, synovial hyperplasia, and infiltration of immune cells [5]. The disease arises from abnormal activation of both innate and adaptive immune responses, leading to persistent local and systemic inflammation [5]. Because chronic inflammation is a feature shared by RA and AD, RA would intuitively be expected to increase the risk of AD [1, 6]. Surprisingly, several recent studies suggest the opposite: RA patients may have a lower risk of developing AD [7, 8]. Understanding the factors behind this unexpected inverse relationship is therefore of great interest.

Treatment for RA aims to reduce inflammation, with immunosuppressants (IS), nonsteroidal anti- inflammatory drugs (NSAIDs), and glucocorticoids (GC) forming the mainstay of therapy [9]. Evidence from observational studies suggests that DMARDs, particularly TNF blockers, may slow cognitive decline or reduce the risk of AD in patients with autoimmune diseases [10, 11]. These findings raise the possibility that immunosuppressive therapy itself could contribute to the lower observed AD risk among RA patients.

Nevertheless, current observational studies suffer from multiple inherent limitations, such as limited sample scale, reverse causality and unadjusted confounding factors. As a powerful analytical approach, Mendelian randomization (MR) leverages genetic variants as instrumental variables to infer causal relationships between clinical indicators and disease phenotypes [12]. It can resolve conflicting findings from observational evidence and make up for the insufficient data derived from randomized controlled trials. In particular, the two- step Mendelian randomization method (TSMR) can examine a factor whether it mediates the association between exposure and outcome [13]. Multivariate Mendelian randomization (MVMR), on the other hand, can be used to simultaneously assess estimates of multiple exposures with potential pleiotropic effects on one outcome [14].

Based on the above theoretical foundation, we first analyzed data from the National Health and Nutrition Examination Survey (NHANES) to examine associations between IS use and cognitive performance in RA patients, controlling for potential confounders. To strengthen causal inference, we also conducted two- sample MR analyses using genome-wide association study (GWAS) data to assess potential causal links between RA and AD, with and without medication- related instrumental variables. A TSMR approach was used to investigate whether IS mediates the RA–AD relationship, and MVMR analyses accounted for pleiotropic effects to estimate the direct effect of IS on AD. This combined observational and genetic approach allows for a more comprehensive understanding of the potential role of immunosuppressants in modulating AD risk in RA patients.

2 | Methods

2.1 | Study Design

This study consisted of two complementary components. First, a population- based observational analysis using NHANES 2011–2014 data was conducted to investigate the association between IS use and cognitive function in patients with RA. Second, a MR framework, incorporating TSMR and MVMR approaches, was used to explore whether genetically predicted RA influences AD risk and whether any such association is mediated by IS use.

2.2 | Population-Based Observational Study

2.2.1 | Study Population

Participants aged ≥ 60 years from two NHANES cycles (2011–2012 and 2013–2014) who self- reported RA, had prescription medication records, and completed all cognitive tests were included ($N = 217$; Figure 1). NHANES is a nationally representative survey of non- institutionalized U.S. adults, including interviews and physical examinations. The study was approved by the NHANES Ethics Review Board, and all participants provided informed consent. As NHANES does not capture clinical AD diagnoses, cognitive tests were used as proxy measures of neurocognitive status. The NHANES data used in this study are publicly available at https://www.cdc.gov/nchs/nhanes/. Data were accessed on January 6, 2025. All NHANES data are de-identified and publicly accessible.

2.2.2 | Cognitive Function

Cognitive performance was assessed using the CERAD Word Learning Test (immediate [CERAD- IRT] and delayed recall [CERAD- DRT]), Animal Fluency Test (AFT), and Digit Symbol Substitution Test (DSST). Individual scores were standardized into *z*- scores, and a global cognition *z*-score (*Z*- score) was calculated as the mean of the three normalized scores [15].

2.2.3 | Rheumatoid Arthritis

RA status was determined from self- reported physician diagnosis. Participants answering "yes" to having arthritis were further asked to specify the type. Only those reporting RA were included.

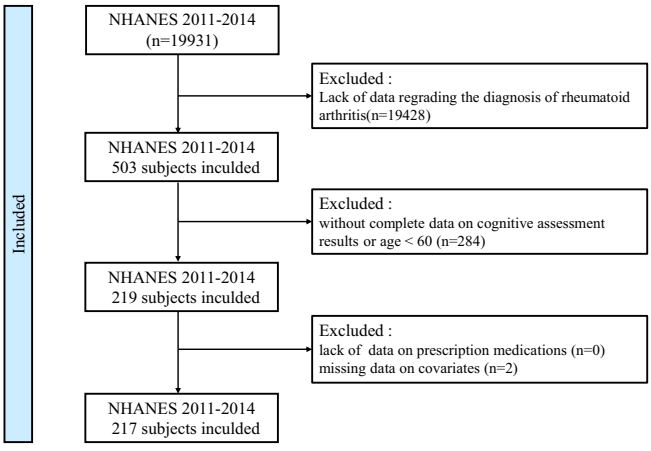

FIGURE 1 | Flowchart of subject selection.

2.2.4 | Use of Immunosuppressants

In NHANES, participants were asked during the interview if there were any prescription medications they had taken in the past 30 days. Those who answered "yes" were asked to show the investigator the containers of all medications. If no container was available, participants need to provide the name of the medication. We defined a participant as using IS if he/she was using any of the following medications: hydroxychloroquine, methotrexate, mycophenolate, azathioprine, tacrolimus, adalimumab, leflunomide, sirolimus, and cyclosporine.

2.2.5 | Covariates

According to previous studies, we included several factors that are risk factors for cognitive functioning as covariates [16]. Covariates used in this study included age (60–79 and 70–79), gender (male and female), marital status (married/living with a partner and widowed/divorced/separated/never married), race (mexican american, non- hispanic black, non-hispanic white, other hispanic and other race), educational attainment (below high school and high school, above high school), poverty- to-income ratios (<1, 1–1.99, 2–3.99, and 4), body mass index (BMI) (normal: $<25\,kg/m^2$, overweight: 25 to $<30\,kg/m^2$, obese: $\geq 30\,kg/m^2$), smoking status (yes and no), alcohol status (yes and no), and diabetes (yes and no).

2.3 | Mendelian Randomization

2.3.1 | Data Sources

Two large RA- related GWAS datasets of Stahl et al. [17] (Cases: 13838, Controls: 33742) and Eyre et al. [18] (Cases: 5539, Controls: 20169) from European ancestry were included in this study. For AD, the GWAS datasets of Schwartzentruber et al. [19] (Total: 472868), Bellenguez et al. [20] (Cases: 39106, Controls: 46828), and Kunkle et al. [21] (Cases: 21982, Controls: 41944) were included, where the GWAS of Schwartzentruber et al. recruited AD patients as well as their family history. For IS, the GWAS summary statistics from the UK Biobank were obtained from Wu et al.'s study [22] (Cases: 17352, Controls: 188348), which also contains datasets for NSAID (Cases: 3954, Controls: 268648) and GC (Cases: 74150, Controls: 90370). GWAS summary statistics were downloaded from IEU OpenGWAS on May 20, 2025, and contained no individual- level identifiers. The GWAS datasets for RA, medication use, and AD were derived from independent study populations with no sample overlap. All of these datasets were restricted to European- ancestry individuals, and the traits were uniformly defined using consistent diagnostic criteria, ensuring comparable genetic variant–exposure associations. The full list of included studies can be found in Table S3.

2.3.2 | Selection of the Genetic Instruments

All instrumental variables (IVs) must satisfy the three core MR assumptions (Figure 2):

1. Relevance: The genetic variants are strongly associated with the exposure (RA or medication use);
2. Independence: The genetic variants are not associated with any confounding factors that influence both the exposure and the outcome (AD);
3. Exclusion restriction: The genetic variants affect the outcome only through the exposure, not via any alternative pathway.

The validity of these assumptions was assessed using multiple methods as described below. At first, we set the threshold of genome- wide significance level ($p<5\times10^{-8}$) to select single nucleotide polymorphisms (SNPs) as IVs. Secondly, to ensure the selected SNPs were independent of exposure, we excluded SNPs with strong linkage disequilibrium (LD) ($r^2<0.001$, aggregation window = 10000 kb). In addition, to minimize the effect of low confidence, we also excluded SNPs with minor allele frequency (MAF) < 0.01 or SNPs with a palindromic effect or *F*-statistics < 10.

2.3.3 | MR Analysis

We first performed two- sample MR to estimate the causal association between RA and AD, both before and after excluding SNPs associated with IS, NSAIDs, or GCs at $p<5\times10^{-8}$. Subsequently, TSMR was used to assess the mediating role of IS in the RA–AD causal pathway (Figure 2A):

- Step 1: Estimate the causal effect of RA on the use of IS, NSAIDs, and GCs;
- Step 2: Estimate the causal effect of each medication class on AD risk.

Finally, MVMR was performed to estimate the direct causal effect of IS on AD risk, adjusting for the effects of NSAIDs and GCs to account for their potential confounding and pleiotropic effects (Figure 2B). This study was not pre- registered.

2.4 | Statistical Analysis

In the first part, following the NHANES analysis guidelines, with the years 2011 through 2014, we selected "wtmec2yr" and calculated these weights with the following formula: wt = WTDR2D × 1/2. Survey weights (wtmec2yr) were applied per NHANES guidelines. Continuous variables are presented as mean ± SD, categorical as *n* (%). Participants were grouped by IS use. Multiple linear regression models were constructed:

- Model 1: unadjusted;
- Model 2: adjusted for age, sex, marital status;
- Model 3: further adjusted for race/ethnicity, education, income, smoking, alcohol, BMI, and diabetes. Variance inflation factors (VIFs) were computed to assess multicollinearity for covariates in Model 3. All estimates were below 5 (range: 1.41–2.63), with no evidence of prominent multicollinearity (Table S2).

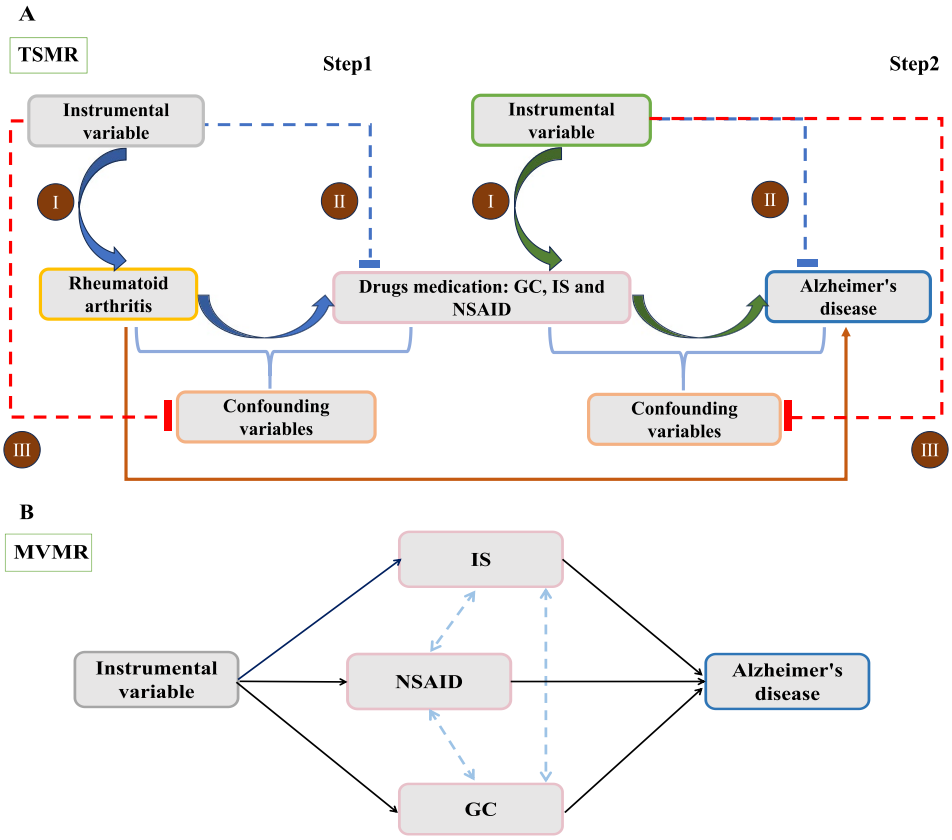

FIGURE 2 | The workflow of the TSMR and MVMR studies and the three corresponding principal hypotheses. (A) The blue arrow flow on the left shows the assumptions for Step 1, namely RA on medications (GC, IS, and NSAIDs), and the causal effect of RA on AD. The green arrow flow on the right shows the assumptions for Step 2, namely medications (GC, IS, and NSAIDs) on AD. (B) Overview of the MVMR analysis for medications (GC, IS, and NSAIDs) on AD. Assumption 1: SNPs associated with the exposure; Assumption 2: SNPs not associated with confounding factors; Assumption 3: SNPs not associated with the outcome. GC, glucocorticoids; IS, immunosuppressants; NSAIDs, non- steroidal antiinflammatory drugs; MVMR, multivariable Mendelian randomization; SNPs, single nucleotide polymorphisms; TSMR, two- step Mendelian randomization.

Two- sample MR, TSMR, and MVMR analyses were applied in the second part. The inverse variance weighted (IVW) method was the primary analysis, which provided the highest efficacy but was not sensitive to horizontal pleiotropy. To correct for polyvalence, the weighted median (WM) method and MR Egger regression were used as secondary methods [23, 24]. To assess potential heterogeneity and horizontal pleiotropy, the MR-PRESSO and Cochran *Q* tests were used [25]. A leave- one- out analysis was also performed to determine whether individual SNPs affected MR estimates. Reverse MR was conducted to evaluate potential bidirectional causality. All statistical analyses were performed with R software version 4.2.1, with two- sided $p < 0.05$ represented statistically significant results. MR analyses were conducted with the TwoSampleMR package (version 0.5.6) and the MendelianRandomization package (version 0.7.0). NHANES data were analyzed with the survey package (version 4.1- 1) to account for the complex sampling design. No formal correction for multiple testing was applied due to the exploratory nature of the analyses and the a priori hypotheses tested. Complete- case analysis was used for NHANES data, as the proportion of missing observations was < 5% for all variables. Summary- level GWAS data contained no individual-level missing data.

3 | Results

3.1 | Basic Characteristics of NHANES

This study included 217 participants in NHANES who self-reported RA. As shown in Table 1, we divided all the participants into two groups, with a mean age was 69.11 years in the group using IS and 70.15 years in the group not using IS. There were no significant differences between the two groups in the distribution of gender, age, marital status, race, education level, smoking status, alcohol status, BMI, and poverty- income ratios, but significant differences existed in diabetes mellitus history. As for the three different cognitive tests (*z. CERAD*, *z.AFT*, and *z.DSST*) and global cognition *z*- score, significant differences were only observed for *z.DSST*.

3.2 | Association Between IS Use and Cognitive Function

As shown in Table 2, IS use was significantly associated with better cognitive performance. In Model 1, better performance in cognitive function was significantly associated with the use of IS. The

TABLE 1 | Characteristics of all participants according to the use of immunosuppressants.

Characteristic	No IS (190)	IS (27)	*p*
Age	70.15 (6.163)	69.11 (7.584)	0.381
Age_group (%)			0.935
60–69	107 (56.32)	16 (59.26)	
70–79	83 (43.68)	11 (40.74)	
Gender (%)			0.773
Male	84 (44.21)	21 (77.78)	
Female	106 (55.79)	6 (22.22)	
Marital status (%)			0.368
Married/living with partner	95 (50.00)	11 (40.74)	
Widowed/ divorced/ separated/never married	95 (50.00)	16 (59.26)	
Race (%)			0.859
Mexican American	19 (10.00)	4 (14.80)	
Non-Hispanic Black	71 (37.37)	8 (29.63)	
Non-Hispanic White	68 (35.79)	11 (40.74)	
Other Hispanic	14 (7.37)	2 (7.41)	
Other Race	18 (9.47)	2 (7.41)	
Educational level (%)			0.399
Below high school and high school	69 (36.32)	7 (25.93)	
Above high school	121 (63.68)	20 (74.07)	
Poverty–income ratio (%)			0.537
< 1	66 (34.74)	10 (37.04)	
1–1.9	60 (31.58)	9 (33.33)	
2–3.9	30 (15.79)	6 (22.22)	
> 4	34 (17.89)	2 (7.41)	
Body mass index (%)			0.629
Normal	37 (19.47)	8 (29.63)	
Obese	75 (39.47)	9 (33.33)	
Overweight	76 (40.00)	10 (37.04)	
Underweight	2 (1.05)	0 (0.00)	
Smoke status (%)			1.000
Yes	29 (15.26)	4 (14.81)	
No	161 (84.74)	23 (85.19)	
Alcohol status (%)			0.858
Yes	120 (63.16)	16 (59.26)	
No	70 (36.84)	11 (40.74)	
Diabetes mellitus (%)	121 (63.68)	20 (74.07)	0.041*
z.DDST	−0.054 (0.983)	0.381 (1.048)	0.034*
z.CERAD	−0.024 (1.020)	0.167 (0.879)	0.379
z.AFT	−0.003 (0.991)	0.022 (1.080)	0.638
Z-score	−0.027 (0.786)	0.190 (0.851)	0.123

Abbreviation: IS, immunosuppressants.
*$p < 0.05$.

β coefficients for *z.DDST*, *z.CERAD* and *Z*- score were 0.535 (95% CI: 0.058–1.012), 0.318 (95% CI: 0.050–0.585), and 0.353 (95% CI: 0.085–0.622), respectively. However, after adjusting for age, gender, and marital status, only *z.DDST* and *Z*- score were revealed to be significantly higher for RA patients in the IS-used group (*z.DDST*: β 0.429, $p = 0.036$, *Z*-score: β 0.259, $p = 0.009$). After adjusting for all covariates, the positive association between the use of IS and *z.DDST* and *Z*- score remained (*z.DDST*: $p = 0.0320$, *Z*-score: $p = 0.0364$). In addition, we performed subgroup analyses, which showed that females have higher *z.CERAD*, whereas males are more advantageous in DDST (Table S1).

3.3 | Causal Effect Between RA and AD

In the two- sample MR analysis, we used the GWAS of Schwartzentruber et al. as the discovery dataset and the other two GWAS as the replication datasets. The results of the IVW method showed that genetically predicted RA was associated with a reduced risk of AD during the discovery phase (Eyre: OR = 0.936, 95% CI: 0.902–0.971, $p = 4.531E{-}04$; Stahl: OR = 0.960, 95% CI: 0.938–0.983, $p = 6.043E{-}04$) (Figure 3). Results from the weighted median method (OR = 0.942, 95% CI: 0.905–0.981, $p = 0.005$) and MR Egger regression (OR = 0.938, 95% CI: 0.887–0.992, $p = 0.026$) were consistent with the IVW results, further supporting a protective effect of RA on A risk. As demonstrated in Figure 3, the causal effect of RA on AD in the replication phase was consistent with the discovery phase. The reverse MR showed that genetically predicted AD was not associated with RA risk ($p > 0.05$) (Table S5). Leave-one-out analysis showed that no single SNP was driving the overall effect, as the OR remained stable after sequentially removing each SNP. Exclusion of SNPs with potential pleiotropic effects also did not alter the main results (Figures S2–S4). Cochran's *Q* test and the MR-PRESSO global test revealed no significant heterogeneity,

TABLE 2 | Multiple linear regression analysis of the relationship between the use of immunosuppressants and cognitive function in rheumatoid arthritis.

	Model 1		Model 2		Model 3	
	β (95% CI)	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
z.DSST						
No IS	Ref		Ref		Ref	
IS	0.535 (0.058, 1.012)	0.036*	0.429 (0.045, 0.814)	0.037*	0.335 (0.036, 0.634)	0.032*
z.CERAD						
No IS	Ref		Ref		Ref	
IS	0.318 (0.050, 0.585)	0.028*	0.202 (−0.050, 0.418)	0.080	0.219 (−0.090, 0.528)	0.143
z.AFT						
No IS	Ref		Ref		Ref	
IS	0.208 (−0.224, 0.640)	0.351	0.144 (−0.243, 0.532)	0.469	0.089 (−0.328, 0.506)	0.639
Z-score						
No IS	Ref		Ref		Ref	
IS	0.353 (0.085, 0.622)	0.015*	0.259 (0.079, 0.439)	0.009**	0.214 (0.017, 0.412)	0.036*

Note: Model 1: unadjusted model; Model 2: adjusted for age, gender, and marital status; Model 3: further adjusted for race, education, income ratio, smoking, alcohol consumption, BMI, and diabetes.
Abbreviation: IS, immunosuppressants.
*$p < 0.05$.
**$p < 0.01$.

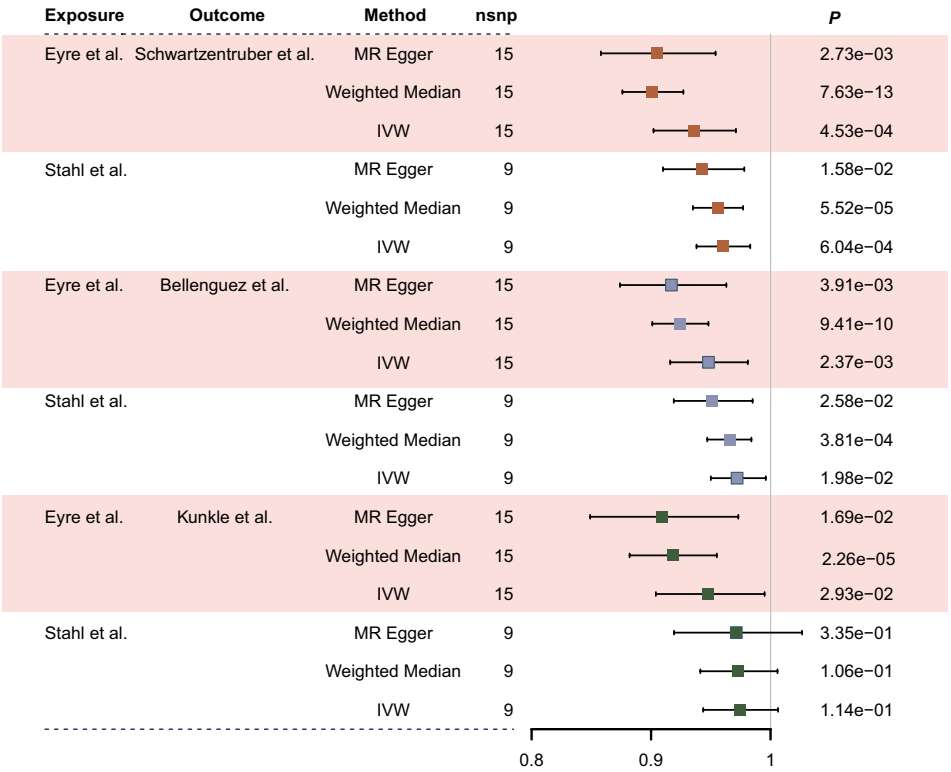

FIGURE 3 | The causal effect of rheumatoid arthritis on Alzheimer's disease in a two- sample Mendelian randomization analysis. AD, Alzheimer's disease; IVW, inverse- variance weighted; RA, rheumatoid arthritis; SNPs, single nucleotide polymorphisms; Exposure: RA: Eyre et al., Stahl et al.; Outcome: AD: Schwartzentruber et al., Bellenguez et al., Kunkle et al.

further supporting the robustness of the causal effect of RA on AD (Table S9). Full details can be found in the Supporting Information.

3.4 | Summary of the TSMR Analysis

A total of 15 SNPs were selected as IVs for RA from the Eyre et al. dataset, and 9 SNPs from the Stahl et al. dataset. For the three RA medication exposures, 4 SNPs were used for IS use, 20 SNPs for GC use, and 4 SNPs for NSAID use, respectively. The F- statistics for all IVs were greater than 10 (range: 10.2–1582.0), confirming strong instrument strength and ruling out weak instrument bias. Detailed information on all IVs is provided in Table S4.

In the first step of the TSMR, we estimated the association between RA and each of the three medications separately and showed that genetically predicted RA was associated with increased use of IS (Eyre: OR = 1.780, 95% CI: 1.664–1.903, $p = 5.581EEE{-}64$; Stahl: OR = 1.489, 95% CI: 1.398–1.585, $p = 3.530E{-}35$) and GC (Eyre: OR = 1.163, 95% CI: 1.098–1.232, $p = 2.961E{-}07$; Stahl: OR = 1.094, 95% CI: 1.059–1.130, $p = 4.372E{-}08$), while there was no causal association with NSAIDs (Eyre: OR = 1.005, 95% CI: 0.981–1.029, $p = 0.703$; Stahl: OR = 1.006, 95% CI: 0.985–1.027, $p = 0.586$) (Figure 4A). The results of the reverse MR of the three medications and RA indicated that only the use of IS was associated with an increased risk of RA development, as detailed in Table S7.

In the second step, we assessed the causal associations between the three medications and AD (Figure 4B). The results of the IVW analyses indicated that the use of IS (OR = 0.854, 95% CI: 0.882–0.887, $p = 3.451E{-}06$), not GC (OR = 0.950, 95% CI: 0.858–1.052, $p = 0.851$), or NSAIDs (OR = 0.979, 95% CI: 0.789–1.216, $p = 0.851$), was associated with a reduced risk of AD during the discovery phase. The results of the validation phase were consistent with them, with the use of IS still being associated with a reduced risk of AD (Bellenguez: OR = 0.879, 95% CI: 0.801–0.954, $p = 0.002$; Kunkle: OR = 0.843, 95% CI: 0.789–0.902, $p = 7.112E{-}07$), whereas there were no causal associations between GC/NSAIDs and AD (all $p > 0.05$). In addition, no reverse causal association was found between IS and AD (Table S8). For the TSMR analysis, no significant heterogeneity was observed for the RA–IS association (all $p > 0.05$) or the IS–AD association in either dataset (Schwartzentruber et al.: Cochran $Q = 1.951$, df = 3, $p = 0.583$; Kunkle et al.: Cochran $Q = 0.855$, df = 2, $p = 0.652$). The reliability of the TSMR analysis was demonstrated by both the forest plot and the leave- one-out analysis (Figures S5–S9).

In addition, there was no causal effect of RA on AD in either the discovery phase or the replication phase after the removal of IS- related SNPs (all$p > 0.05$) (Figure S1, Table S6). These results provide strong evidence that the inverse association between RA and AD risk is largely mediated by IS use.

3.5 | Summary of the MVMR Analysis

To exclude bias due to the existence of interactions among IS, GC, and NSAIDs, we used IS, GC, and NSAIDs as exposures simultaneously, and extracted 5, 20, and 4 SNPs as IVs for MVMR analysis, respectively. The results of the MVMR indicated that only IS use was associated with a reduced risk of AD (Schwartzentruber: OR = 0.884, 95% CI: 0.827–0.944, $p = 0.003$; Bellenguez: OR = 0.912, 95% CI: 0.853–0.976, $p = 0.008$; Kunkle: OR = 0.877, 95% CI: 0.797–0.964, $p = 0.007$) (Table S10).

For the MVMR analysis, the global Q statistic was 18.3 ($p > 0.05$), indicating no significant heterogeneity across the multiple exposures. After adjusting for the effects of GCs and NSAIDs, the direct effect of IS use on AD risk remained statistically significant, confirming that the protective effect is specific to IS and not confounded by other RA medications.

Taken together, these results demonstrate a consistent inverse causal association between RA and AD risk that is largely mediated by IS use. The findings are robust across multiple MR methods, sensitivity analyses, and independent replication datasets.

4 | Discussion

In this study, we combined observational data from the NHANES with large- scale genetic analyses to investigate the role of IS in the relationship between RA and AD. In the NHANES cohort, RA patients using IS performed better on the DSST and on global cognitive z- scores, suggesting that IS use is associated with better cognitive performance. MR analyses further supported an inverse association between RA and AD risk that was largely mediated by IS, whereas GCs and NSAIDs showed no significant protective effects against AD.

Existing MR evidence on the causal relationship between RA and AD is not entirely consistent. Some analyses report a protective effect [8, 26], while others observe a positive association [27, 28]. Such heterogeneity may stem from the confounding effect of IS therapy: uncontrolled, highly active RA may elevate AD risk, whereas effective suppression of systemic inflammation via IS could confer neuroprotection. These findings underscore the necessity of treating IS as a key mediating variable.

Chronic systemic inflammation is a shared pathological hallmark of RA and AD, and its inadequate control may drive cognitive decline [29, 30]. Our study demonstrates that IS, rather than GCs or NSAIDs, plays a critical mediating role in the RA–AD association, consistent with large- scale clinical studies reporting reduced dementia or AD risk in RA patients receiving anti- TNF therapy [10, 31]. Subgroup analyses further identified sex- specific disparities: IS use was associated with higher DSST scores in males and higher CERAD scores in females, which may be attributed to sex- divergent brain structural/functional features and differential susceptibility to inflammation-mediated cognitive impairmen[t] [32, 33].

Several complementary mechanisms may underlie the neuroprotective effects of IS. First, IS mitigate systemic inflammation, including TNF- α and other pro-inflammatory cytokines that trigger neuroinflammation and neuronal damage in AD [34]. By limiting excessive peripheral immune activation, IS may block pro- inflammatory mediators from crossing the blood–brain barrier and initiating central neuroinflammatory

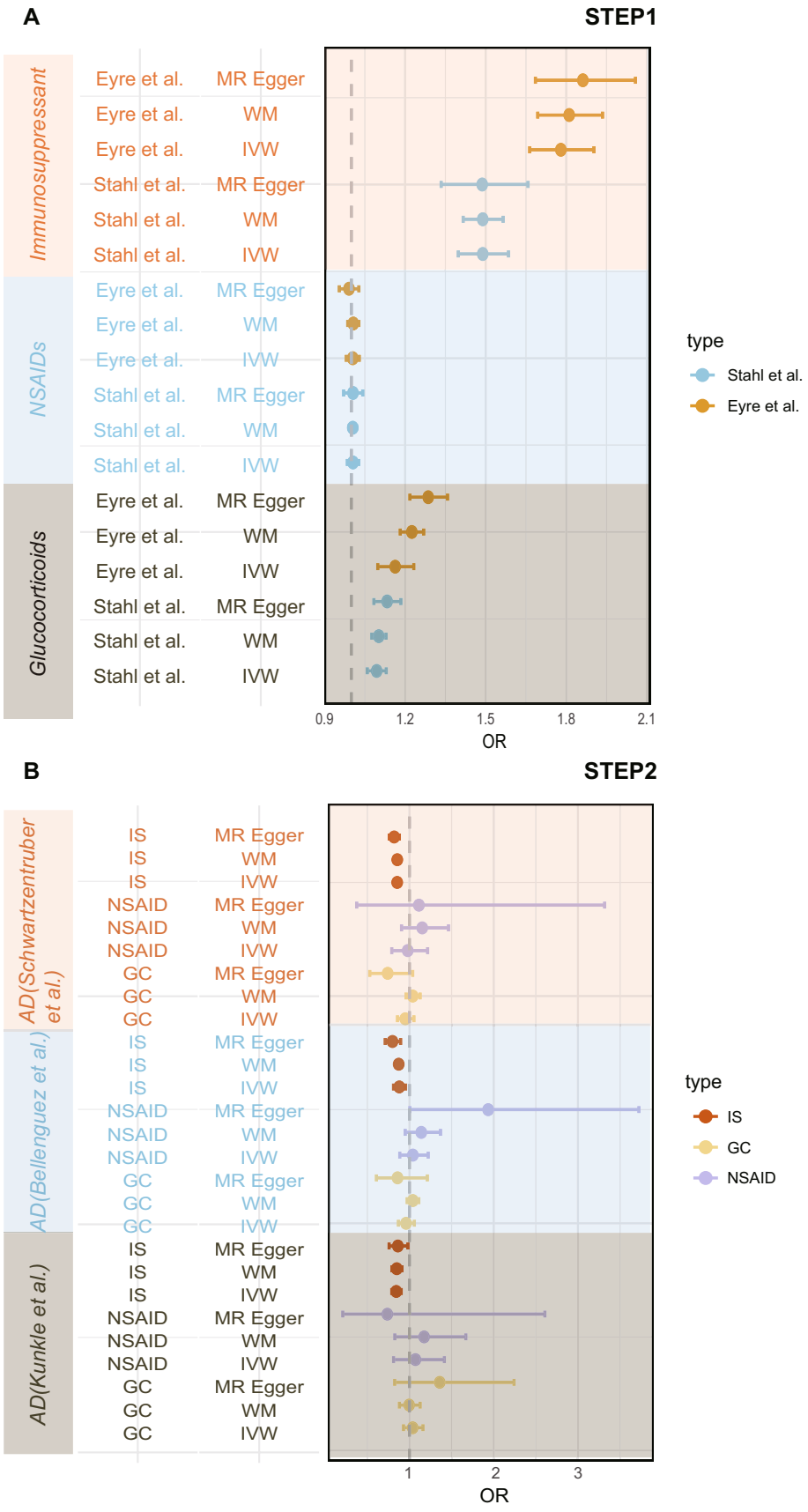

FIGURE 4 | The causal effects between rheumatoid arthritis and medications (step 1) (A) and between medications and Alzheimer's disease (step 2) (B) in two- step Mendelian randomization. AD, Alzheimer's disease; IVW, inverse-variance weighted; RA, rheumatoid arthritis; SNPs, single nucleotide polymorphisms; RA: Eyre et al., Stahl et al.; AD: Schwartzentruber et al., Bellenguez et al., Kunkle et al.

cascades, thereby preserving neuronal integrity and cognitive function [29, 35, 36]. Second, IS may protect cerebral microvessels from inflammation- induced endothelial injury and apoptosis, maintaining cerebral blood perfusion and oxygen delivery to metabolically active brain regions such as the frontal and temporal lobes [37, 38]. Chronic cerebral hypoperfusion, a well-documented complication of RA, impairs neuronal survival and synaptic function in these areas [39]. Third, IS modulate adaptive immunity by suppressing pathogenic autoantibodies (e.g., anti- cyclic citrullinated peptide [anti-CCP] antibodies), which are detectable in cerebrospinal fluid and exert neurotoxicity via microglial activation and local pro- inflammatory cytokine induction [40–42]. These mechanisms align with evidence that RA- related cognitive impairment involves both peripheral and central inflammatory cascades—including MAO- B-driven neuroinflammation—that can be attenuated by IS [43].

In contrast, GCs and NSAIDs showed no significant protective associations in our analyses. Long- term glucocorticoid exposure impairs memory and disrupts hippocampal circadian rhythms [44], while the protective efficacy of NSAIDs against AD remains inconsistent [45]. Although the recent Rotterdam Study linked long- term NSAID use (>24 months) to a 12% lower risk of all- cause dementia [46], randomized controlled trials including the ADAPT trial have failed to confirm its preventive role, and the optimal intervention window remains speculative [47]. This discrepancy highlights IS as a targeted immunomodulatory strategy that directly alleviates neuroinflammation and preserves cognitive function in RA patients.

Our study has notable strengths. The triangulation of NHANES observational data and MR analyses strengthened causal inference. Cognitive function was comprehensively assessed via multiple standardized tests (DSST, AFT, CERAD) and composite *z*- scores, with adjustment for key confounders. Parallel evaluation of IS, GCs, and NSAIDs in MR models disentangled their distinct effects, and large- scale GWAS datasets ensured sufficient statistical power.

Several limitations should be acknowledged. First, NHANES measures cognitive performance rather than confirmed AD diagnoses, which may introduce outcome misclassification bias toward the null. However, the cognitive tests used are well-validated and widely accepted as proxy measures of neurocognitive status in population- based studies. Second, self-reported medication use over the preceding 30 days limits dose–response assessment and may have missed certain IS agents (e.g., etanercept), potentially leading to exposure misclassification. Third, MR analyses were restricted to individuals of European ancestry, limiting generalizability to other ethnic populations. Fourth, IS were analyzed collectively as a single class. Genetic instruments for IS use were derived from the general population rather than RA patients. Accordingly, drug- specific effects and the precise magnitude of the mediated effect within RA populations require further investigation. Fifth, while we used multiple methods to assess horizontal pleiotropy, residual pleiotropy cannot be completely ruled out. Despite these limitations, consistent findings across observational and genetic approaches support the validity of our conclusions.

5 | Conclusions

In summary, IS use mediates the inverse association between RA and AD risk and confers cognitive benefits in RA patients. These results highlight the neuroprotective potential of targeted immunosuppressive therapy and suggest that optimized RA treatment may provide additional protection against neurodegeneration. Future studies are warranted to explore specific IS agents, dose–response relationships, long- term cognitive outcomes, and the underlying molecular mechanisms.

Author Contributions

All authors contributed to the study conception and design. Hai- Feng Pan, Peng Wang and Sha- Sha Tao conceived the present idea and were responsible for the design of the study. Cong Chen performed the statistical analyses and manuscript writing. Jian Cheng and Min Yang participated in acquisition of data and data analyses. Ji- Ping Li participated in modification of English. Literature search by Sha- Sha Tao. All authors read and approved the final manuscript.

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Ethics Statement

The original research for the NHANES (2011–2014) was approved by the National Center for Health Statistics (NCHS) Ethics Review Board and followed the principles of the Declaration of Helsinki. The published genome- wide association studies (GWAS) used in the Mendelian randomization study had received ethical approval for the original study.

Consent

The NHANES data used in this study are publicly available and anonymized, and all participants in the original study signed an informed consent form and the original study was ethically reviewed. Genetic data used in the Mendelian randomization study were obtained from published genome- wide association studies (GWAS), and informed consent was obtained from the participants for the original study, and ethical approvals were made available. This study did not involve the collection of new human samples or direct interventions and therefore no additional informed consent was required.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and material that support the findings of this study are available upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** The results of a subgroup analysis of the use of immunosuppressants and cognitive function by gender in rheumatoid arthritis. **Table S2:** Variance inflation factors for covariates in Model 3. **Table S3:** Summary of the GWAS datasets included in this study. **Table S4:** Instrumental variables used in the Mendelian randomization analysis. **Table S5:** Causal effect of Alzheimer's disease on rheumatoid arthritis in two- sample Mendelian randomization analysis. **Table S6:** Causal effect of rheumatoid arthritis on Alzheimer's disease in two- sample Mendelian randomization analysis after removing single nucleotide polymorphisms associated with Medications (GC, IS, and NSAIDs). **Table S7:** Two- sample Mendelian randomization estimates the causal effect of medications on rheumatoid arthritis. **Table S8:** Two- sample Mendelian randomization estimates the causal effect of AD on IS. **Table S9:** The sensitivity analysis of the causal effect of rheumatoid arthritis on Alzheimer's disease in the discovery and replication phases. **Table S10:** Multivariable Mendelian randomization results of glucocorticoids, immunosuppressants, and non- steroidal anti-inflammatory drugs on Alzheimer's disease risk. **Figure S1:** Causal effect of rheumatoid arthritis on Alzheimer's disease in two- sample Mendelian randomization analysis after removing single nucleotide polymorphisms associated with IS. **Figure S2:** Forest plots of the causal effects of rheumatoid arthritis on Alzheimer's disease in discovery and replication phases. **Figure S3:** Forest plots of leave- one out sensitivity analysis of rheumatoid arthritis on Alzheimer's disease in discovery and replication phases. **Figure S4:** Scatter plots of MR analyses showing the causal effects of rheumatoid arthritis on Alzheimer's disease in discovery and replication phases. **Figure S5:** Forest plots of the causal effects of rheumatoid arthritis on medications (GC, IS, and NSAIDs). **Figure S6:** Forest plots of leave- one out sensitivity analysis of rheumatoid arthritis on medications (GC, IS, and NSAIDs). **Figure S7:** Forest plots of the causal effects of medications (GC, IS, and NSAIDs) on Alzheimer's disease. **Figure S8:** Forest plots of leave- one out sensitivity analysis of medications (GC, IS, and NSAIDs) on Alzheimer's disease. **Figure S9:** Scatter plots of MR analyses showing the causal effects of medications (GC, IS, and NSAIDs) on Alzheimer's disease.

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EDITORIAL

Antigen-Specific Tolerization in Rheumatoid Arthritis: Evaluating the Translational Potential of hPAD4 Plasmid Therapy

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1 | Introduction: The Challenge of Autoimmune Disease in the Era of Precision Medicine

The incidence and clinical burden of autoimmune inflammatory diseases, particularly rheumatoid arthritis (RA), continue to rise and place profound strains on global healthcare systems [1]. Beyond the immediate impacts of chronic joint inflammation, RA significantly elevates the risk of secondary complications, including a marked increase in the development of secondary osteoarthritis, underscoring the urgency of arresting inflammatory cascades before irreversible structural damage occurs [2]. While the current therapeutic armamentarium—ranging from conventional early aggressive interventions like methotrexate [3] to targeted biologic agents like adalimumab [4]—has improved patient outcomes, the field is navigating the complex realities of treating a super-elderly society. Consequently, there is an acute need to transition toward therapies that induce long-term remission without compromising systemic immunocompetence [5]. A recent study by Pagni et al., published in *Arthritis & Rheumatology*, introduces a compelling strategy to meet this need: halting the production of anti-citrullinated protein antibodies (ACPAs) by inducing targeted immune tolerance [6].

2 | A Paradigm Shift in Understanding ACPA Production

The pathophysiological hallmark of RA is the generation of autoantibodies directed against citrullinated proteins. Historically, the "shared epitope binds citrullinated peptide" hypothesis posited that RA-associated HLA-DRB1 molecules preferentially bind these altered peptides, subsequently driving B cell responses. However, because ACPAs recognize up to 100 000 distinct citrullinated peptides, this mechanism would require an implausibly massive repertoire of specific helper T cells. To address this, Pagni et al. advocate for a paradigm-shifting alternative: the hapten/carrier mechanism [6]. In this model, human peptidyl arginine deiminase 4 (hPAD4) acts as the carrier protein, and the multiple citrullinated proteins serve as haptens. T cell recognition of hPAD4 provides the necessary help for B cells to produce ACPAs. Consequently, inducing tolerance to the single hPAD4 protein presents a mathematically and immunologically feasible target to block responses to thousands of downstream citrullinated antigens [6].

3 | The Mechanism of the Tolerogenic Plasmid

To intervene precisely at this immune fulcrum, the researchers engineered a multicistronic tolerogenic plasmid encoding hPAD4 alongside three active murine anti-inflammatory cytokines: transforming growth factor (TGF) β1, interleukin (IL) 10, and IL-2 [6]. Transfecting antigen-presenting cells with this plasmid forces the co-expression of the autoantigen and suppressive signals, theoretically recruiting regulatory T cells to extinguish the hPAD4-reactive T cell response. This highly specific action starkly contrasts with the broad immune modulation seen in traditional treatments like methotrexate or systemic glucocorticoids,

which leave patients vulnerable to severe adverse events [7, 8], including elevated risks of sepsis. In hPAD4-immunized C3H mice, the administration of this specific plasmid significantly inhibited T cell activation against hPAD4 and drastically reduced the production of antibodies targeting both arginine and citrullinated peptides [6].

4 | Translational Validity of the Murine Model

The translational promise of this intervention is bolstered by the precise selection of the animal model. The C3H mice utilized in the study express an IE βk molecule whose amino acid sequence (PEFLEQKRAEVDTVC) shares remarkable homology with the shared epitope region of the human HLA-DRB1*04:01 allele—the primary genetic susceptibility locus for RA [6]. This major histocompatibility complex background ensures that the immunological conflicts observed in the murine model closely mirror the genotypic risk architecture seen in human clinical populations.

5 | Addressing Limitations and Unresolved Mechanisms

Despite its promise, advancing this therapy from the bench to the bedside requires addressing several limitations outlined in the study. First, the suppression of ACPA production following plasmid injection exhibited notable heterogeneity among the treated mice, suggesting that an optimized dosing regimen with increased injection frequency may be necessary [6]. Furthermore, the exact in vivo mechanism of action remains incompletely defined; the investigators were unable to detect a definitive increase in the frequency of Foxp3+ regulatory T cells under their experimental conditions [6]. Clarifying these cellular dynamics is imperative for future clinical trial designs, much like how ongoing real-world observational studies continuously refine our understanding of the clinical effects of current biologics like olokizumab [9].

6 | Clinical Implications and the "Window of Opportunity"

The ultimate goal of managing rheumatic diseases is the preservation of physical function and quality of life [10]. Because ACPAs can be detected in patient serum several years before the onset of clinical joint symptoms, they present a distinct therapeutic "window of opportunity." If hPAD4-specific tolerization can be safely deployed during this pre-clinical phase, it holds the potential to fundamentally switch off ACPA production [6]. While optimization is required, this targeted approach marks a vital step forward in the pursuit of the prevention of rheumatoid arthritis [11].

Author Contributions

Tang-Kai Cheng: conceptualization, writing – original draft. **Hideto Kameda:** writing – review and editing. **James Cheng-Chung Wei:** conceptualization, writing – review and editing, supervision.

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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

From Scrotal Ulcer to Respiratory Failure: An Unusual Presentation of Anti-MDA5 Dermatomyositis

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Dear Editor,

Anti–melanoma differentiation–associated gene 5 positive (Anti-MDA5) dermatomyositis (DM) is a distinct subset of idiopathic inflammatory myopathies with well-described cutaneous features and a high prevalence of rapidly progressive interstitial lung disease (RP-ILD). We report a rare case of scrotal ulcerations as the sole initial cutaneous manifestation of anti-MDA5 DM with fulminant RP-ILD.

A 49-year-old Bangladeshi man, a shipyard worker, presented with 12 days of prolonged fever with non-productive cough and 1 week of scrotal ulcers. These began as "pimples" but evolved into three discrete, painless, well-circumscribed, and punched-out ulcers, measuring 0.5–1 cm in diameter (Figure 1).

Initial investigations prioritized a search for infectious causes, given the acuity of presentation, fever, and genital ulcers. Herpes simplex virus polymerase chain reaction (PCR) swab of the ulcers, human immunodeficiency virus and syphilis screen were negative. A nasopharyngeal respiratory virus panel, leptospirosis PCR, rickettsia serologies, and tuberculosis PCR, smear and culture of the sputum were also negative. White blood cell count was 6.32×10^9/L (Ref range: $4.30–10.40 \times 10^9$/L), C-reactive protein (CRP) was 17.4 mg/L (Ref range: 0.0–5.0 mg/L). Erythrocyte sedimentation rate (ESR) was 12 mm/h. Chest radiograph showed bilateral lower zone airspace opacities. Computed tomography (CT) of the thorax showed predominantly ground-glass scattered changes in dependent areas of the lung, reported as possibly infective or inflammatory in nature. A diagnosis of community-acquired pneumonia was made, and he was discharged on oral antibiotics.

Ten days later, he returned with persistent fever, myalgia, exertional dyspnea, and hypoxemia requiring supplemental oxygen at 2 L/min via nasal cannula. He also developed new cutaneous features including: Gottron papules with ulceration over the hands, heliotrope rash, shawl sign, and violaceous papules with erythema over the extensors of the elbows, deltoids, buttocks, and knees. The scrotal ulcers remained unchanged.

Investigations revealed elevated inflammatory markers (CRP 13.2 mg/L, ESR 42 mm/h, ferritin 1025 μg/L) and muscle enzymes (creatine kinase (CK) 881 U/L, lactate dehydrogenase (LDH) 560 U/L), despite preserved motor power throughout. CT pulmonary angiogram excluded pulmonary embolism but showed worsening bilateral pulmonary infiltrates with features consistent with organizing pneumonia (Figure 2). Transthoracic echocardiogram was normal. A Myositis-specific antibody panel was sent. Given the constellation of classical cutaneous features, elevated ferritin, myositis and progressive respiratory signs and radiological findings suggestive of RP-ILD, a clinical diagnosis of amyopathic DM with high suspicion of anti-MDA5 disease was made. Empirical immunosuppressive treatment was commenced pending antibody confirmation. Skin and muscle biopsy were deferred given the rapidity of respiratory decline, sufficient clinical evidence to support the diagnosis, and the need to prioritize urgent treatment.

On Day 1 of the second admission, the patient received pulse intravenous methylprednisolone 1 g daily for 3 doses, followed by intravenous hydrocortisone 100 mg 8 hourly. Rituximab 1 g was administered on Day 3. Broad-spectrum antibiotics were commenced concurrently, given the ongoing concern for

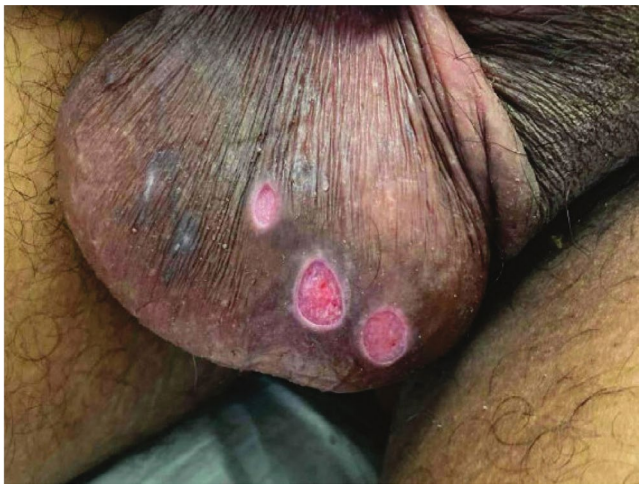

FIGURE 1 | Three well- circumscribed, punched-out, clean based scrotal ulcers.

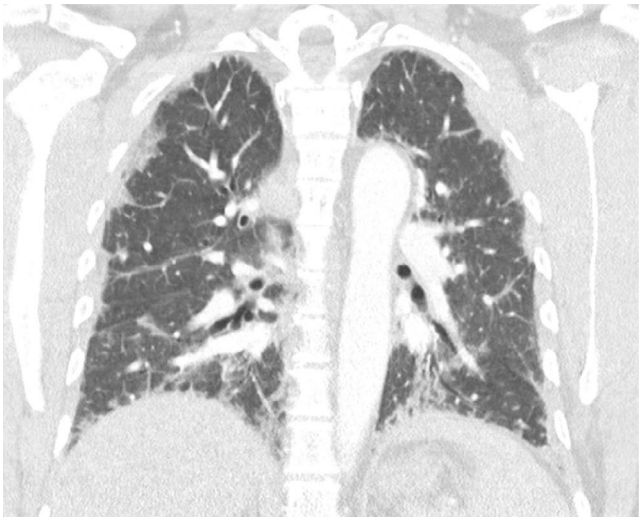

FIGURE 2 | Bilateral patchy opacities and ground glass changes on CT Lung (coronal view), worse in the bases and subpleural peripheries, suggestive of organizing pneumonia.

superimposed infection. An infectious disease specialist was consulted and guided antimicrobial therapy in view of concurrent potent immunosuppression.

However, on Day 4 the patient developed a new spike fever and worsening hypoxemia, requiring escalation of oxygen therapy to high flow nasal cannula at FiO2 50%, flow rate 50 L/min. Chest radiograph showed increased air space shadowing in the bilateral lower zones. CRP was 1.8 mg/L, Ferritin 1700 μg/L. A repeat infective workup with bacterial blood cultures remained negative; procalcitonin was 0.1 μg/L. On Day 6, oxygen requirement escalated to FiO2 60%, flow rate 60 L/min, with chest radiograph showing worsening bilateral lower zone opacities and interstitial thickening. Antibiotics were escalated to piperacillin-tazobactam.

On Day 6, intravenous immunoglobulin (IVIG) 2 g/kg (140 g total over 2 days), and tofacitinib 10 mg twice daily were started when repeat bacterial blood cultures returned negative. Myositis panel sent on admission subsequently returned: Anti- MDA5 was 106 signal intensity unit on immunoblot (strong positive > 50 SIU), confirming the diagnosis. Anti- Ro 52 was negative.

On Day 9, tacrolimus 1 mg twice daily was added. Plasma exchange was initiated on Day 10; however, he developed a new fever spike, and his oxygen requirements increased to FiO2 70%. Ferritin was 3500 μg/L, procalcitonin 0.1 μg/L, and CRP 1.2 mg/L. Intravenous antibiotics were escalated to meropenem. He was intubated on the same day. Ventilator settings were high, requiring FiO2 100% and positive end- expiratory pressure (PEEP) of 10 cm water. Despite deep sedation and neuromuscular blockade, the PaO2/FiO2 ratio post- paralysis was 87 mmHg, consistent with severe adult respiratory distress syndrome by Berlin criteria. The patient continued to deteriorate and died on Day 14. Endotracheal tube aspirates on this day eventually grew aspergillus fumigatus.

We describe a challenging case of anti- MDA5 DM presenting with fever, an initial skin manifestation of solely scrotal ulcers, and lung involvement misdiagnosed as pneumonia.

Scrotal involvement in DM is rare. Prior cases reported in the literature include pyoderma gangrenosum [1], calcinosis [2], shallow erosions [3], and deep ulcerations [4]. However, there is only one case report describing painful scrotal erosions as the initial predominant presentation [3]. Briefly, this was a 35- year-old man presenting with proximal muscle weakness, Gottron's sign, and painful scrotal erythema, edema, and shallow erosions. He had elevated ESR and CK levels but a negative myositis panel. Unlike our patient, there was no respiratory involvement, and the patient was treated with prednisone (1 mg/kg/day) with good response.

Scrotal ulcers usually prompt consideration for infections first, particularly sexually transmitted ones. Other differential diagnoses include vasculitis (e.g., Behçet's disease), malignancy, Crohn's disease, and inflammatory dermatoses such as pyoderma gangrenosum, hidradenitis suppurativa, and immunobullous disorders like bullous pemphigoid [5]. While cutaneous ulceration is characteristic of anti- MDA5 DM, such ulcers are typically located overlying Gottron's signs at the metacarpophalangeal and proximal interphalangeal joints, arms, shoulders, elbows, knees and ankles [6], rarely at the genitalia. Cutaneous ulceration is associated with RP- ILD [7] and thus are markers for severe disease. Scrotal ulcers are atypical and even rarer as a sole initial cutaneous feature.

Beyond the peculiar cutaneous ulcer, the diagnosis of anti-MDA5 DM was missed during the first admission for various reasons. The masquerade as community acquired pneumonia delayed the eventual diagnosis. Although certain atypical features were noted during initial presentation (i.e., prolonged fever, bilateral pulmonary infiltrates), prompting the CT thorax to be performed, the lack of the typical DM rashes that are distinctively photo- distributed and involving the extensor surfaces was misleading. Interestingly ESR was not elevated initially, and this may have given false assurance that the patient did not have an inflammatory illness. Anti- MDA5 DM is predominantly driven by type 1 interferon pathway with macrophage mediated inflammation. Downstream effects include activation

of interferon signaling genes with cytokines representative of this pathway include ferritin and LDH [8]. ESR reflects acute phase fibrinogen- driven erythrocyte rouleaux formation, a process that lags behind acute inflammation and may be falsely reassuring early in the disease course [9]. In our patient, unfortunately the inflammatory markers were performed too early in his disease course. It is also not usual practice to request for ferritin and LDH in common infections like pneumonia.

Anti- MDA5 DM with lung involvement is known to have a mortality rate exceeding 50% [10]. Poor prognostic factors include fever, RP- ILD, high ferritin, anti-MDA5 and LDH levels [10], all of which were present in our patient. In addition, the delay in diagnosis in our patient had led to delay in definitive treatment and dismal outcome. Despite an aggressive, stepwise immunosuppressive regimen including high- dose corticosteroids, rituximab, IVIG, tofacitinib, tacrolimus, and plasmapheresis, our patient's disease course was fulminant. There are no randomized controlled trials to guide treatment of this rare disease, and established clinical guidelines remain sparse [11]. In hindsight, concurrent rather than sequential immunosuppressive therapy could have been employed during the initial small window of opportunity. We could have prioritized Janus kinase inhibitors and plasmapheresis upfront for rapid reduction of type 1 interferon signaling and circulating pathogenic antibodies, respectively. Parallel B- cell depletion should be initiated, but the response of treatment to this modality is slower.

Apparent treatment resistance may reflect undiagnosed infection. Opportunistic and nosocomial infections as a result of intensive immunosuppression required for anti-MDA5 DM can further increase mortality risk, as illustrated in our patient. Fever can reflect disease activity, superimposed infection or both, rendering clinical decision making particularly challenging. The fever spikes on Days 4 and 10 coincided with clinical deterioration and were attributed to the disease rather than infection, although this distinction is extremely difficult in such circumstances. Therefore, multidisciplinary subspecialty consultation with the infectious disease team from the outset is crucial in initiating antimicrobial prophylaxis and surveillance microbiological tests.

This case highlights that unexplained scrotal ulcers with fever and pulmonary infiltrates should raise a high index of suspicion of anti- MDA5 DM and prompt urgent lung evaluation. Given the fulminant nature of RP- ILD, delay in diagnosis portends a bleak outcome.

Author Contributions

Xin Hui Chew and Sujatha Narayanan contributed to the case presentation. Gim Gee Teng and Phang Kee Fong contributed to the discussion segment.

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 – Anti-HMGCR-Associated Immune-Mediated Necrotizing Myopathy and Interstitial Lung Disease With Long-Term Use of Ezetimibe and Low-Dose Atorvastatin

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Dear Editor,

Inflammatory myopathies (IM), also known as idiopathic inflammatory myopathies (IIM) and commonly referred to as myositis, form a group of autoimmune diseases that mostly affect striated muscle [1]. Non-muscular manifestations such as rash, arthritis, interstitial lung disease (ILD) or cardiac disease are common. Five different forms of IM are usually distinguished. These include dermatomyositis, anti-synthetase syndrome, overlap myositis, sporadic inclusion body myositis, and immune-mediated necrotizing myopathy (IMNM) [1]. Organ manifestations, response to treatment, and prognosis vary considerably between these subtypes, suggesting different pathophysiologic mechanisms in each subtype [1]. IMs may or may not be associated with myositis-specific antibodies (MSA) or myositis-associated antibodies (MAA) [2]. The diagnosis of IMs can be made by various methods, including history, clinical and physical examination, blood tests, electromyography (EMG), magnetic resonance imaging (MRI) or by muscle or skin biopsy. Subgroups defined by MSA/MAA may help to define the underlying pathophysiology and will also be important in future clinical trials for the development of targeted therapies and for identifying biomarkers to inform treatment decisions and monitor treatment response in individual patients [1].

IMNMs are clinically characterized by a predominant proximal weakness and elevated creatine kinase (CK) [3]. They may be associated with autoantibodies (e.g., anti-3-hydroxy-3-methyl-glutaryl coenzyme A reductase [HMGCR], anti-signal recognition peptide [SRP]), triggered by statin use (e.g., anti-HMGCR-associated IMNM), associated with cancer or idiopathic [3]. Immunotherapy is required to improve strength and reduce CK levels. However, no therapies for IMNM are currently approved by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA), as the optimal treatment strategy for IMNM is currently unknown. In practice, there is wide variation in the treatment of IMNMs, but certain therapies may be more effective for the different serologic subtypes of IMNMs [3]. Anti-HMGCR-associated IMNM often responds well to intravenous immunoglobulin (IVIG), even as monotherapy. SRP and seronegative IMNM usually require combination immunotherapy, usually consisting of an oral immunosuppressant, corticosteroids and IVIG or rituximab (RTX) [3]. Patients often require immunotherapy for years, and relapses frequently occur when immunotherapy is discontinued. To our knowledge, no patient with anti-HMGCR-associated IMNM and ILD treated with ezetimibe and low-dose atorvastatin over a prolonged period has been reported. The patient's verbal consent to the publication of the case was obtained.

The patient is a Caucasian woman in her eighties, who developed gait disturbances and exertional dyspnoea 23 days prior to admission (Table S1). Two weeks later, her internist recorded a CK level of 7900 U/L leading to the suspicion of heart attack. However, coronary angiography revealed only a 50% stenosis of the right coronary artery. Her medical history was positive for arterial hypertension, thrombendarterectomy (TEA) of a 90% stenosis of the right internal carotid artery at the age of 80, polyarthrosis, recurrent moderate renal failure, cataract surgery and hyperlipidemia. The family history was negative for neuromuscular disorders. She had been taking lisinopril (10 mg/day),

acetylsalicylic acid (100 mg/day), triazolam (0.25 mg/day) and ezetimibe/atorvastatin (10 mg/20 mg/day) regularly for 2 years. On admission, CK had risen to 10 669 U/L (Table S2). Clinical neurological examination revealed proximal quadriparesis (M5-), weak anteflexion of the head (M1) and weak retroflexion (M4). The left palpebral fissure was wider than the right due to a previous left eye trauma. Investigation for suspected myositis revealed positive HMGCR- antibodies. HMGCR-antibodies were detected using the combination immunoblot from Euroimmun (Euroline autoimmune inflammatory myopathies 16 + 2 Ag (IgG)). An MRI of the thigh muscles with contrast showed high STIR- signals in the anterior, medial and posterior thigh mus culature with surrounding edema compatible with myositis (Figure 1). Anti- HMGCR-associated IMNM was diagnosed.

Investigation for malignancy, including thoracic and abdominal CT, gastroscopy, colonoscopy, gynecologic examination, and whole- body fluorodeoxy-glucose positron emission tomog raphy (FDG-PET), revealed only diverticulosis but no malignancy. High- resolution chest-CT showed reticular densities and ground- glass areas posterolaterally in both upper and lower lobes, being interpreted as early ILD (Figure 2). Pulmonary function testing revealed a restrictive ventilatory disorder (TLC 4.0 L, FVC 1.7 L). The diffusion capacity of the lung for carbon monoxide was 46%. Video cinematography of the swallowing act showed marked vallecular and piriform sinus retention with postdeglutitive aspiration. However, MRI of the neck and pharyngeal muscles showed no signs of myositis. The cardiologic examination was unremarkable.

From the first day of hospitalization, the patient was treated with prednisolone, which led to a rapid reduction in CK and transaminases, but CRP and leukocytes remained elevated (Table S2). The patient also benefited from the steroids in terms of quadriparesis, gait disturbance, and exertional dyspnea. To further enhance the therapeutic effect, she received 2 g/kg body weight of IVIG (30 g each on 4 consecutive days [120 g in total]). To avoid long- term steroid use, it was decided to also administer RTX because of the ILD and dysphagia (Table S1). She was given calcium, vitamin- D, and zoledronic acid to prevent osteoporosis. On hospital day 39, the patient was discharged on a regimen of lisinopril (10 mg/day), prednisolone (12.5 mg/day), amlodipine (10 mg/day), furosemide/spironolactone (20/50 mg/day), acetylsalicylic acid (100 mg/day), and pantoprazole (40 mg/day). Fourteen days after discharge, she received a second IVIG cycle (25 g each on 4 consecutive days [100 g in total]). As a result of these therapies, the patient regained her pre- illness condition and reported that she was no longer restricted in her daily life. Six months after discharge, the patient received the third dose of RTX. One month later, the patient was readmitted due to right heart failure.

The patient presented is interesting for several reasons. First, the patient had anti- HMGCR-associated IMNM that responded to prednisolone, IVIGs and RTX. Previously reported patients with anti-HMGCR-associated IMNM [4, 5] presented with proximal predominant muscle weakness, myalgia, elevated CK, and myofiber necrosis with minimal inflammatory infiltrates on muscle biopsy [6]. There are also patients, like the index patient, who do not complain of myalgia. Some patients, but not the index patient, also have cardiac involvement, which may manifest as acute systolic dysfunction. In rare cases, HMGCR- associated IMNM may resemble facioscapulohumeral dystrophy [7]. Some patients also present with atypical skin lesions, such as rashes with ash- like scales or non-scaly red patches and nodules [8]. The prevalence of anti- HMGCR-associated IMNM is estimated at 2–3/100 000 [9]. If IMNM is refractory to glucocorticoids, IVIG, plasmapheresis, and RTX may be beneficial [10]. There is also a case of anti- HMGCR-associated IMNM that responded favorably to efgartigimod [11].

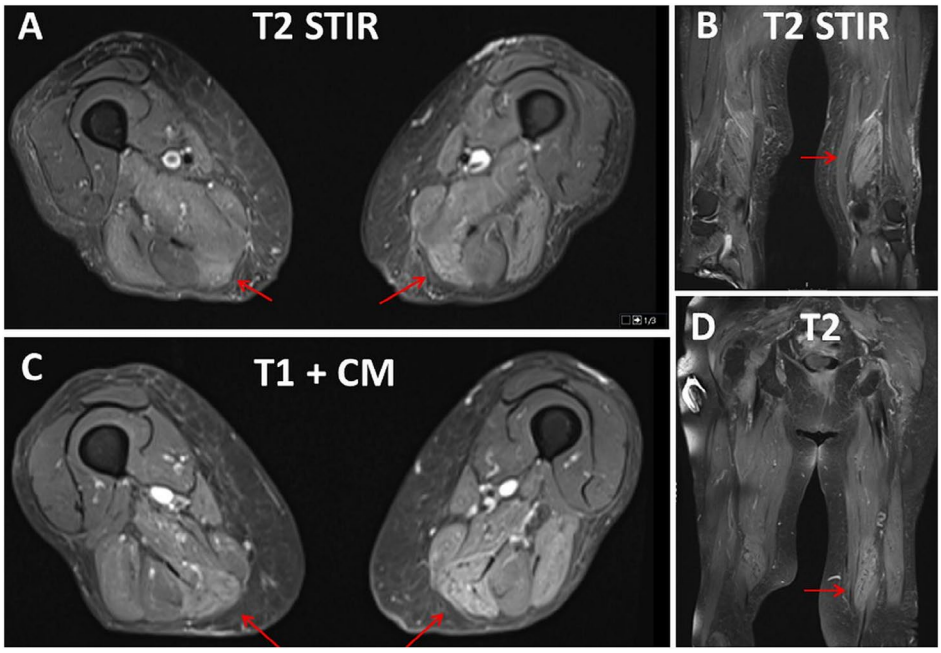

FIGURE 1 | Magnetic resonance imaging of thighs bilaterally showing high short tau inversion recovery (STIR) signals in the anterior, medial, and posterior thigh musculature with surrounding edema compatible with myositis. Axial STIR images (panel A), coronal STIR images (panel B). On T1 with contrast, the muscles were enhanced (panel C). On coronal T1 images without contrast, the muscles appear isointense (panel D).

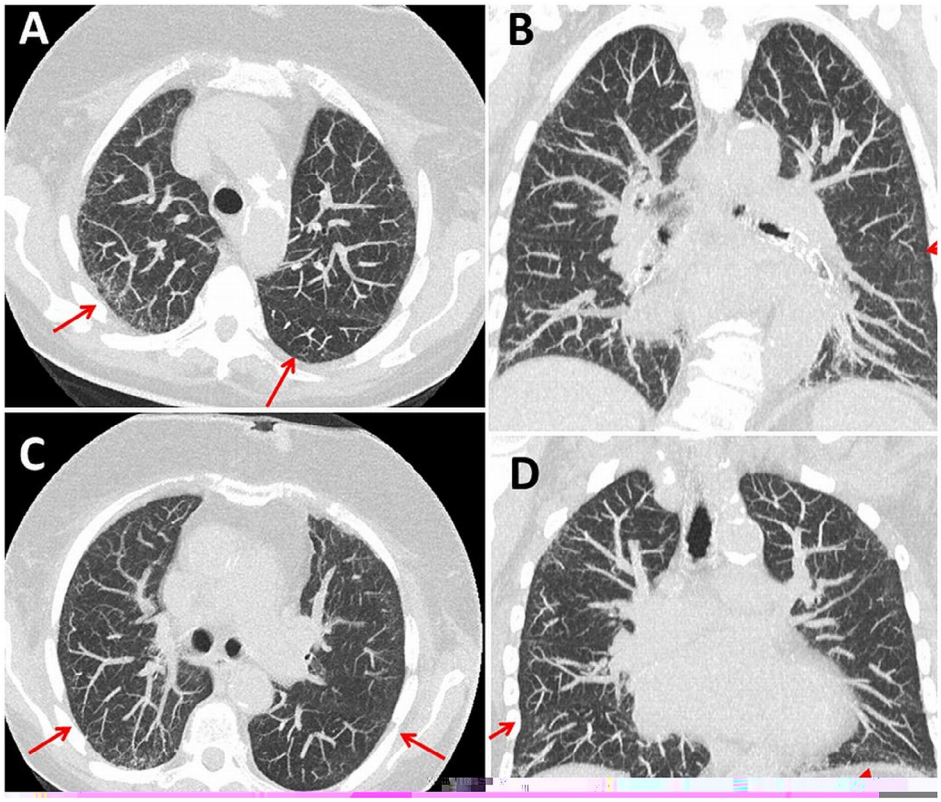

FIGURE 2 | High- resolution chest computed tomography showing reticular densities and ground-glass areas posterolaterally in both upper and lower lobes, being interpreted as early interstitial lung disease (ILD) (panels A–D).

Second, the putative trigger of anti- HMGCR-associated IMNM in the index patient was low- dose (20 mg/day) atorvastatin. Although statins are considered the most common trigger of anti- HMGCR-associated IMNM, there are rarely other causes or no obvious cause for the disease. In a 47- year-old woman who was not taking statins, the trigger of anti- HMGCR-associated IMNM was a viral respiratory infection followed by dengue fever [6]. There is also evidence that statin- naïve anti-HMGCR-associated IMNM in particular is associated with certain HLA types, such as HLA- DRB1*11.01 [12]. There is also a case of hereditary anti- HMGCR-associated IMNM [8]. Anti-HMGCR-associated IMNM can also be a complication of malignant diseases, especially lymphoma, lung cancer, or breast cancer [12]. Most likely, atorvastatin triggered the myositis in the index patient. There was no evidence of a viral or bacterial infection prior to the onset of the first myositis symptoms.

Thirdly, the patient had early ILD in addition to myositis. ILD describes a group of conditions that cause inflammation and scarring of the lungs [13]. The symptoms commonly reported in ILD include difficulty in breathing and a hacking cough. ILD can be associated with radiation therapy, connective tissue diseases, inhaling toxic substances, or side effects of certain medications. Lung damage caused by ILD is often irreversible [14]. ILD is classified as major (idiopathic pulmonary fibrosis, idiopathic nonspecific interstitial pneumonia, respiratory bronchiolitis-interstitial lung disease, desquamative interstitial pneumonia, cryptogenic organizing pneumonia, acute interstitial pneumonia), rare (idiopathic lymphoid interstitial pneumonia, idiopathic pleuroparenchymal fibroelastosis) and unclassified [15]. Idiopathic pulmonary fibrosis is the most lethal among the ILDs and presents high heterogeneity in clinical behavior [15]. Since some of the ILDs respond to steroids, IVIGs, and RTX, it is likely that the index patient's ILD also responded to immunosuppressive treatment for myositis.

Fourth, the patient developed delayed right heart failure 7 months after discharge. Since left heart failure has been occasionally reported as a complication of anti- HMGCR-associated IMNM [16]. It is conceivable that the right heart failure in the index patient was also a complication of myositis. However, right heart failure has not yet been described in anti- HMGCR-associated IMNM. The long latency period between the diagnosis of myositis and the occurrence of heart failure also argues against a causal relationship. Alternatively, the right heart failure could also have been caused by ILD. ILD patients are known to have an increased risk of pulmonary hypertension and thus heart failure, as was found in the index patient. It should also not be neglected that heart failure in the index patient could be a side effect of RTX, as has been repeatedly reported in the literature. The treatment of ILD includes antifibrotic therapy or lung transplantation.

In summary, this case shows that anti- HMGCR-associated IMNM can occur together with ILD, that it can occur without muscle pain, that it can occur together with the combination of ezetimibe and low- dose atorvastatin, and that anti-HMGCR-associated IMNM responds favorably to steroids, IVIGs, and RTX. Patients with quadriparesis, rhabdomyolysis and under long- term treatment with ezetimibe/atorvastatin should be screened for anti- HMGCR-associated IMNM and concomitant lung involvement.

Author Contributions

J.F. was responsible for the design and conception, discussed available data with coauthors, wrote the first draft, and gave final approval. J.F.: contributed to literature search, discussion, correction, and final approval.

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Funding

The author has nothing to report.

Ethics Statement

The study was approved by the Institutional Review Board.

Consent

Consent was obtained from the patient for participation and the publication of this case report.

Conflicts of Interest

The author declares 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:** Disease course before, during and after hospitalization. **Table S2:** Results of blood work before, during, and after hospitalization.

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ORIGINAL ARTICLE

Comparative Outcomes of Electroacupuncture, Warm Acupuncture, and Their Combination in Patients Receiving Standard Treatment for Knee Osteoarthritis: A Retrospective Cohort Study

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ABSTRACT

Background: Knee osteoarthritis (KOA) causes pain and functional limitation. Electroacupuncture (EA) and warm acupuncture (WA) are widely used, but the comparative effectiveness of their combined use remains unclear. This study compared usual care, EA plus usual care, WA plus usual care, and combined EA + WA plus usual care for KOA.

Methods: In this retrospective cohort, 351 patients with symptomatic KOA were analyzed: usual care ($n = 73$), EA plus usual care ($n = 73$), WA plus usual care ($n = 87$), and EA + WA plus usual care ($n = 118$). Acupuncture-treated patients received five sessions weekly for 4 weeks. Outcomes included pain, knee function, physical performance, quantitative sensory testing, surface electromyograph, serum inflammatory, oxidative, and cartilage-related biomarkers, and safety. Adjusted models, logistic regression, and correlation analyses were used.

Results: All groups improved, with the greatest benefits observed in the EA + WA group. At week 4, EA + WA showed the lowest visual analogue scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores, the highest clinical response rate, better physical performance, greater pressure pain threshold (PPT) and conditioned pain modulation (CPM) improvement, greater temporal summation (TS) reduction, and more favorable exploratory inflammatory, oxidative, and cartilage-related biomarker profiles. After multivariable adjustment, EA + WA was associated with more favorable week-4 outcomes than usual care, EA, or WA. No serious adverse events occurred, although minor local or thermal events were more common in acupuncture-treated groups.

Conclusion: EA + WA plus usual care was associated with greater multidomain improvement and acceptable tolerability in KOA. Prospective randomized, sham-controlled studies are needed to confirm effectiveness, durability, safety, and potential mechanisms.

1 | Introduction

Knee osteoarthritis (KOA) is one of the most prevalent degenerative joint disorders among middle-aged and elderly populations worldwide, which is characterized by progressive degeneration of articular cartilage, subchondral bone sclerosis, osteophyte formation, and chronic synovial inflammation [1]. According to recent epidemiological evidence, the global burden of KOA has increased substantially alongside population aging and increasing obesity prevalence [2]. In 2019 year

alone, over 364 million individuals globally were affected by KOA, and the prevalence continues to rise each year. Current KOA management includes education, exercise- based rehabilitation, pharmacological analgesia including non- steroidal anti- inflammatory drugs (NSAIDs), intra-articular treatments in selected patients, and joint replacement for advanced disease [3, 4]. However, these approaches have significant limitations. For instance, NSAIDs are often associated with gastrointestinal, renal, and cardiovascular side effects when used long- term, and surgical treatments are costly and not always feasible for elderly or comorbid patients [4, 5]. Therefore, there is a growing demand for complementary and alternative therapies with a favorable safety profile and sustainable therapeutic effect. Among complementary approaches, acupuncture- based interventions have been evaluated in randomized trials for symptom management in KOA [6–8].

EA involves the application of electrical stimulation through acupuncture needles inserted into specific acupoints [9]. Regarding WA, which integrates moxibustion (thermal stimulation) with traditional needle insertion, it has been traditionally used in Chinese medicine to dispel cold and dampness, enhance qi and blood flow, and relieve pain in musculoskeletal disorders [10]. Although EA and WA have each been used for KOA management, comparative effectiveness across acupuncture approaches and against usual care remains incompletely defined, particularly in routine clinical settings [11, 12]. In particular, limited evidence is available on whether EA, WA, or their combined application provides additional clinical benefit beyond usual care, and whether the combined approach offers greater improvement than either modality alone [1, 13, 14]. From a biological perspective, EA and WA have been hypothesized to influence different but overlapping processes relevant to KOA symptoms. EA has been associated with modulation of nociceptive signaling and inflammatory activity, whereas WA may provide local thermal stimulation that could affect microcirculation, muscle tension, and inflammatory responses [10, 15–17]. However, these proposed pathways remain uncertain, and the present study was not designed to establish mechanisms, cartilage protection, or disease- modifying effects. The potential involvement of thermal, immune, metabolic, and other systemic pathways requires confirmation in prospectively designed mechanistic studies.

However, most available studies have focused on symptom scores alone, and fewer have integrated clinical outcomes, sensory testing, inflammatory and oxidative biomarkers, and cartilage- related indicators within the same real-world comparative framework. Therefore, the present study aimed to evaluate the real- world comparative effectiveness and safety of usual care, EA, WA, and combined EA + WA in patients with symptomatic KOA. Using a re- collected clinical dataset, we compared changes in pain intensity, knee function, physical performance, quantitative sensory testing parameters, inflammatory markers, oxidative stress indicators, and cartilage- related biomarkers across treatment groups. We further explored whether changes in sensory and biochemical indicators were associated with clinical improvement.

2 | Materials and Methods

2.1 | Participant Enrollment and Study Design

A total of 351 participants aged between 45 and 80 years were recruited in our hospital from January 2022 to December 2024. Patients were diagnosed with KOA according to the 1995 revised American College of Rheumatology (ACR) criteria and had knee radiographs consistent with Kellgren-Lawrence (KL) grades II–III. Inclusion criteria were: chronic knee pain ≥ 3 months, VAS score ≥ 4, and ability to comply with the protocol. Both unilateral and bilateral symptomatic KOA were eligible. Exclusion criteria included: rheumatoid arthritis or other autoimmune diseases, severe cardiovascular/hepatic/renal comorbidities, history of joint replacement, intra-articular corticosteroid injection within the past 3 months, and current enrollment in other clinical trials. The retrospective dataset included 164 patients with unilateral KOA (86 right- sided and 78 left-sided) and 187 with bilateral KOA. Clinical outcomes were recorded and analyzed at the patient level. For patients with bilateral KOA, no prespecified index knee or separate knee-level outcome data were available. The study was approved by the Ethics Committee of our hospital and followed the guidelines of the Declaration of Helsinki. All participants provided written informed consent prior to enrollment.

2.2 | Intervention

Participants were assigned based on the treating strategies that they had received during their previous treatments. Group A received usual care alone, Group B received electroacupuncture plus usual care, Group C received warm acupuncture plus usual care, and Group D received combined EA + WA plus usual care. Usual care included routine health education, knee- protection guidance, home- based rehabilitation exercise, and oral or topical non- steroidal anti-inflammatory drugs when clinically required. All acupuncture- treated groups underwent a 4-week treatment course consisting of five sessions per week (total 20 sessions). Each session lasted 30 min. For EA treatment, disposable sterile needles (0.30 mm × 40 mm) were inserted at ST35 (Dubi), ST36 (Zusanli), SP9 (Yinlingquan), GB34 (Yanglingquan), EX- LE5 (Neixiyan), and Ashi points around the patella (Figure 1). For patients receiving bilateral EA treatment, electrical stimulation was applied to both knees using a G6805- II EA stimulator with dense- disperse wave mode (2/100 Hz) and intensity adjusted to patient tolerance (1.5–2.5 mA) (Figure 1). Two pairs of electrodes were connected: ST36–SP9 and GB34–EX- LE5. For WA treatment, treatment was administered using traditional moxibustion in conjunction with acupuncture. Needles were inserted into the same points as in the EA group. Moxa rolls (4:1 ratio compressed Ai Ye) were affixed to the needle handles and ignited, producing heat conducted through the needle to the subcutaneous tissues. One moxa roll per acupoint was used for approximately 10–12 min until burned two- thirds, and needles were retained for the entire 30- min session. Patients in the combined group received electroacupuncture at ST36 and GB34 while simultaneously undergoing warm acupuncture at ST35 and EX- LE5. EA stimulation and moxa ignition were started concurrently. Treatment balance was ensured by alternating EA

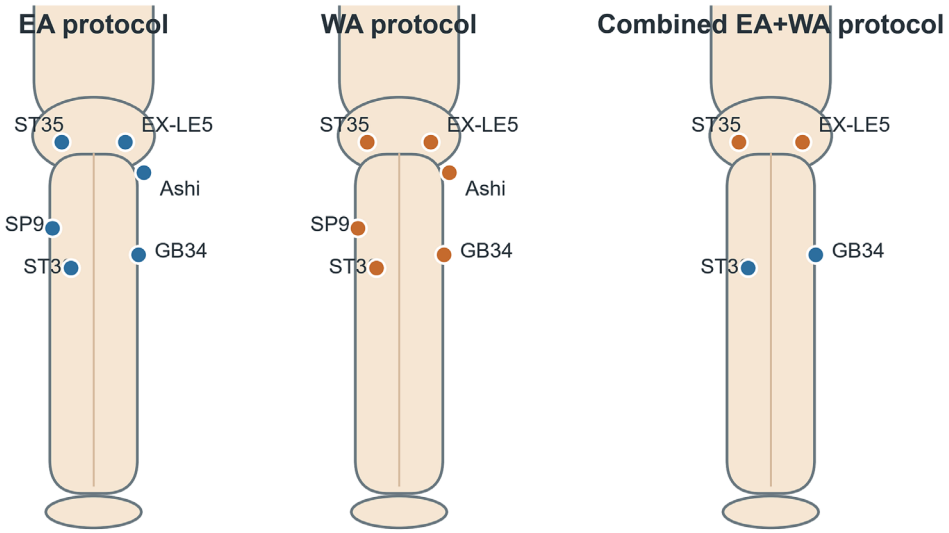

FIGURE 1 | Schematic illustration of acupuncture- point locations and treatment allocations. The EA and WA protocols used ST35 (Dubi), EX-LE5 (Neixiyan), ST36 (Zusanli), SP9 (Yinlingquan), GB34 (Yanglingquan), and Ashi points around the patella. In the combined EA + WA protocol, EA was applied at ST36 and GB34, whereas WA was applied at ST35 and EX- LE5. Blue indicates EA and orange indicates WA. Points were applied bilaterally; locations are schematic and not to scale.

and WA acupoint pairs in different limbs to prevent thermal or electrical overload at any single site (Figure 1). Treatment expectation, exercise adherence, oral NSAID use, topical NSAID use, rescue medication use, and the number of acupuncture sessions completed during weeks 0–4 were extracted from the medical records for subsequent adjusted analyses.

2.3 | Clinical Outcome Measures

2.3.1 | Pain and Functional Assessment

Pain intensity was measured using a 10- cm visual analogue scale (VAS) at baseline, post- treatment (week 4), follow-up (week 8), and month 3. Knee function was assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Performance- based tests included the Timed Up- and-Go Test (TUGT) and the 15-m walk test. Patient global satisfaction and symptom recurrence were evaluated at follow-up and month 3. The primary endpoint was the change in WOMAC total score from baseline to week 4. Secondary endpoints included changes in VAS, TUGT, 15- m walk time, satisfaction score, recurrence, and the proportion of patients achieving a $\geq 30\%$ reduction in VAS at week 4.

2.3.2 | Temporal Summation (TS) and Conditioned Pain Modulation (CPM)

TS was evaluated using 10 repeated pressure stimuli at the patellar tendon, and the TS index was calculated as the difference between the first and the last pressure pain threshold (PPT) response. CPM was assessed using a cold-pressor conditioning stimulus (10°C water immersion for 60 s), and the CPM effect was defined as the change in PPT at ST36 before and during conditioning.

2.3.3 | Surface Electromyography (sEMG)

Quadriceps muscle activation was evaluated using a surface EMG system (Noraxon, USA). Root mean square (RMS) amplitude was recorded during standardized isometric knee extension tasks. Pre- and post- intervention RMS values were analyzed as indicators of neuromuscular improvement. *All QST and sEMG assessments were conducted at baseline and immediately post-treatment.*

2.3.4 | Pressure Pain Threshold (PPT)

Algometry was conducted using a handheld digital pressure algometer (Wagner Force Ten FDX) applied over the medial joint line, patellar tendon, and ST36. Pressure was gradually increased until patients reported discomfort. The average of three readings per site was used.

2.3.5 | Serum Inflammatory Markers

Fasting venous blood samples were collected at baseline and week 4 between 8:00 and 10:00 AM, centrifuged, and stored at −80°C until assay. All serum biomarkers were quantified in duplicate using standardized commercial kits following manufacturer instructions. Inflammatory cytokines, including CRP, TNF- α, IL-1β, and IL-6, as well as the anti-inflammatory cytokine IL- 10, were measured using ELISA kits (R&D Systems, USA). Cartilage metabolism markers, including COMP, CTX- II, and MMP- 3, were also assessed using ELISA. Oxidative stress indicators (MDA and TAC) and antioxidant enzyme activity (SOD) were determined using colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, China). All assays were performed by trained laboratory technicians blind to group

assignment, and mean values of duplicate measurements were used for analysis.

2.3.6 | Safety Assessment

Adverse events during treatment were extracted from the medical records, including local pain, bleeding or hematoma, dizziness, burn, skin allergy, infection, serious adverse events, and treatment discontinuation.

2.3.7 | Statistical Analysis

All statistical analyses were performed using SPSS 26.0 (IBM Corp., USA) and R software version 4.4.0. Continuous variables were tested for normality using the Shapiro–Wilk test and presented as mean ± SD or median (IQR), while categorical variables were expressed as frequencies and percentages. Between- group comparisons were conducted using one-way ANOVA with Bonferroni post hoc tests or Kruskal–Wallis tests when appropriate; categorical variables were compared using chi- square or Fisher's exact tests. Week-4 outcomes were compared using adjusted models controlling for baseline outcome values and clinically relevant baseline and treatment- context covariates. Because this was a retrospective non- randomized study, adjusted models included clinically relevant baseline and treatment- context covariates, including age, sex, BMI, KOA duration, Kellgren–Lawrence grade, affected side, prior acupuncture exposure, baseline treatment expectation, physical activity, exercise adherence, oral NSAID use, topical NSAID use, and rescue medication use. The primary endpoint was the change in WOMAC total score from baseline to week 4. No formal adjustment for multiple comparisons was applied. Secondary clinical, functional, sensory, and biomarker outcomes were considered exploratory, and their p values should therefore be interpreted cautiously. Logistic regression was used to identify predictors of treatment response (≥ 30% VAS reduction), and model performance was assessed by AUC. Pearson correlation matrices were constructed to examine multidomain associations among changes in clinical outcomes, sensory measures, inflammatory markers, oxidative stress indicators, and cartilage- related biomarkers. Given the retrospective observational design, correlation findings were interpreted as exploratory and not as evidence of biological causality. Statistical significance was set at $p < 0.05$ (two-sided).

3 | Results

3.1 | Participant Characteristics and Treatment Context

A total of 351 patients with symptomatic KOA were included in the final analysis, including 73 patients in the usual- care group, 73 in the EA + usual care group, 87 in the WA + usual care group, and 118 in the EA + WA + usual care group. Baseline demographic and disease- related characteristics were generally comparable among the four groups. No significant between-group differences were observed in age, sex distribution, KOA duration, Kellgren–Lawrence grade, affected side, hypertension, diabetes, smoking status, baseline VAS, baseline WOMAC, baseline TUGT, baseline PPT at ST36, CRP, IL- 6, or COMP levels (all $p > 0.05$; Table 1). Baseline VAS scores were 7.35 ± 0.94, 7.12 ± 0.98, 7.08 ± 0.99, and 7.00 ± 1.10 in the usual- care, EA, WA, and EA + WA groups, respectively ($p = 0.136$). Baseline WOMAC total scores were also similar among groups ($p = 0.973$).

However, several treatment- context variables differed significantly. BMI showed a modest between- group difference ($p = 0.030$). Prior acupuncture exposure was more frequent in the acupuncture- treated groups, especially in the EA + WA group ($p < 0.001$). Baseline treatment expectation was also higher in the EA, WA, and EA + WA groups than in the usual- care group ($p < 0.001$). Exercise adherence differed significantly among groups ($p < 0.001$). In addition, oral NSAID use, topical NSAID use, and rescue medication use during weeks 0–4 were more frequent in the usual- care group than in the acupuncture-treated groups (all $p < 0.001$; Table 1). Therefore, adjusted analyses were performed to account for these baseline and treatment- context differences.

3.2 | Changes in Pain, Function, and Clinical Response

All four groups showed some degree of improvement after treatment, but the magnitude of improvement differed substantially among groups (Table 2). At week 4, VAS decreased from 7.35 ± 0.94 to 6.28 ± 1.25 in the usual- care group, from 7.12 ± 0.98 to 4.37 ± 1.46 in the EA group, from 7.08 ± 0.99 to 4.45 ± 1.42 in the WA group, and from 7.00 ± 1.10 to 3.12 ± 1.50 in the EA + WA group ($p < 0.001$). The corresponding week- 4 VAS improvement was greatest in the EA + WA group (3.88 ± 0.87), followed by the EA group (2.75 ± 0.98), WA group (2.62 ± 0.83), and usual- care group (1.07 ± 0.92; $p < 0.001$).

The longitudinal trajectory of VAS also showed that the EA + WA group maintained the lowest pain scores at week 8 and month 3 (Figure S1A). At month 3, VAS scores were 6.97 ± 1.32 in the usual- care group, 5.04 ± 1.53 in the EA group, 5.02 ± 1.49 in the WA group, and 3.36 ± 1.58 in the EA + WA group ($p < 0.001$; Table 2).

WOMAC total score showed a similar pattern. At week 4, WOMAC decreased to 46.85 ± 10.69 in the usual- care group, 34.08 ± 11.19 in the EA group, 36.01 ± 10.82 in the WA group, and 27.62 ± 11.07 in the EA + WA group ($p < 0.001$). The week- 4 WOMAC improvement was 6.98 ± 5.84, 19.23 ± 6.88, 17.79 ± 6.99, and 25.88 ± 6.50, respectively ($p < 0.001$; Table 2). These differences remained evident at week 8 and month 3, and the WOMAC trajectory consistently favored EA + WA treatment (Figure S1B).

Functional performance also improved most clearly in the EA + WA group. At week 4, TUGT time was 13.23 ± 2.42 s in the usual- care group, 11.28 ± 2.28 s in the EA group, 11.60 ± 2.50 s in the WA group, and 10.38 ± 2.74 s in the EA + WA group ($p < 0.001$). The 15- m walk test showed the same trend, with week- 4 values of 16.33 ± 2.69 s, 14.63 ± 3.03 s, 15.16 ± 3.05 s, and 13.44 ± 2.98 s, respectively ($p < 0.001$; Table 2).

TABLE 1 | Baseline characteristics of the patients.

Variable	Usual care ($n=73$)	EA ($n=73$)	WA ($n=87$)	EA + WA ($n=118$)	p
Age, years	63.9 ± 7.2	64.0 ± 6.5	62.3 ± 7.1	62.7 ± 6.8	0.301
Female, *n* (%)	53 (72.6%)	46 (63.0%)	57 (65.5%)	67 (56.8%)	0.167
BMI, kg/m^2	26.8 ± 2.6	26.0 ± 2.5	25.5 ± 2.9	25.9 ± 2.8	0.030
KOA duration, years	6.8 ± 3.1	6.4 ± 2.9	6.4 ± 2.4	6.4 ± 3.0	0.698
KL grade III, *n* (%)	37 (50.7%)	38 (52.1%)	40 (46.0%)	55 (46.6%)	0.826
Affected side: bilateral, *n* (%)	35 (47.9%)	40 (54.8%)	49 (56.3%)	63 (53.4%)	0.869
Prior acupuncture, *n* (%)	8 (11.0%)	15 (20.5%)	22 (25.3%)	43 (36.4%)	< 0.001
Hypertension, *n* (%)	33 (45.2%)	26 (35.6%)	28 (32.2%)	44 (37.3%)	0.390
Diabetes, *n* (%)	5 (6.8%)	14 (19.2%)	19 (21.8%)	21 (17.8%)	0.068
Current smoking, *n* (%)	23 (31.5%)	18 (24.7%)	27 (31.0%)	26 (22.0%)	0.368
Baseline treatment expectation (0–10)	4.8 ± 1.2	6.6 ± 1.4	6.5 ± 1.3	7.1 ± 1.4	< 0.001
Exercise adherence, %	75.1 ± 13.0	86.5 ± 10.1	82.3 ± 11.5	83.3 ± 12.2	< 0.001
Acupuncture sessions completed	0.0 ± 0.0	18.1 ± 1.5	18.0 ± 1.7	18.2 ± 1.7	< 0.001
Oral NSAID days during weeks 0–4	11.9 ± 5.6	9.7 ± 4.7	9.7 ± 5.9	7.0 ± 5.2	< 0.001
Topical NSAID days during weeks 0–4	14.2 ± 7.2	10.3 ± 6.3	10.2 ± 5.8	8.3 ± 5.4	< 0.001
Rescue medication used, *n* (%)	65 (89.0%)	57 (78.1%)	64 (73.6%)	72 (61.0%)	< 0.001
VAS baseline	7.35 ± 0.94	7.12 ± 0.98	7.08 ± 0.99	7.00 ± 1.10	0.136
WOMAC baseline	53.84 ± 9.10	53.31 ± 8.28	53.80 ± 8.01	53.49 ± 7.78	0.973
TUGT baseline, s	14.10 ± 2.07	13.61 ± 1.78	13.70 ± 2.10	13.62 ± 2.34	0.423
PPT ST36 baseline, kg/cm^2	3.73 ± 0.63	3.81 ± 0.73	3.82 ± 0.68	3.87 ± 0.60	0.569
CRP baseline, mg/L	6.70 ± 1.97	6.70 ± 1.89	6.71 ± 1.79	6.81 ± 1.97	0.968
IL- 6 baseline, pg/mL	13.01 ± 2.01	12.76 ± 2.43	12.88 ± 2.44	12.30 ± 2.10	0.131
COMP baseline, ng/mL	18.71 ± 3.08	18.51 ± 3.01	18.09 ± 2.26	18.73 ± 2.48	0.337

The proportion of patients achieving a ≥ 30% reduction in VAS at week 4 was 11.0% in the usual- care group, 75.3% in the EA group, 73.6% in the WA group, and 94.1% in the EA + WA group ($p < 0.001$; Table 2 and Figure S2). Patient satisfaction was also highest in the EA + WA group at both week 8 and month 3. Recurrence at week 8 did not differ significantly among groups ($p = 0.495$), whereas month-3 recurrence was lowest in the EA + WA group and highest in the usual- care group (1.7% vs. 12.3%, $p = 0.014$; Table 2 and Figure S2).

3.3 | Changes in Quantitative Sensory Testing and Neuromuscular Function

Baseline QST and sEMG parameters were comparable among the four groups (Table 3). After treatment, the acupuncture-treated groups showed greater improvements in sensory modulation than the usual- care group, with the most pronounced changes observed in the EA + WA group.

At week 4, PPT at ST36 increased to 4.17 ± 0.76 kg/cm^2 in the usual- care group, 4.71 ± 0.90 kg/cm^2 in the EA group, 4.79 ± 0.86 kg/cm^2 in the WA group, and 5.29 ± 0.78 kg/cm^2 in the EA + WA group ($p < 0.001$). The corresponding week- 4 increase in PPT at ST36 was 0.43 ± 0.40, 0.90 ± 0.43, 0.97 ± 0.48, and 1.41 ± 0.41 kg/cm^2, respectively ($p < 0.001$). Similar between-group differences were observed for PPT at the medial joint line and patellar tendon.

Temporal summation decreased after treatment, with the lowest week- 4 value in the EA + WA group. The week- 4 temporal summation index was 0.501 ± 0.110 in the usual- care group, 0.441 ± 0.116 in the EA group, 0.445 ± 0.096 in the WA group, and 0.401 ± 0.103 in the EA + WA group ($p < 0.001$). CPM increased most clearly in the EA + WA group, reaching 0.519 ± 0.127 kg/cm^2 at week 4 compared with 0.289 ± 0.111 kg/cm^2 in the usual-care group ($p < 0.001$; Table 3).

Quadriceps sEMG RMS amplitude also improved after treatment. At week 4, sEMG RMS was 46.28 ± 7.15 μV in the usual-care group, 49.25 ± 7.08 μV in the EA group, 48.32 ± 7.19 μV in the WA group, and 53.40 ± 7.74 μV in the EA + WA group ($p < 0.001$; Table 3).

TABLE 2 | Effects of different treatment strategies on the longitudinal clinical outcomes.

Outcome/timepoint	Usual care ($n=73$)	EA ($n=73$)	WA ($n=87$)	EA + WA ($n=118$)	p
VAS baseline	7.35 ± 0.94	7.12 ± 0.98	7.08 ± 0.99	7.00 ± 1.10	0.136
VAS week 4	6.28 ± 1.25	4.37 ± 1.46	4.45 ± 1.42	3.12 ± 1.50	< 0.001
VAS week 8	6.74 ± 1.34	4.55 ± 1.57	4.75 ± 1.43	3.29 ± 1.57	< 0.001
VAS month 3	6.97 ± 1.32	5.04 ± 1.53	5.02 ± 1.49	3.36 ± 1.58	< 0.001
VAS improvement week 4	1.07 ± 0.92	2.75 ± 0.98	2.62 ± 0.83	3.88 ± 0.87	< 0.001
WOMAC baseline	53.84 ± 9.10	53.31 ± 8.28	53.80 ± 8.01	53.49 ± 7.78	0.973
WOMAC week 4	46.85 ± 10.69	34.08 ± 11.19	36.01 ± 10.82	27.62 ± 11.07	< 0.001
WOMAC week 8	47.31 ± 11.67	34.63 ± 11.89	36.90 ± 10.89	28.09 ± 11.79	< 0.001
WOMAC month 3	49.92 ± 10.83	37.01 ± 11.90	38.00 ± 10.83	28.36 ± 11.53	< 0.001
WOMAC improvement week 4	6.98 ± 5.84	19.23 ± 6.88	17.79 ± 6.99	25.88 ± 6.50	< 0.001
TUGT baseline, s	14.10 ± 2.07	13.61 ± 1.78	13.70 ± 2.10	13.62 ± 2.34	0.423
TUGT week 4, s	13.23 ± 2.42	11.28 ± 2.28	11.60 ± 2.50	10.38 ± 2.74	< 0.001
TUGT improvement week 4, s	0.87 ± 1.25	2.34 ± 1.43	2.10 ± 1.33	3.23 ± 1.23	< 0.001
15- m walk baseline, s	17.37 ± 2.60	17.35 ± 2.67	17.26 ± 2.81	17.12 ± 2.78	0.915
15- m walk week 4, s	16.33 ± 2.69	14.63 ± 3.03	15.16 ± 3.05	13.44 ± 2.98	< 0.001
15- m walk improvement week 4, s	1.04 ± 1.30	2.72 ± 1.36	2.10 ± 1.27	3.68 ± 1.43	< 0.001
≥ 30% VAS reduction at week 4, *n* (%)	8 (11.0%)	55 (75.3%)	64 (73.6%)	111 (94.1%)	< 0.001
Satisfaction week 8 (0–10)	6.31 ± 1.03	7.86 ± 1.20	7.59 ± 0.96	8.64 ± 0.91	< 0.001
Satisfaction month 3 (0–10)	5.67 ± 1.15	7.08 ± 1.15	6.95 ± 1.03	8.01 ± 1.02	< 0.001
Recurrence week 8, *n* (%)	8 (11.0%)	4 (5.5%)	8 (9.2%)	7 (5.9%)	0.495
Recurrence month 3, *n* (%)	9 (12.3%)	7 (9.6%)	4 (4.6%)	2 (1.7%)	0.014

TABLE 3 | Effects of different treatment strategies on the quantitative sensory testing (QST) and sEMG.

Variable/timepoint	Usual care ($n=73$)	EA ($n=73$)	WA ($n=87$)	EA + WA ($n=118$)	p
PPT ST36 baseline, kg/cm^2	3.73 ± 0.63	3.81 ± 0.73	3.82 ± 0.68	3.87 ± 0.60	0.569
PPT ST36 week 4, kg/cm^2	4.17 ± 0.76	4.71 ± 0.90	4.79 ± 0.86	5.29 ± 0.78	< 0.001
PPT ST36 change week 4	0.43 ± 0.40	0.90 ± 0.43	0.97 ± 0.48	1.41 ± 0.41	< 0.001
PPT medial baseline, kg/cm^2	3.33 ± 0.65	3.31 ± 0.66	3.30 ± 0.66	3.28 ± 0.62	0.957
PPT medial week 4, kg/cm^2	3.65 ± 0.76	3.97 ± 0.67	4.03 ± 0.81	4.32 ± 0.76	< 0.001
PPT patellar baseline, kg/cm^2	3.46 ± 0.68	3.54 ± 0.58	3.51 ± 0.65	3.40 ± 0.62	0.465
PPT patellar week 4, kg/cm^2	3.80 ± 0.74	4.21 ± 0.68	4.23 ± 0.78	4.47 ± 0.75	< 0.001
Temporal summation baseline	0.539 ± 0.096	0.532 ± 0.096	0.526 ± 0.075	0.532 ± 0.090	0.832
Temporal summation week 4	0.501 ± 0.110	0.441 ± 0.116	0.445 ± 0.096	0.401 ± 0.103	< 0.001
Temporal summation reduction week 4	0.038 ± 0.051	0.092 ± 0.060	0.080 ± 0.056	0.131 ± 0.053	< 0.001
CPM baseline, ΔPPT kg/cm^2	0.239 ± 0.085	0.262 ± 0.104	0.246 ± 0.099	0.241 ± 0.103	0.458
CPM week 4, ΔPPT kg/cm^2	0.289 ± 0.111	0.417 ± 0.132	0.392 ± 0.144	0.519 ± 0.127	< 0.001
CPM increase week 4	0.049 ± 0.073	0.154 ± 0.083	0.146 ± 0.088	0.278 ± 0.085	< 0.001
sEMG RMS baseline, μV	43.44 ± 5.43	42.26 ± 5.29	42.50 ± 5.99	42.58 ± 6.12	0.621
sEMG RMS week 4, μV	46.28 ± 7.15	49.25 ± 7.08	48.32 ± 7.19	53.40 ± 7.74	< 0.001

TABLE 4 | Effects of different treatment strategies on biomarker changes.

Biomarker/timepoint	Usual care ($n=73$)	EA ($n=73$)	WA ($n=87$)	EA + WA ($n=118$)	p
CRP baseline, mg/L	6.70 ± 1.97	6.70 ± 1.89	6.71 ± 1.79	6.81 ± 1.97	0.968
CRP week 4, mg/L	6.17 ± 2.27	5.77 ± 2.05	5.72 ± 1.96	5.13 ± 2.34	0.011
TNF- α baseline, pg/mL	28.18 ± 3.15	27.79 ± 2.77	27.64 ± 3.06	27.87 ± 3.21	0.740
TNF- α week 4, pg/mL	27.20 ± 3.35	25.48 ± 3.43	24.86 ± 3.38	23.89 ± 3.88	< 0.001
IL- 1β baseline, pg/mL	15.30 ± 3.17	15.07 ± 2.87	15.60 ± 2.68	15.39 ± 2.57	0.693
IL- 1β week 4, pg/mL	14.61 ± 3.46	13.40 ± 3.29	13.86 ± 3.14	12.83 ± 3.27	0.003
IL- 6 baseline, pg/mL	13.01 ± 2.01	12.76 ± 2.43	12.88 ± 2.44	12.30 ± 2.10	0.131
IL- 6week 4, pg/mL	12.27 ± 2.17	11.04 ± 2.87	11.03 ± 2.62	9.48 ± 2.30	< 0.001
IL- 6 reduction week 4	0.73 ± 0.97	1.72 ± 1.12	1.85 ± 1.12	2.82 ± 1.14	< 0.001
IL- 10 baseline, pg/mL	4.11 ± 0.80	4.21 ± 0.71	4.18 ± 0.97	4.13 ± 0.74	0.868
IL- 10week 4, pg/mL	4.28 ± 0.85	4.52 ± 0.81	4.53 ± 1.03	4.67 ± 0.88	0.035
COMP baseline, ng/mL	18.71 ± 3.08	18.51 ± 3.01	18.09 ± 2.26	18.73 ± 2.48	0.337
COMP week 4, ng/mL	17.68 ± 3.12	16.57 ± 3.51	16.35 ± 2.70	15.05 ± 2.96	< 0.001
COMP reduction week 4	1.04 ± 1.29	1.94 ± 1.47	1.74 ± 1.43	3.68 ± 1.54	< 0.001
CTX- II baseline, ng/mL	0.617 ± 0.115	0.644 ± 0.112	0.633 ± 0.134	0.634 ± 0.123	0.615
CTX- II week 4, ng/mL	0.590 ± 0.126	0.599 ± 0.135	0.589 ± 0.136	0.546 ± 0.148	0.029
MMP- 3 baseline, ng/mL	12.03 ± 2.73	11.51 ± 2.47	11.34 ± 2.40	11.24 ± 2.63	0.196
MMP- 3week 4, ng/mL	11.46 ± 2.87	11.09 ± 2.84	10.65 ± 2.65	10.13 ± 2.92	0.010
MDA baseline, μmol/L	4.86 ± 0.79	4.73 ± 0.89	4.92 ± 0.83	4.76 ± 0.87	0.450
MDA week 4, μmol/L	4.67 ± 0.95	4.24 ± 1.06	4.47 ± 0.91	4.00 ± 1.03	< 0.001
MDA reduction week 4	0.19 ± 0.40	0.50 ± 0.43	0.45 ± 0.43	0.77 ± 0.46	< 0.001
SOD baseline, U/mL	90.63 ± 14.17	91.25 ± 13.65	89.45 ± 14.40	91.70 ± 13.20	0.699
SOD week 4, U/mL	95.55 ± 18.35	101.67 ± 16.55	103.54 ± 17.87	110.87 ± 16.96	< 0.001
SOD increase week 4	4.92 ± 10.83	10.42 ± 11.48	14.10 ± 11.16	19.16 ± 12.56	< 0.001
TAC baseline, mmol Trolox/L	1.138 ± 0.210	1.170 ± 0.211	1.165 ± 0.199	1.129 ± 0.192	0.429
TAC week 4, mmol Trolox/L	1.177 ± 0.230	1.241 ± 0.212	1.243 ± 0.224	1.242 ± 0.221	0.170

3.4 | Changes in Inflammatory, Oxidative, and Cartilage-Related Biomarkers

Baseline inflammatory, oxidative, and cartilage- related bio markers were largely comparable among groups (Table 4). At week 4, inflammatory markers showed greater reductions in the acupuncture- treated groups, especially in the EA+ WA group.

CRP at week 4 was 6.17 ± 2.27 mg/L in the usual- care group, 5.77 ± 2.05 mg/L in the EA group, 5.72 ± 1.96 mg/L in the WA group, and 5.13 ± 2.34 mg/L in the EA + WA group ($p = 0.011$). TNF- α decreased to 27.20 ± 3.35, 25.48 ± 3.43, 24.86 ± 3.38, and 23.89 ± 3.88 pg/mL, respectively ($p < 0.001$). IL- 6 reduction was also greatest in the EA + WA group, with week- 4 reductions of 0.73 ± 0.97, 1.72 ± 1.12, 1.85 ± 1.12, and 2.82 ± 1.14 pg/mL across the four groups, respectively ($p < 0.001$). IL-10 increased modestly and differed significantly among groups at week 4 ($p = 0.035$).

Cartilage- related biomarkers showed similar trends. COMP at week 4 was 17.68 ± 3.12 ng/mL in the usual- care group, 16.57 ± 3.51 ng/mL in the EA group, 16.35 ± 2.70 ng/mL in the WA group, and 15.05 ± 2.96 ng/mL in the EA + WA group ($p < 0.001$). COMP reduction was most evident in the EA + WA group (3.68 ± 1.54 ng/mL; $p < 0.001$). CTX- II and MMP-3 were also significantly lower at week 4 in the acupuncture- treated groups, with the most favorable values generally observed in the EA + WA group.

For oxidative stress indicators, MDA was lowest and SOD was highest in the EA + WA group at week 4. MDA values were 4.67 ± 0.95, 4.24 ± 1.06, 4.47 ± 0.91, and 4.00 ± 1.03 μmol/L in the usual- care, EA, WA, and EA+ WA groups, respectively ($p < 0.001$). SOD values were 95.55 ± 18.35, 101.67 ± 16.55, 103.54 ± 17.87, and 110.87 ± 16.96 U/mL, respectively ($p < 0.001$). TAC did not differ significantly among groups at week 4 ($p = 0.170$; Table 4). Because no formal adjustment for multiple

comparisons was applied, these biomarker findings should be interpreted as exploratory.

3.5 | Adjusted Comparative Analysis

Because several baseline and treatment- context variables differed among groups, adjusted models were used to compare week- 4 outcomes (Table5). After adjustment for baseline outcome values and clinically relevant covariates, EA, WA, and EA + WA remained significantly associated with better week- 4 clinical outcomes than usual care.

Compared with usual care, the adjusted differences in week- 4 VAS were −1.50 for EA, −1.38 for WA, and −2.56 for EA + WA (all $p < 0.001$). EA + WA also showed greater VAS reduction than EA alone and WA alone, with adjusted differences of −1.06 and −1.18, respectively (both $p < 0.001$). For week- 4 WOMAC, the adjusted differences compared with usual care were −10.96 for EA, −9.49 for WA, and −17.26 for EA + WA (all $p < 0.001$). EA + WA was also associated with lower week- 4 WOMAC scores than EA or WA.

Adjusted analyses of functional, sensory, inflammatory, oxidative, and cartilage- related outcomes were consistent with the clinical findings. EA + WA was associated with greater improvements in TUGT, 15- m walk time, PPT-ST36, temporal summation, CPM, IL- 6, MDA, SOD, and COMP than usual care and than either EA or WA alone (Table 5). These associations persisted after multivariable adjustment for measured covariates; however, residual and unmeasured confounding cannot be excluded.

3.6 | Predictors of Clinical Response

Clinical response was defined as a ≥ 30% reduction in VAS at week 4. In the adjusted logistic regression model, all three acupuncture- based treatments were significantly associated with a higher likelihood of clinical response compared with usual care (Table S1). The adjusted odds ratios were 29.67 for EA versus usual care, 24.90 for WA versus usual care, and 139.82 for EA + WA versus usual care (all $p < 0.001$). The model showed good discriminative performance, with an AUC of 0.897.

Among baseline covariates, higher BMI was associated with a greater likelihood of clinical response (OR = 1.15, 95% CI: 1.02–1.30; $p = 0.027$), whereas higher baseline VAS was associated with a lower likelihood of achieving ≥ 30% VAS reduction (OR = 0.39, 95% CI: 0.26–0.59; $p < 0.001$). Age, sex, KOA duration, Kellgren–Lawrence grade, prior acupuncture exposure, treatment expectation, exercise adherence, NSAID use, and rescue medication use were not independently associated with response in the adjusted model.

3.7 | Correlation Analysis

Correlation analysis demonstrated multidomain associations between clinical improvement, sensory modulation, inflammation, oxidative stress, and cartilage- related biomarkers (Figure S3). Greater VAS improvement was positively associated with greater PPT- ST36 improvement, IL-6 reduction, MDA reduction, and COMP reduction. WOMAC improvement showed similar associations with sensory and biochemical changes. These findings suggest that improvements in pain and function were accompanied by parallel improvements in pressure pain sensitivity, inflammatory activity, oxidative stress, and cartilage- related biomarker profiles. However, these associations should be interpreted as exploratory and do not establish causal mechanisms.

3.8 | Safety Outcomes

No serious adverse events or infections occurred in any group (Table S2). Treatment discontinuation did not differ significantly among groups, occurring in 11.0% of patients in the usual- care group, 4.1% in the EA group, 6.9% in the WA group, and 4.2% in the EA + WA group ($p = 0.241$).

Minor adverse events were more common in the acupuncture-treated groups. Local pain occurred in 9.6% of patients in the EA group, 6.9% in the WA group, and 11.9% in the EA + WA group, but not in the usual- care group ($p = 0.024$). Bleeding or hematoma occurred in 6.8%, 9.2%, and 2.5% of patients in the EA, WA, and EA + WA groups, respectively ($p = 0.021$). Burn events were observed only in the WA and EA + WA groups, occurring in 2.3% and 6.8% of patients, respectively ($p = 0.012$). Dizziness/syncope and skin allergy were uncommon and did not differ significantly among groups. Overall, EA, WA, and EA + WA were generally tolerated, although local pain, minor bleeding, and thermal stimulation- related burns should be monitored during acupuncture-based treatment.

4 | Discussion

The current study compared usual care, electroacupuncture plus usual care, warm acupuncture plus usual care, and combined electroacupuncture plus warm acupuncture plus usual care in patients with KOA. In this retrospective cohort, EA + WA plus usual care was associated with more favorable clinical, sensory, neuromuscular, and biomarker profiles. These multidomain differences do not establish specific treatment effects or a direct systemic mechanism. The observed multidomain differences may inform hypotheses for future research but do not establish therapeutic superiority or biological mechanisms. These findings are in line with emerging evidence supporting the integration of multiple acupuncture modalities in KOA care [12, 18].

Pain relief and functional improvement were greater in the EA + WA group than in the other treatment groups. Previous literature has proposed pain- modulatory pathways for acupuncture [9], and the QST findings may be compatible with altered pain processing. However, the retrospective design and QST assessments cannot establish a central or peripheral pain-modulatory mechanism. These findings should therefore be regarded as exploratory and hypothesis- generating. Systematic reviews suggest that EA and WA may be associated with improvements in pain and function in KOA, although the certainty and comparative evidence remain limited [10, 12].

TABLE 5 | Adjusted comparative analysis of week- 4 outcomes.

Outcome	EA vs. usual care	WA vs. usual care	EA + WA vs. usual care	EA + WA vs. EA	EA + WA vs. WA	Model R^2
VAS week 4	−1.50 (−1.84 to −1.16); < 0.001	−1.38 (−1.71 to −1.06); < 0.001	−2.56 (−2.91 to −2.21); < 0.001	−1.06 (−1.34 to −0.79); < 0.001	−1.18 (−1.44 to −0.92); < 0.001	0.780
WOMAC week 4	−10.96 (−13.44 to −8.49); < 0.001	−9.49 (−11.86 to −7.12); < 0.001	−17.26 (−19.82 to −14.71); < 0.001	−6.30 (−8.30 to −4.30); < 0.001	−7.77 (−9.67 to −5.88); < 0.001	0.766
TUGT week 4	−1.43 (−1.94 to −0.93); < 0.001	−1.21 (−1.69 to −0.73); < 0.001	−2.33 (−2.85 to −1.81); < 0.001	−0.89 (−1.30 to −0.49); < 0.001	−1.12 (−1.51 to −0.74); < 0.001	0.781
15- m walk week 4	−1.33 (−1.84 to −0.82); < 0.001	−0.71 (−1.20 to −0.22); 0.005	−2.16 (−2.69 to −1.63); < 0.001	−0.83 (−1.24 to −0.41); < 0.001	−1.45 (−1.84 to −1.06); < 0.001	0.828
PPT ST36 week 4	0.44 (0.28 to 0.60); < 0.001	0.51 (0.35 to 0.67); < 0.001	0.96 (0.79 to 1.13); < 0.001	0.52 (0.39 to 0.66); < 0.001	0.45 (0.33 to 0.58); < 0.001	0.792
Temporal summation week 4	−0.05 (−0.07 to −0.03); < 0.001	−0.03 (−0.05 to −0.01); 0.001	−0.08 (−0.10 to −0.06); < 0.001	−0.03 (−0.05 to −0.02); < 0.001	−0.05 (−0.06 to −0.03); < 0.001	0.769
CPM week 4	0.09 (0.06 to 0.12); < 0.001	0.08 (0.05 to 0.11); < 0.001	0.20 (0.17 to 0.24); < 0.001	0.11 (0.09 to 0.14); < 0.001	0.12 (0.10 to 0.15); < 0.001	0.727
IL-6 week 4	−0.85 (−1.27 to −0.42); < 0.001	−1.02 (−1.43 to −0.62); < 0.001	−1.93 (−2.37 to −1.49); < 0.001	−1.08 (−1.42 to −0.74); < 0.001	−0.90 (−1.23 to −0.58); < 0.001	0.840
MDA week 4	−0.23 (−0.40 to −0.06); 0.007	−0.21 (−0.37 to −0.05); 0.009	−0.49 (−0.66 to −0.31); < 0.001	−0.26 (−0.39 to −0.12); < 0.001	−0.28 (−0.40 to −0.15); < 0.001	0.830
SOD week 4	6.69 (2.22 to 11.15); 0.003	9.80 (5.54 to 14.07); < 0.001	15.92 (11.31 to 20.54); < 0.001	9.23 (5.64 to 12.83); < 0.001	6.12 (2.69 to 9.54); < 0.001	0.617
COMP week 4	−1.10 (−1.65 to −0.54); < 0.001	−0.82 (−1.35 to −0.28); 0.003	−2.82 (−3.40 to −2.25); < 0.001	−1.73 (−2.17 to −1.28); < 0.001	−2.01 (−2.44 to −1.58); < 0.001	0.806

Note: Values are adjusted mean differences (95% CI) from multivariable models. *p* values are two- sided and unadjusted for multiple comparisons. The WOMAC change from baseline to week 4 was the primary endpoint; all other outcomes were exploratory.

The EA + WA group also showed more favorable values for selected inflammatory, oxidative, and cartilage- related biomarkers at week 4. These biomarkers are relevant to inflammation, oxidative stress, and cartilage metabolism in KOA [19]. However, the absence of structural imaging, histological assessment, synovial- tissue analysis, and direct mechanistic measurements prevents conclusions regarding anti- inflammatory activity, cartilage protection, or disease modification. The biomarker findings should be interpreted as exploratory associations requiring prospective mechanistic confirmation. At week 8 and month 3, the EA + WA group retained the most favorable pain and function scores and had the lowest reported recurrence. Treatment frequency may influence clinical outcomes in KOA [20]. Nevertheless, the present follow-up data cannot establish durability or the optimal treatment regimen. No serious adverse events or infections occurred, although local pain, bleeding or hematoma, and thermal burns were more frequent in the acupuncture- treated groups. These findings support the need for careful safety monitoring, particularly when thermal stimulation is used [5, 21].

Despite the strengths of this study, including multimodal outcome assessment and biomarker integration, several limitations should be acknowledged. First, this was a retrospective, nonrandomized cohort study in which treatment allocation was determined by routine clinical practice. Selection bias and confounding by baseline characteristics, including BMI, previous acupuncture exposure, and treatment expectations, may therefore have influenced the observed associations. Differences in exercise adherence, NSAID use, and rescue medication use during follow-up may also have contributed to between- group differences. Although the adjusted models included measured covariates, residual and unmeasured confounding cannot be excluded. Second, the absence of a sham- acupuncture control group prevents separation of specific acupuncture effects from placebo, expectation, attention, and other contextual effects. Third, no formal adjustment was applied for multiple comparisons across secondary and biomarker outcomes. These findings should therefore be regarded as exploratory and may be susceptible to false- positive results. Fourth, follow-up was limited to 3 months, and no structural imaging endpoints were assessed; consequently, the durability of the observed associations and their relation to structural progression remain uncertain. Finally, the study did not include direct mechanistic assessments, such as synovial- tissue analysis, advanced cytokine profiling, multi- omics analyses, or neuroimaging. Accordingly, the findings should be interpreted as observational and hypothesis-generating, rather than as evidence of treatment superiority, causality, cartilage protection, or disease modification.

In this retrospective cohort, EA + WA plus usual care was associated with greater improvements in pain, knee function, physical performance, sensory measures, and selected biomarker profiles than the other treatment strategies. However, in the absence of randomization and a sham- acupuncture control, these findings should be interpreted as observational associations and cannot establish treatment superiority, specific acupuncture effects, or causality. Combined treatment was generally tolerated, although minor local and thermal adverse events require clinical attention. Prospective randomized trials with sham or active controls, longer follow-up, and structural or imaging endpoints are needed to confirm effectiveness, durability, safety, and potential mechanisms.

Author Contributions

Y.F. performed data curation, formal analysis, and wrote and revised the draft of the work. Y.Y. and H.Y.L. performed data curation and formal analysis.

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The authors have nothing to report.

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Ethics Statement

The study was approved by the Ethics Committee of Sichuan Province Orthopedic Hospital (approval no. 2019-11-13-1) and followed the guidelines of the Declaration of Helsinki.

Consent

All participants provided written informed consent prior to enrollment.

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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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Predictors of treatment response (≥ 30% VAS reduction). **Table S2:** Safety outcomes. **Figure S1:** Longitudinal changes in pain intensity and knee function among the four treatment groups. (A) Changes in visual analogue scale (VAS) scores from baseline to week 4, week 8, and month 3 in the usual- care, electroacupuncture plus usual care (EA), warm acupuncture plus usual care (WA), and combined electroacupuncture plus warm acupuncture plus usual care (EA + WA) groups. Lower VAS scores indicate less pain. (B) Changes in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total scores over the same follow- up period. Lower WOMAC scores indicate better knee symptoms and function. The EA + WA group showed the greatest and most sustained improvements in both pain intensity and knee function. **Figure S2:** Clinical response and recurrence across treatment groups. It presents the proportion of patients achieving a clinical response, defined as a ≥ 30% reduction in VAS score at week 4, and the recurrence rates at week 8 and month 3 among the usual- care, EA, WA, and EA+ WA groups. The EA + WA group had the highest response rate and the lowest month- 3 recurrence rate, suggesting more durable symptom improvement. **Figure S3:** Correlation heatmap of clinical, sensory, inflammatory, oxidative, and cartilage- related changes. The heatmap shows correlations among changes in pain, function, quantitative sensory testing parameters, inflammatory markers, oxidative stress indicators, and cartilage- related biomarkers. Greater improvements in VAS and WOMAC were associated with increased pressure pain threshold and with reductions in IL- 6, MDA, and COMP. These associations suggest parallel changes across clinical, sensory, and biochemical domains, but do not establish causal mechanisms.

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ORIGINAL ARTICLE

Longitudinal Course of Muscle Status in Patients With Rheumatoid Arthritis

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ABSTRACT

Objective: Muscle impairment is a common complication of rheumatoid arthritis (RA), but longitudinal data regarding its clinical course remain limited. This study aimed to evaluate the prevalence of muscle impairment and longitudinal changes in muscle status in patients with RA and to identify factors associated with muscle impairment.

Methods: This retrospective cohort study included 75 patients with RA who underwent assessment of skeletal muscle mass index (SMI) using bioelectrical impedance analysis and handgrip strength at baseline and follow-up. Muscle status was classified according to the Asian Working Group for Sarcopenia 2025 criteria using handgrip strength and height-adjusted SMI as normal muscle status, low muscle mass, possible sarcopenia, or sarcopenia. Patients with low muscle mass, possible sarcopenia, or sarcopenia were categorized as having muscle impairment. Baseline characteristics associated with muscle impairment were evaluated using logistic regression analysis. Longitudinal changes in SMI, handgrip strength, and muscle status were assessed over a median follow-up of 29 months.

Results: At baseline, 20 patients (26.7%) had normal muscle status, whereas 55 (73.3%) had muscle impairment, including 30 (40.0%) with sarcopenia. Patients with muscle impairment were older and had lower body mass index (BMI), more advanced Steinbrocker stage, worse physical function, and lower health-related quality of life than those with normal muscle status. In multivariable logistic regression analysis, older age (odds ratio [OR] 1.10, 95% confidence interval [CI] 1.03–1.20, $p = 0.009$) and lower BMI (OR 0.79, 95% CI 0.65–0.92, $p = 0.005$) were independently associated with muscle impairment. No significant changes in SMI or handgrip strength were observed during follow-up. Among patients with normal muscle status at baseline, 78% remained normal at follow-up, whereas 86% of those with muscle impairment continued to exhibit impaired muscle status.

Conclusion: Muscle impairment was highly prevalent in patients with RA and was independently associated with older age and lower BMI. No significant longitudinal changes in muscle status were detected during follow-up, with most patients retaining their baseline muscle status classification. These findings provide longitudinal evidence that muscle impairment tends to persist in patients with established RA.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by persistent synovial inflammation, joint destruction, and functional impairment [1–3]. In addition to joint manifestations, RA is associated with systemic complications, including reduced skeletal muscle mass and muscle strength [4]. Muscle impairment in patients with RA has attracted increasing attention because it contributes to physical disability, impaired quality of life, and frailty [5, 6]. Previous studies have reported

a high prevalence of sarcopenia in patients with RA; however, longitudinal data regarding changes in muscle status over time remain limited.

Muscle impairment in RA is considered a multifactorial condition. In addition to chronic systemic inflammation, several factors may contribute to reduced muscle mass and muscle strength, including aging, physical inactivity, pain, joint dysfunction, malnutrition, and long disease duration [4, 7, 8]. Furthermore, decreased physical activity associated with disability and fear of pain may accelerate muscle decline in patients with RA [7]. Although advances in RA treatment have improved disease control and reduced joint destruction, muscle impairment and physical dysfunction remain common even among patients with low disease activity or remission [9]. Previous studies have reported a high prevalence of sarcopenia and muscle dysfunction in patients with RA [6, 9–16]; however, most available studies have focused on cross- sectional associations or clinical outcomes such as falls and fractures [17–19]. Therefore, longitudinal changes in muscle status over time remain insufficiently characterized.

Therefore, the present study aimed to evaluate muscle status, including skeletal muscle mass and muscle strength, in patients with RA, to compare clinical characteristics between patients with normal muscle status and those with muscle impairment, and to assess longitudinal changes during a median follow-up period of 29 months.

2 | Material and Method

2.1 | Study Population

This retrospective observational study included patients with RA who attended the outpatient clinic of our institution between January and March 2023. Patients fulfilled either the 1987 American College of Rheumatology classification criteria or the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for RA. During routine clinical practice, skeletal muscle mass was assessed using bioelectrical impedance analysis (BIA) in 177 consecutive patients. Among these patients, 96 provided written informed consent to participate in a follow-up study evaluating changes in muscle status after routine lifestyle and exercise recommendations. Of these, 77 patients underwent reassessment of skeletal muscle mass after a median follow-up of 29 months (IQR, 28–31 months). Two patients lacked baseline handgrip strength measurements and were excluded. Therefore, 75 patients with available baseline skeletal muscle mass and handgrip strength data were included in the present analysis (Figure 1). Ethical approval for this study was obtained from the Kyushu University Institutional Review Board (approval number: 22267). Written informed consent was obtained from all participants, and the study was conducted in accordance with the Declaration of Helsinki.

2.2 | Clinical Information

Baseline clinical information included age, sex, disease duration, body mass index (BMI), and disease activity. Disease activity was evaluated using the Disease Activity Score in 28 joints with C- reactive protein (DAS28-CRP). Functional status and health- related quality of life were assessed using the modified Health Assessment Questionnaire (mHAQ) [20]. and the EuroQol 5- Dimension (EQ-5D) [21, 22], respectively. Serological status was assessed using rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti- CCP) antibodies. Steinbrocker stage was determined by radiographic abnormalities of the hand in patients with RA as follows: stage I, no destructive changes; stage II, slight cartilage and/or subchondral bone destruction and osteoporosis; stage III, cartilage and/or bone destruction and osteoporosis; and stage IV, osseous ankylosis [23, 24]. Data on serological markers were unavailable in some patients because these measurements had not been performed at our institution or were not available in the medical records. Information regarding pharmacological treatment, including glucocorticoid use, methotrexate use, and biologic or targeted synthetic disease- modifying antirheumatic drugs (b/tsDMARDs), was obtained from medical records. Physical activity at follow-up was evaluated using the University of California, Los Angeles (UCLA) Activity Score.

2.3 | Clinical and Body Composition Assessments

Body composition was assessed using BIA with the InBody 570 device (InBody Japan Co. Ltd., Tokyo, Japan) as part of routine clinical practice. BMI was calculated as body weight divided by height squared (kg/m^2). The following body composition parameters were obtained: skeletal muscle mass index (SMI, kg/m^2). SMI was calculated as appendicular skeletal muscle (ASM) mass divided by height squared [25]. Handgrip strength was measured at baseline and at the follow-up visit using a handheld dynamometer. Measurements were obtained from both hands, and the highest value was used for analysis. Handgrip strength was analyzed as a continuous variable and was used as an indicator of muscle strength.

2.4 | Sarcopenia Definition

Muscle status was classified according to the Asian Working Group for Sarcopenia (AWGS) 2025 criteria [26, 27]. using handgrip strength and SMI measured by BIA. Low handgrip strength was defined as <34 kg for men aged 50–64 years, <28 kg for men aged ≥ 65 years, <20 kg for women aged 50–64 years, and <18 kg for women aged ≥ 65 years. Low muscle mass was defined as an SMI <7.6 kg/m^2 for men aged 50–64 years, <7.0 kg/m^2 for men aged ≥ 65 years, and <5.7 kg/m^2 for women. Because only a small number of participants were younger than 50 years, these patients were evaluated using the cutoff values for the 50–64 year age category. Participants were categorized as having normal muscle status, possible sarcopenia (low handgrip strength alone), low muscle mass (low SMI alone), or sarcopenia (both low handgrip strength and low muscle mass) (Figure 1). For subsequent analyses, patients were further classified into two groups: normal muscle status and muscle impairment, the latter including possible sarcopenia, low muscle mass, and sarcopenia. Because the AWGS 2025 additionally recommends consideration of BMI- adjusted ASM/BMI cutoffs in individuals with

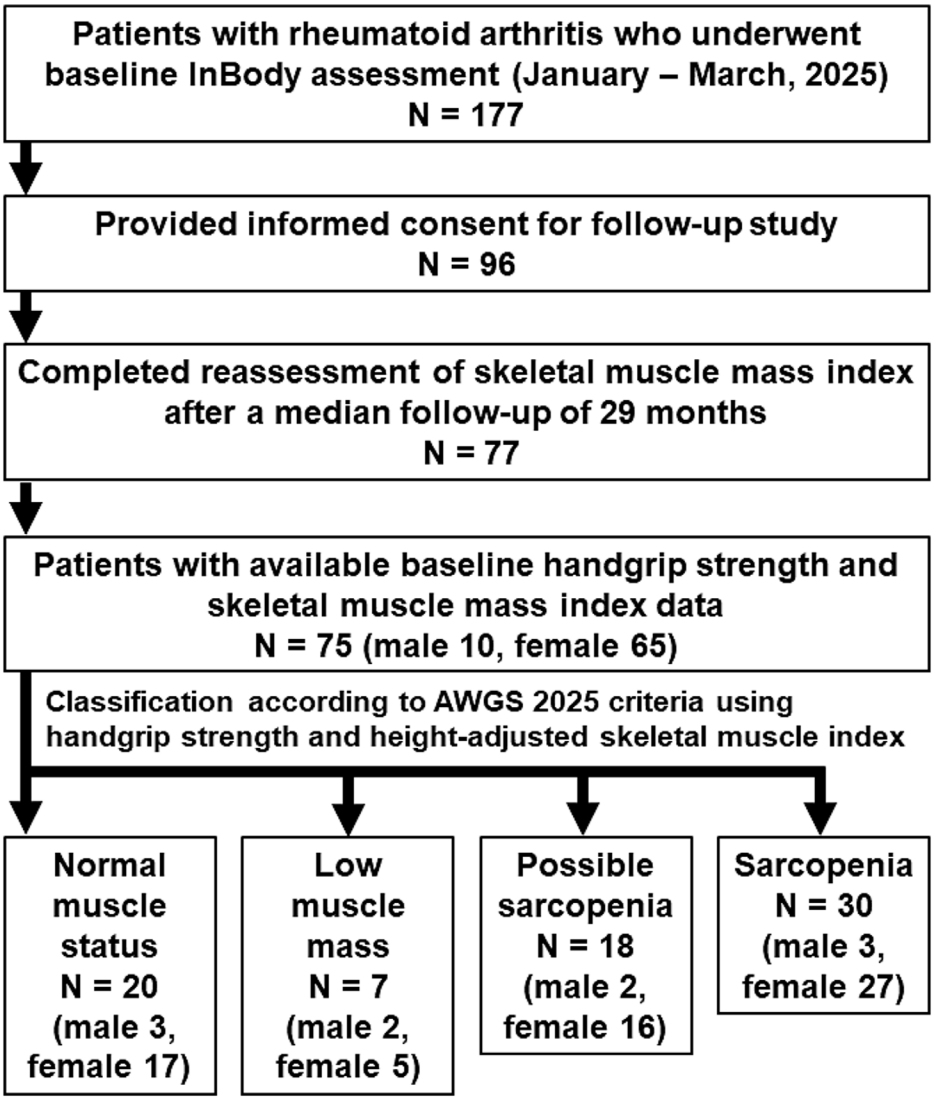

FIGURE 1 | Flow diagram of patient selection and classification of muscle status. Among 177 patients with rheumatoid arthritis who underwent baseline bioelectrical impedance analysis between January and March 2023, 96 provided informed consent for follow- up assessment. Seventy-seven patients underwent repeat skeletal muscle mass assessment after a median follow- up of 29months. Of these, Seventy- five patients had both baseline skeletal muscle mass index (SMI) and handgrip strength measurements available and were included in the analysis. Muscle status was classified according to the Asian Working Group for Sarcopenia (AWGS) 2025 criteria using handgrip strength and height- adjusted SMI. Patients were categorized as normal muscle status, low muscle mass, possible sarcopenia, or sarcopenia.

BMI $\geq 24\,kg/m^2$, a supplementary sensitivity analysis was performed in which participants meeting this BMI criterion were reclassified using the BMI- adjusted criteria. The results of this reclassification are presented in Table S1.

2.5 | Outcome Definition

The primary outcome of this study was the prevalence of muscle impairment at baseline, defined according to the AWGS 2025 criteria. The primary objective was to compare the clinical characteristics of patients with normal muscle status and those with muscle impairment. Secondary outcomes included longitudinal changes in SMI and handgrip strength during a median follow-up period of 29 months and transitions in muscle status categories between baseline and follow-up assessments.

2.6 | Statistical Analysis

All statistical analyses were performed using JMP Student Edition version 19 (SAS Institute Inc., Cary, NC, USA). Continuous variables are presented as median and interquartile range (IQR), and categorical variables are presented as numbers and percentages. Baseline characteristics were compared between patients with normal muscle status and those with muscle impairment using the Mann–Whitney *U* test for continuous variables and the chi- square test or Fisher's exact test for cate gorical variables, as appropriate. Changes in SMI and handgrip strength between baseline and the follow-up were evaluated using paired *t*- tests and are presented graphically as spaghetti plots. Longitudinal changes in SMI and handgrip strength between baseline and follow-up were evaluated using paired non- parametric analyses. To identify factors independently associated with muscle impairment at baseline, multivariable

logistic regression analysis was performed. Because of the limited sample size, the multivariable model was restricted to a limited number of clinically relevant variables to minimize the risk of model overfitting. Variables were selected a priori based on clinical relevance and statistical considerations and included age at assessment, disease duration, BMI, Steinbrocker stage, and DAS28- CRP. Steinbrocker stage was dichotomized as stages I–II and stages III–IV for multivariable logistic regression analysis. Other clinically relevant variables, including disease activity and treatment- related factors, were not simultaneously included because of the limited sample size. Odds ratios (OR) and 95% confidence interval (CI) were calculated. Transitions in muscle status categories between baseline and follow-up were summarized descriptively, and associations between baseline and follow-up muscle status were evaluated using the chi- square test. All tests were two- sided, and a p value <0.05 was considered statistically significant.

3 | Result

3.1 | Baseline Characteristics

A total of 75 patients with RA who had available baseline measurements of both SMI and handgrip strength were included in the analysis. According to the AWGS 2025 criteria, 20 patients (26.7%) were classified as having normal muscle status and 55 patients (73.3%) as having muscle impairment, including possible sarcopenia ($N=18$), low muscle mass ($N=7$), or sarcopenia ($N=30$) (Figure 1 and Table 1). Compared with patients with

TABLE 1 | Baseline characteristics of patients with rheumatoid arthritis according to muscle status ($N=75$).

	Total ($N=75$)	Normal muscle status ($N=20$)	Muscle impairment ($N=55$)	p
Age at the time of assessment, year	67 (58–72)	65 (50–69)	68 (59–74)	0.027
Female sex, N (%)	65 (87)	17 (85)	48 (87)	0.798
Disease duration, year	14 (7–21)	11 (4–12)	16 (8–22)	0.008
BMI, kg/m^2	22.6 (20.0–25.2)	23.7 (21.5–27.9)	21.8 (19.4–25.0)	0.012
RF, IU/mL	29 (7–78)	12 (5–55)	31 (7–93)	0.248
Anti- CCP antibodies, U/mL	15.6 (0.6–100.0)	3.9 (0.6–39.3)	28.2 (3.9–100.0)	0.081
Steinbrocker				
Stage I, N (%)	11 (15)	6 (30)	5 (9)	0.036
Stage II, N (%)	24 (32)	8 (40)	16 (29)	
Stage III, N (%)	18 (24)	4 (20)	14 (25)	
Stage IV, N (%)	22 (29)	2 (10)	20 (36)	
DAS28-CRP	1.52 (1.23–2.22)	1.41 (1.22–2.21)	1.56 (1.25–2.26)	0.928
mHAQ	0 (0–0.38)	0 (0–0.13)	0.13 (0–0.75)	0.024
EQ-5D	0.69 (0.62–0.81)	0.77 (0.71–0.81)	0.69 (0.61–0.81)	0.085
Treatment				
Prednisolone use, N (%)	31 (44)	9 (45)	22 (40)	0.697
Prednisolone dose, mg/day	2.5 (2.5–5.0)	3.0 (2.5–10.0)	2.5 (2.5–5.0)	0.317
Methotrexate use, N (%)	56 (75)	15 (75)	41 (75)	0.968
Methotrexate dose, mg/week	8 (6–10)	8 (6–10)	8 (6–10)	0.753
b/tsDMARDs use	34 (48)	6 (30)	28 (51)	0.108
UCLA activity score	5 (4–6)	6 (4–6)	5 (4–6)	0.168
SMI, kg/m^2	5.9 (5.2–6.5)	6.4 (6.0–7.1)	5.5 (5.1–6.3)	0.002
Handgrip strength, kg	17.7 (12.7–22.2)	23.2 (20.1–31.1)	14.8 (11.4–18.0)	<0.001

Note: Muscle impairment was defined as low handgrip strength and/or low skeletal muscle mass index according to the Asian Working Group for Sarcopenia 2025 criteria. Values are presented as median (interquartile range [IQR]) or number (%). p values were calculated using the Mann–Whitney U test or χ^2 test, as appropriate (normal muscle status vs. muscle impairment). Missing data were observed for anti- CCP antibody ($N=17$), EQ- 5D ($N=1$), mHAQ ($N=1$), and UCLA activity score ($N=9$). bDMARDs included abatacept ($N=2$), adalimumab ($N=4$), certolizumab pegol ($N=1$), etanercept ($N=12$), golimumab ($N=1$), infliximab ($N=3$), sarilumab ($N=5$), and tocilizumab ($N=6$). No patients received targeted synthetic DMARDs.

Abbreviations: b/tsDMARDs, biologic or targeted synthetic DMARDs; BMI, body mass index; CCP, cyclic citrullinated peptide; DAS28- CRP, disease activity score in 28 joints with C- reactive protein; DMARDs, disease modifying anti-rheumatic drugs; EQ-5D, EuroQol 5-Dimension; mHAQ, modified Health Assessment Questionnaire; RF, rheumatoid factor; SMI, skeletal muscle index; UCLA, University of California, Los Angeles.

normal muscle status, those with muscle impairment were significantly older (median 65 [IQR 50–69] vs. 68 [59–74] years, $p = 0.027$), had longer disease duration (median 11 [4–13]. vs. 16 [9–22]. years, $p = 0.029$), more advanced Steinbrocker stage ($p = 0.036$), and had lower BMI (median 23.7 [21.5–27.9] vs. 21.8 [19.4–25.0] kg/m^2, $p = 0.012$). Patients with muscle impairment also had worse functional status as assessed by mHAQ (median 0 [0–0.13] vs. 0.13 [0–0.75] years, $p = 0.024$). As expected, SMI and handgrip strength were significantly lower in the muscle impairment group (both $p < 0.001$). No significant differences were observed in disease activity or other clinical characteristics between the groups.

As a supplementary analysis, participants with BMI ≥ 24 kg/m^2 ($n = 25$) were reclassified according to the BMI- adjusted ASM/BMI criteria recommended in the AWGS 2025 [26, 27]. Compared with the primary height- adjusted SMI classification, six participants were reclassified from normal muscle status to low muscle mass and nine participants from possible sarcopenia to sarcopenia, whereas no other changes in classification were observed (Table S1). These reclassifications did not materially change the overall interpretation of the longitudinal findings. In addition, to assess potential selection bias, baseline characteristics of patients included and not included in the longitudinal analysis were compared (Table S2). Overall, no major differences in baseline demographic or clinical characteristics were observed between the two groups.

3.2 | Factors Associated With Muscle Impairment

Variables significantly associated with muscle impairment in univariable analyses were entered into a multivariable logistic regression model. Older age (OR 1.10, 95% CI 1.03–1.20, $p = 0.009$) and lower BMI (OR 0.79, 95% CI 0.65–0.92, $p = 0.005$) were independently associated with muscle impairment, whereas disease duration, Steinbrocker stage, and DAS28- CRP were not (Table 2).

3.3 | Longitudinal Changes in Muscle Status

At the follow-up assessment, SMI measurements were available for all 75 patients, whereas handgrip strength measurements were available for 68 patients. No significant changes in SMI were observed between baseline and follow-up in the overall cohort or when patients were analyzed according to baseline muscle status (Figure 2A). Similarly, handgrip strength remained largely unchanged over the follow-up period in both groups (Figure 2B). No significant difference in DAS28- CRP was observed between groups, suggesting that current inflammatory activity alone may not fully explain muscle impairment. Among the 68 patients with complete data for muscle status classification at both time points, 14 of 18 patients (77.8%) with normal muscle status at baseline remained normal at follow-up, whereas 43 of 50 patients (86.0%) with baseline muscle impairment continued to exhibit muscle impairment. Baseline muscle status was significantly associated with muscle status at follow-up ($p < 0.001$) (Table 3). Absolute changes in SMI and handgrip strength are also summarized in Table 3. Median changes in SMI were −0.1 kg/m^2 (IQR, −0.3 to 0.1) in the normal muscle group and 0.0 kg/m^2 (IQR, −0.3 to 0.2) in the muscle impairment group. Corresponding median changes in handgrip strength were −0.3 kg (IQR, −2.7 to 1.9) and −0.2 kg (IQR, −3.2 to 3.4), respectively.

TABLE 2 | Multivariable logistic regression analysis for muscle impairment at baseline ($N = 75$).

Variable	OR	95% CI	*p*
Age at the time of assessment, year	1.08	1.01–1.17	0.036
Disease duration, year	1.05	0.97–1.15	0.235
BMI, kg/m^2	0.78	0.65–0.91	0.005
Steinbrocker Stage III–IV	2.32	0.55–10.87	0.260
DAS28-CRP	0.83	0.33–2.09	0.691

Note: variance inflation factors; BMI, body mass index; DAS28- CRP, disease activity score in 28 joints with C- reactive protein.
Abbreviations: CI, confidence interval; OR, odds ratio.

4 | Discussion

In this cohort study of patients with RA, approximately one-quarter of patients maintained normal muscle status according to the AWGS 2025 criteria, whereas the remaining three- quarters exhibited some degree of muscle impairment. Notably, no significant longitudinal change in muscle status was detected during a median follow-up period of 29 months, and most patients retained their baseline muscle classification. Older age and lower BMI were independently associated with muscle impairment in the multivariable analysis, consistent with previous studies in patients with RA. However, these findings should be interpreted cautiously because the relatively small sample size limited adjustment for additional clinically relevant variables, including disease activity, treatment characteristics, and structural joint damage. Therefore, residual confounding cannot be excluded.

Muscle impairment is a well- recognized complication of RA and is commonly referred to as rheumatoid cachexia or sarcopenia associated with RA [5, 6]. In the present study, 40% of patients fulfilled the AWGS 2025 criteria for sarcopenia. This prevalence is higher than that reported in community- dwelling older adults and is generally consistent with previous studies demonstrating an increased burden of sarcopenia among patients with RA [10, 12, 13, 28–30]. Chronic systemic inflammation plays a central role in muscle catabolism through increased production of pro- inflammatory cytokines such as tumor necrosis factor-α and interleukin- 6, which promote protein degradation and impair muscle protein synthesis [4, 31, 32]. In addition, reduced physical activity due to joint pain, functional limitation, and fatigue may contribute to progressive declines in muscle mass and muscle strength in patients with RA [9, 33, 34]. Notably, approximately one- quarter of patients in our cohort maintained normal muscle status despite established RA. This finding suggests that preservation of normal muscle status is possible in a subset of patients with RA, even in those with longstanding disease. Consistent with previous reports, older age and lower

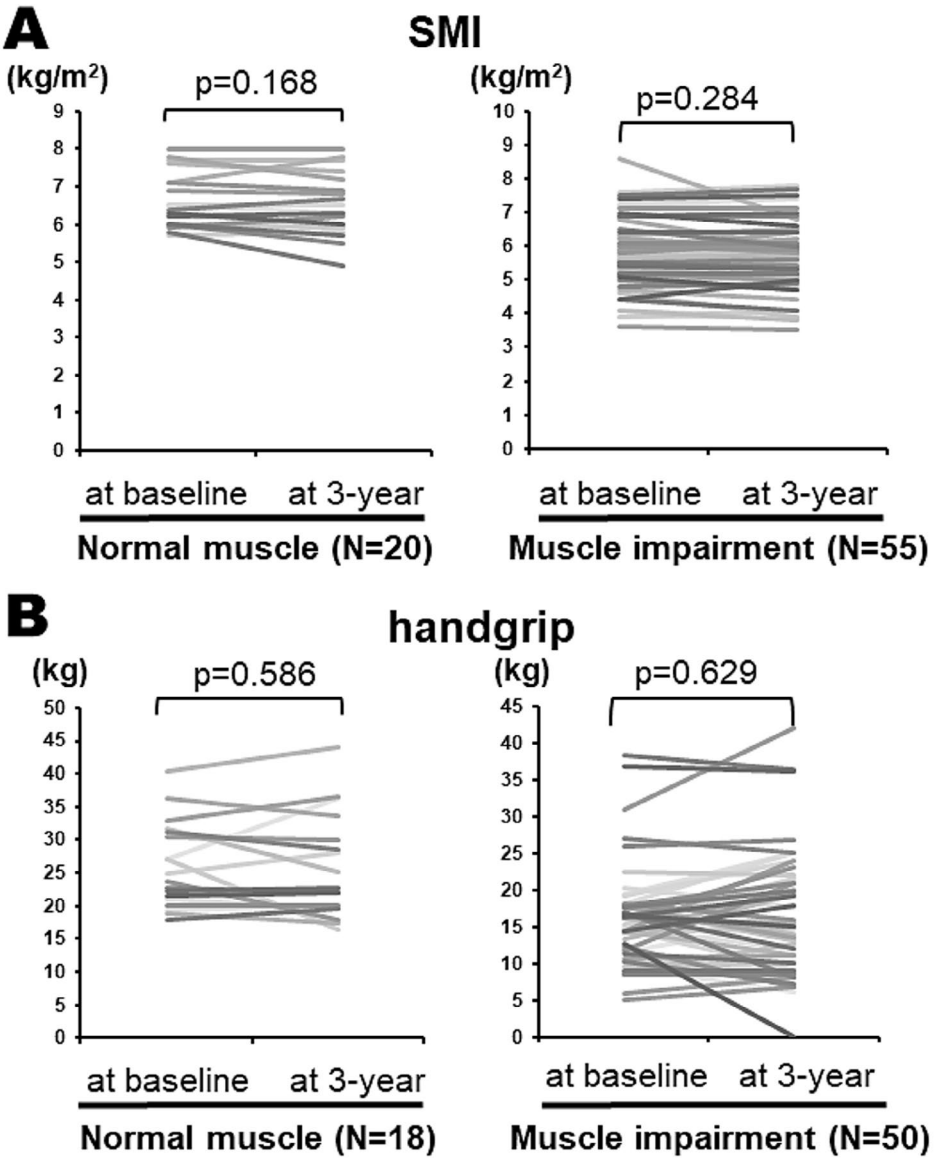

FIGURE 2 | Longitudinal changes in skeletal muscle mass index and handgrip strength according to baseline muscle status. Spaghetti plots showing individual longitudinal changes in (A) skeletal muscle mass index (SMI) and (B) handgrip strength from baseline to follow- up. Patients were stratified according to baseline muscle status as normal muscle status or muscle impairment, defined according to the Asian Working Group for Sarcopenia (AWGS) 2025 criteria. Each line represents an individual patient. No significant changes in SMI or handgrip strength were observed during follow- up in either group as assessed by paired*t*-tests.

BMI were independently associated with muscle impairment [14, 30, 33, 35], whereas DAS28- CRP was not independently associated with muscle impairment in the multivariable analysis. Although advanced Steinbrocker stage was more frequent among patients with muscle impairment, this association was not independent after adjustment for age and BMI. These findings should be interpreted cautiously because the relatively small sample size limited adjustment for additional clinically relevant variables, including treatment characteristics and cumulative inflammatory burden; therefore, residual confounding cannot be excluded.

Interestingly, approximately 78% of patients with normal muscle status at baseline remained normal after a median follow-up of 29 months, whereas 86% of those with muscle impairment continued to exhibit impaired muscle status at follow-up. Consistent with these findings, neither SMI nor handgrip strength showed significant changes during the observation period. Together, these results suggest that no significant longitudinal changes in muscle status were detected in patients with established RA over the median follow-up period of 29 months. Our findings are consistent with a recent longitudinal study evaluating sarcopenia in RA [36]. In a prospective cohort study with a median follow-up of 6.4 years, the prevalence of sarcopenia remained largely unchanged, and no new cases of sarcopenia were observed during follow-up. The persistence of muscle impairment in our cohort may indicate that muscle loss and weakness, once established, are difficult to reverse through routine clinical management alone. Conversely, the high rate of preserved normal muscle status suggests that maintenance of muscle health is achievable in a subset of patients with RA. Although the factors contributing to long- term preservation of normal muscle status remain

TABLE 3 | Clinical characteristics and muscle status at 3- year follow-up according to baseline muscle status (*N* = 75).

	Total (*N* = 75)	Normal muscle status at baseline (*N* = 20)	Muscle impairment at baseline (*N* = 55)	*p*
Age at the time of assessment, year	70 (60–75)	68 (52–71)	71 (62–76)	0.023
BMI, kg/m^2	22.6 (19.8–24.9)	23.4 (21.8–28.4)	21.7 (19.2–24.1)	0.021
DAS28-CRP	1.42 (1.31–1.82)	1.42 (1.32–1.76)	1.47 (1.31–1.82)	0.871
UCLA activity score	5 (4–6)	6 (4–6)	4 (4–6)	0.083
SMI, kg/m^2	5.9 (5.2–6.5)	6.3 (5.9–7.1)	5.6 (5.0–6.2)	0.001
Change in SMI, kg/m^2	0 (−0.3–0.1)	−0.1 (−0.3–0.1)	0 (−0.3–0.2)	0.300
Handgrip strength, kg	17.9 (12–23.7)	22.5 (19.5–30.9)	15.0 (10.0–21.1)	< 0.001
Change in handgrip strength, kg	−0.2 (−2.9–3.1)	−0.3 (−2.7–1.9)	−0.2 (−3.2–3.4)	0.835
Muscle status,				
Normal, *n* (%)	21 (31)	14 (78)	7 (14)	< 0.001
Impairment, *n* (%)	47 (69)	4 (22)	43 (86)	
Treatment				
Prednisolone use, *N* (%)	25 (33)	4 (20)	21 (38)	0.140
Prednisolone dose, mg/day	2.5 (1.6–3.0)	4.0 (1.5–5.0)	2.5 (1.6–2.8)	0.263
Methotrexate use, *N* (%)	52 (69)	16 (80)	36 (65)	0.227
Methotrexate dose, mg/week	8 (6–10)	8 (6–8)	8 (6–10)	0.607
b/tsDMARDs use	37 (49)	7 (35)	30 (55)	0.134
Follow- up duration, month	29 (28–31)	29 (28–31)	29 (28–32)	0.716

Note: Muscle impairment was defined as low handgrip strength and/or low skeletal muscle mass index according to the Asian Working Group for Sarcopenia 2025 criteria. Values are presented as median (interquartile range [IQR]) or number (%). *p* values were calculated using the Mann–Whitney *U* test, as appropriate (normal muscle status vs. muscle impairment). Missing data were observed for UCLA activity score (*N* = 2), handgrip strength (*N* = 7), and muscle status (*N* = 7). b/tsDMARDs included abatacept (*N* = 2), adalimumab (*N* = 4), certolizumab pegol (*N* = 1), etanercept (*N* = 12), ozoralizumab (*N* = 1), infliximab (*N* = 2), sarilumab (*N* = 6), tocilizumab (*N* = 7), and filgotinib (*N* = 2).

Abbreviations: b/tsDMARDs, biologic or targeted synthetic disease modifying anti- rheumatic drugs; BMI, body mass index; SMI, skeletal muscle index; UCLA, University of California, Los Angeles.

unclear, our findings highlight the potential importance of early identification of patients at risk for muscle impairment and implementation of preventive strategies before substantial muscle loss develops. In the present study, physical activity assessed using the UCLA activity score likewise showed little change over time, consistent with previous reports evaluating UCLA activity scores in patients undergoing total joint arthroplasty [37]. Although the relationship between physical activity and muscle status could not be fully evaluated, no significant longitudinal changes were detected in either muscle status or habitual physical activity during the follow-up period.

From a clinical perspective, these findings highlight the importance of understanding the long- term course of muscle status in patients with RA. A previous study has suggested that sarcopenia and muscle dysfunction may improve following effective treatment in patients with early RA [38]. In contrast, the patients included in the present study had established RA with a relatively long disease duration, and muscle status remained largely unchanged throughout the follow-up period. Together with the high persistence of muscle impairment observed in our cohort, these findings suggest that no significant longitudinal improvement in muscle mass or muscle strength was detected once muscle impairment had developed in patients with long-standing RA. Because longitudinal studies evaluating muscle status in RA remain limited, our findings provide additional evidence regarding the natural course of muscle impairment in contemporary RA management. Further studies with larger cohorts and longer follow-up periods are needed to clarify factors associated with preservation or improvement of muscle status and to determine whether targeted interventions can modify its long-term trajectory.

This study has several limitations. First, this was a single- center study with a relatively small sample size, which may limit the generalizability of our findings. In addition, the longitudinal analysis included only patients who agreed to undergo follow-up body composition assessments, which may have introduced selection bias. Patients who participated in follow-up evaluations may have differed from those who did not in terms of health status, physical function, or motivation. Second, although disease activity was assessed using DAS28- CRP at baseline and follow-up, cumulative inflammatory burden during the observation period was not evaluated. Therefore, residual confounding

related to long- term disease activity may remain. Third, physical activity was assessed using the UCLA activity score only at the follow-up visit. Because baseline physical activity data were unavailable, we could not determine whether changes in physical activity influenced longitudinal changes in muscle status. In addition, patient- reported outcomes such as mHAQ and EQ- 5D were evaluated primarily at baseline, limiting our ability to examine their longitudinal relationship with muscle status. Fourth, handgrip strength measurements were unavailable in several patients at follow-up, resulting in a reduced number of patients who could be classified according to the AWGS 2025 criteria. Consequently, longitudinal transitions in muscle status may have been underestimated or misclassified in some cases. Finally, body composition was assessed using BIA rather than dual- energy X-ray absorptiometry, which is generally considered the reference method for evaluating skeletal muscle mass. However, BIA is widely used in clinical practice and has been validated as a reliable and non- invasive tool for assessing skeletal muscle mass in patients with RA. Despite these limitations, this study provides longitudinal data on muscle status, including skeletal muscle mass and muscle strength, in patients with RA. Our findings indicate that no significant longitudinal changes in muscle status were detected over a median follow-up period of 29 months and highlight the importance of identifying factors associated with preserved muscle health in this population.

5 | Conclusion

Muscle impairment was common among patients with RA, whereas approximately one- quarter of patients maintained normal muscle status according to the AWGS 2025 criteria. Older age and lower BMI were independently associated with muscle impairment. During a median follow-up period of 29 months, muscle status remained largely unchanged, with most patients maintaining their baseline classification. These findings indicate that no significant longitudinal changes in muscle status were detected over a median follow-up period of 29 months. Given the limited longitudinal evidence currently available, further prospective studies are warranted to clarify the determinants of long- term changes in muscle status in patients with RA.

Author Contributions

The authors confirm contribution to the paper as follows: Conceptualization, Toshifumi Fujiwara, Hidetoshi Tsushima and Osamu Tokunaga; Acquisition of data, Toshifumi Fujiwara, Hidetoshi Tsushima, Osamu Tokunaga, Daisuke Hara, Ryosuke Yamaguchi, and Yukio Akasaki; Analysis of data, Toshifumi Fujiwara, Hidetoshi Tsushima and Osamu Tokunaga; Interpretation of data, Toshifumi Fujiwara and Hidetoshi Tsushima; Original draft preparation, Toshifumi Fujiwara; Writing – reviewing and editing, Toshifumi Fujiwara, Hidetoshi Tsushima, Daisuke Hara, Ryosuke Yamaguchi, Ichiro Kurakazu, and Yukio Akasaki; Supervision, Toshifumi Fujiwara and Yasuharu Nakashima; All authors approved the final version of the manuscript.

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During the preparation of this work the authors used ChatGPT (OpenAI) in order to assist with English language editing and refinement of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Ethics Statement

Ethical approval for this study was obtained from the Kyushu University Institutional Review Board (approval number: 22267). The written informed consent was obtained from all participants prior to participation. 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 data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Cross- classification of muscle status according to height- adjusted SMI and BMI-adjusted ASM/BMI criteria (AWGS 2025) ($N = 75$). **Table S2:** Comparison of baseline characteristics between patients included and not included in the longitudinal analysis. (N = 177).

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ORIGINAL ARTICLE

Elevated Histamine Receptor 2 and Toll-Like Receptor 7 Expression in Rheumatoid Arthritis: Insights Into Inflammatory Mechanisms and Potential Therapeutic Strategies

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovial inflammation and joint damage. Toll-like receptor 7 (TLR7), one of the innate immunity receptors, plays a role in releasing inflammatory mediators like cytokines and histamine. These mediators are crucial in inducing and maintaining chronic inflammation in the joints. This study examines the relationship between histamine receptor 2 (H2R) and TLR7 expression in peripheral blood mononuclear cells (PBMCs) and evaluates plasma levels of histamine, IL-10, and TNF-α in RA patients.

Methods: The study included 60 RA patients (22 newly diagnosed and 38 undergoing treatment) and 30 healthy controls. H2R and TLR7 gene expression in PBMCs was measured using Real-time PCR (RT-PCR), while plasma levels of histamine, TNF-α, and IL-10 were assessed using ELISA.

Results: H2R and TLR7 gene expression in PBMCs was significantly higher in RA patients than in controls ($p = 0.0013$, $p = 0.0057$), with newly diagnosed patients showing increased H2R expression compared to treated individuals ($p = 0.0004$). Plasma levels of histamine, TNF-α, and IL-10 were elevated in RA patients, with higher histamine and TNF-α levels in newly diagnosed cases and increased IL-10 in treated patients ($p = 0.0253$). Significant correlations were observed between H2R and TLR7 expression ($R = 0.68$, $p < 0.0001$) and between H2R expression and histamine levels ($R = 0.34$, $p = 0.01$).

Conclusions: Our study suggests a potential link between H2R signaling and TLR7 activation in RA. The therapeutic potential of H2R inhibitors and TLR7 antagonists in mitigating RA progression warrants further investigation.

1 | Introduction

Rheumatic diseases encompass a range of conditions affecting joints, bones, and muscles, often causing pain, inflammation, and stiffness. Rheumatoid arthritis (RA) is an autoimmune rheumatic disease where the body's immune system mistakenly attacks its joints, leading to chronic inflammation [1]. This inflammation is a key driver of RA pathogenesis. Histamine,

a biogenic amine and crucial mediator in inflammatory processes, is found in high quantities during these processes and plays a significant role in RA [2]. Histamine acts through four G protein- coupled receptors (H1R, H2R, H3R, and H4R) [3]. While H1 receptors primarily drive vascular effects and H2 receptors influence gastric secretion [4] H4 receptors on hematopoietic cells are important in inflammatory conditions, including RA [5]. H3 receptors also play a role in inflammation, modulating histamine release and other neurotransmitter systems, although their precise role in RA is still being investigated. Histamine and its receptors (H1R- H4R) are implicated in RA pathogenesis and are potential therapeutic targets. Specifically, H2 receptors are expressed on various immune cells, including PBMCs, and play a role in modulating immune responses in inflammatory conditions like RA [6]. Although recent research has predominantly focused on H4 receptors due to their established role in immune cell recruitment and inflammation, the immunomodulatory functions of H2R—particularly in regulating cytokine production and immune balance—remain relatively underexplored in RA. This highlights a critical gap in current knowledge and provides a rationale for further investigation of H2R in the context of RA pathogenesis.

The Toll- like receptor (TLR) family initiates innate and adaptive immune responses and plays a significant role in the inflammatory processes characteristic of RA [7]. TLRs identify pathogen- associated and damage-associated molecular patterns (PAMPs and DAMPs, respectively) [8]. TLR7, found in hematopoietic and non- immune cells, responds to its ligand within endosomes, and its expression is elevated in RA [9]. TLR7 activation can contribute to pro- inflammatory cytokine production, further exacerbating RA's inflammatory cascade. Both histamine signaling via H2R and TLR7 activation are implicated in the complex inflammatory mechanisms driving RA [10].

Cytokines modulate immune responses and contribute to systemic disorders [11]. IL- 10, produced by TH2 lymphocytes, T regulatory cells, and macrophages, is an anti- inflammatory factor [12]. TNF- α, a pro-inflammatory cytokine, plays roles in immune responses, tumor cytotoxicity, and conditions like diabetes and rheumatoid arthritis [13, 14]. Both histamine signaling and TLR activation can influence the production and release of these cytokines. For instance, through its receptors (particularly H2R), histamine can modulate the production of pro- inflammatory cytokines like TNF-α and anti- inflammatory cytokines like IL-10, contributing to the complex cytokine balance in inflammatory conditions [15]. Similarly, TLR7 activation is known to induce the production of pro- inflammatory cytokines, including TNF-α, which further amplifies the inflammatory cascade [16]. This interplay between histamine signaling, TLR activation, and cytokine production is crucial in the pathogenesis of RA. This study aimed to investigate the correlation of histamine receptor 2 (H2R) and TLR7 expression in peripheral blood mononuclear cells of RA patients. Crucially, rather than evaluating cytokines in isolation, we also assessed plasma levels of TNF- α and IL- 10 to serve as functional, downstream indicators of the pro- and anti- inflammatory balance associated with the activation of this receptor axis across different stages of treatment status.

2 | Materials and Methods

2.1 | Sample Collection

This case–control study included a patient group of 60 individuals diagnosed with rheumatoid arthritis (RA), among whom approximately 22 were newly diagnosed cases, ranging in age from 20 to 90 years. A rheumatologist confirmed RA diagnoses based on the American College of Rheumatology (ACR) criteria, and disease activity was evaluated using the Clinical Disease Activity Index (CDAI). The control group consisted of 30 healthy individuals, free from rheumatoid arthritis or other chronic illnesses, selected to be partially matched with the RA group. All participants provided informed consent before inclusion in the study, and none of the patients had comorbid conditions. Patient recruitment took place between August 2022 and April 2023 at Sayad Shirazi Hospital in Gorgan City.

2.2 | RNA Extraction and Real–Time PCR (RT-PCR)

The blood samples were collected and divided into two 5 mL tubes, one for isolating PBMCs and then RNA extraction and cDNA synthesis for RT-PCR. Total RNA was isolated from frozen PBMC samples by RNX-PLUS (Sinaclon, Cat. No: EX6101), and the cDNA was synthesized by a cDNA kit (Sinaclon, Cat. No: RT5210). Gene- specific primers were designed using oligoanalyzer software and the NCBI website. Primer sequences specific for the TLR7 and H2R genes are presented in Table 1.

To evaluate mRNA expression level, RT-PCR was performed using specific primers (Table 1), and the relative amount of the genes of interest and the s18 reference gene was measured in two independent assays. Specific amplification of the PCR products was analyzed using melting curve analysis. The data were presented as the relative expression of the genes of interest compared with the internal control gene as determined by the $2^{-\Delta CT}$ method. Histamine levels, tumor necrosis factor- alpha (TNF-α), and interleukin- 10 (IL-10) were measured in plasma using ELISA kits from Biolegend (Cat. No: 430204) and Zellbio GmbH (Cat. No: RK00012), respectively.

2.3 | Statistical Analysis

Continuous variables (cytokines and gene expression levels) were expressed as (means ± SE). Group comparisons were conducted using the Student's *t*- test (or Mann–Whitney test). For analyses involving more than two groups, the Kruskal- Wallis ANOVA test was utilized. Correlations between variables were assessed using Spearman's test. Statistical analysis was performed using GraphPad Prism, with a significance threshold set at $p \leq 0.05$.

3 | Results

3.1 | Demographic Analysis

The results showed that the highest percentage of RA- affected individuals was in the 46–55 age group (41.60%), followed by the

TABLE 1 | Primer sequences specific for the TLR- 7 and H2R genes.

Oligo-name	Seq. (5–3)	M.W	T.M	GC%	Mer
TLR-7-F	AGCCACAACCAACTGACCAC	6009	59.35	55	20
TLR-7-R	ATCGCAACTGGAAGGCATCTTG	6759.4	60.25	50	22
H2R-F	CAGTTCGGGTCGCCATCTC	5755.7	60.98	63.16	19
H2R-R	CTGGTCTCGTTCCTGCTGCTGTTC	6346.1	61.78	57.14	21
18srRNA-F	CAGCCACCCGAGATTGAGCA	6096	63	60	20
18srRNA-R	TAGTAGCGACGGGCGGTGTG	6254	65	65	20

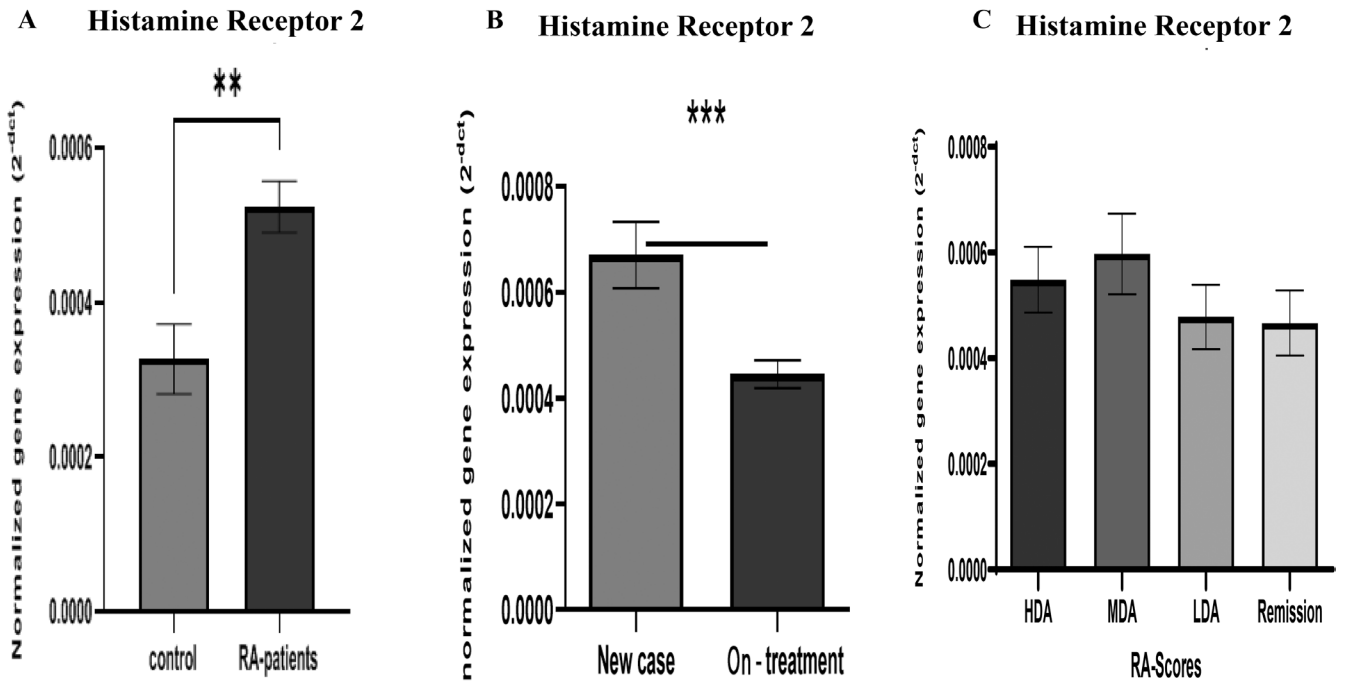

FIGURE 1 | The histamine receptor 2 gene expression in PBMCs of RA patients and controls. (A) The gene expression of H2R in RA patients is significantly higher than in the control group ($p = 0.0013$). (B) The H2R gene expression is significantly lower in RA patients who are under treatment in comparison to new case patients ($p = 0.0004$). (C) The comparison between H2R gene expression with RA scores showed no significant differences. Significance was calculated using the unpaired *t*- test and one-way ANOVA/Kruskal–Wallis test. Data are displayed as Means ± SEM. (**$p \leq 0.01$. ***$p \leq 0.001$).

56–65 age group (30%). The 26–35 and 36–45 age groups had 11.60%, while the 15–25 and 86–95 age groups each had the lowest percentage (1.60%). The sex distribution of the patient group was predominantly female (80%) and male (20%). Based on CDAI, 26% of patients had high disease activity, 21% had moderate disease activity, 23% had low disease activity, and 25% were in remission.

3.2 | H2R Gene Expression

The histamine receptor 2 (H2R) gene expression was evaluated and compared with a control group. As shown in Figure 1A–C, H2R gene expression was significantly higher in RA patients than in controls ($p = 0.0013$) (Figure 1A). New cases showed significantly higher H2R expression than on- treatment cases ($p = 0.0004$) (Figure 1B). Comparison of H2R expression between RA- Score groups showed no significant differences (Figure 1C).

3.3 | TLR7 Gene Expression

TLR7 gene expression was significantly higher in RA patients than in controls ($p = 0.0057$) (Figure 2A). New cases showed significantly higher expression than on- treatment RA patients ($p = 0.0010$) (Figure 2B). Comparison of gene expression across RA- Score groups (one-way ANOVA/Kruskal–Wallis test) showed significant differences ($p = 0.0426$) (Figure 2C). Post hoc Mann–Whitney tests revealed significantly higher expression in the high disease activity (HDA) group compared to the remission group ($p = 0.0248$) and in the moderate disease activity (MDA) group compared to the remission group ($p = 0.0286$).

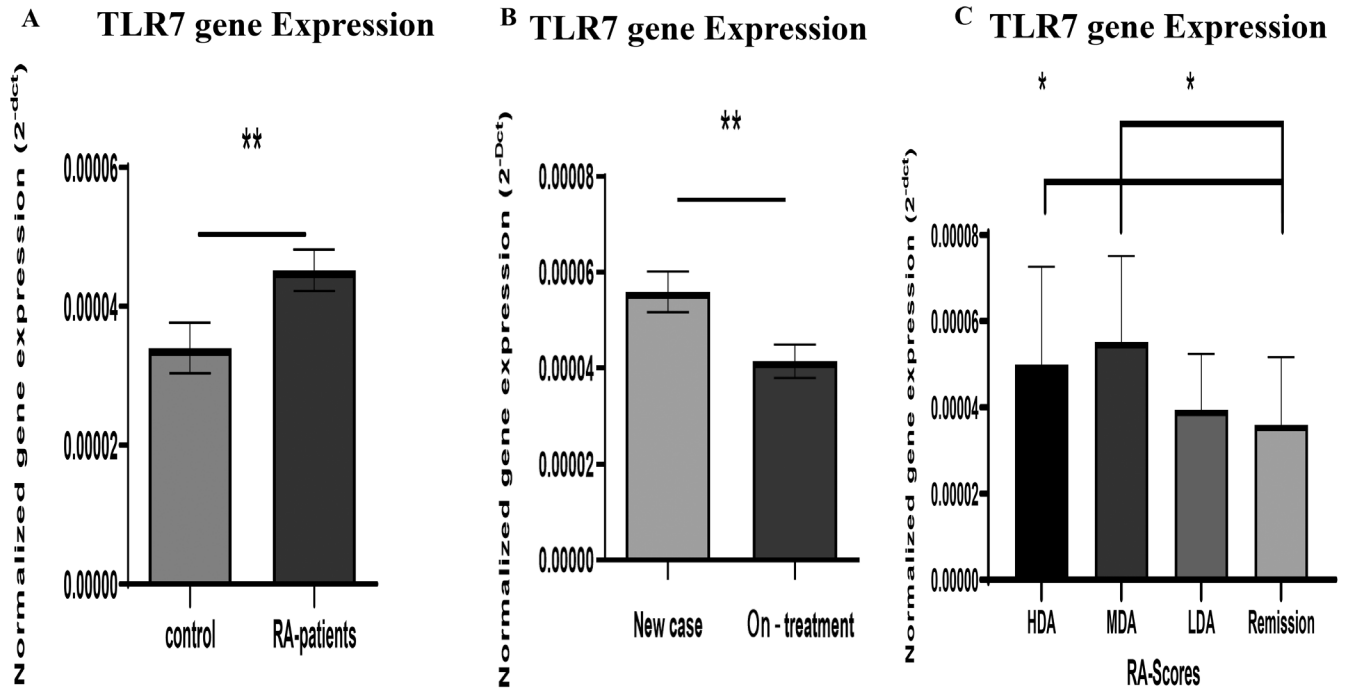

FIGURE 2 | The toll-like receptor 7 gene expression in PBMCs of RA patients and controls. (A) TLR- 7 gene expression was significantly higher in RA patients than in healthy controls ($p = 0.0057$). (B) New cases patients showed significantly higher expression than under- treatment RA patients ($p = 0.0010$). (C) Comparison of TLR- 7 gene expression with RA-Score groups (one-way ANOVA/Kruskal–Wallis test) showed significant differenes ($p = 0.0426$). Also, analysis showed significantly higher TLR- 7 expression in the high disease activity (HDA) group compared to the remission group ($p = 0.0248$) and in the moderate disease activity (MDA) group compared to the remission group ($p = 0.0286$). Significance was calculated using the unpaired *t*- test, one-way ANOVA/Kruskal–Wallis test, and post hoc Mann–Whitney test. Data are displayed as Means ± SEM. (**$p \leq 0.01$. ***$p \leq 0.001$).

3.4 | Detection of Histamine Levels by ELISA

Histamine levels were significantly higher in RA patients than in controls ($p = 0.0497$) (Figure 3A). Newly diagnosed RA patients had significantly higher histamine levels than on- treatment patients ($p = 0.0277$) (Figure 3B). Comparison of histamine levels across RA- Score groups (one-way ANOVA/Kruskal–Wallis test) showed no significant differences (Figure 3C).

3.5 | Detection of TNF-α Levels by ELISA

TNF- α levels were significantly higher in RA patients compared to controls ($p < 0.0001$) (Figure 4A). Newly diagnosed RA patients had higher TNF- α levels than on-treatment patients ($p = 0.0151$) (Figure 4B). Comparison of TNF- α levels across RA- score groups (one-way ANOVA/Kruskal–Wallis test) showed significant differences. Post hoc Mann–Whitney tests revealed significant differences between HDA and remission ($p = 0.0004$) and between HDA and LDA ($p = 0.0443$), with no significant differences between other groups (Figure 4C).

3.6 | Detection of IL-10 Levels by ELISA

IL- 10 levels were significantly higher in RA patients than in controls ($p = 0.0196$) (Figure 5A). On- treated RA patients had higher IL- 10 levels than newly diagnosed RA patients ($p = 0.0253$) (Figure 5B). Comparison of IL- 10 levels across RA-score groups showed no significant differences (Figure 5C).

3.7 | Correlation Between H2R and TLR7 Genes Expression

Correlation analysis revealed a strong positive correlation between H2R and TLR7 (Spearman's Rho $r = 0.68$, $p = 2.78$). A slight positive correlation was found between H2R gene expression and histamine levels ($r = 0.34$, $p = 0.01$).

4 | Discussion

Our findings highlight a potential relationship between histamine signaling and TLR7 activation in rheumatoid arthritis (RA), especially during the early stages of the disease. Increased expression of H2R and TLR7 in the peripheral blood mononuclear cells (PBMCs) of RA patients, particularly those newly diagnosed, coupled with higher levels of plasma histamine, TNF- α, and IL-10 suggests a potential association with disease progression. The positive association between H2R and TLR7 expression, as well as between H2R and histamine levels, may indicate a potential role of histamine signaling in immune activation.

The increased H2R gene expression in RA PBMCs, especially in newly diagnosed RA patients, is a novel finding. While Kim et al. [5] reported elevated H4R expression in RA PBMCs, the

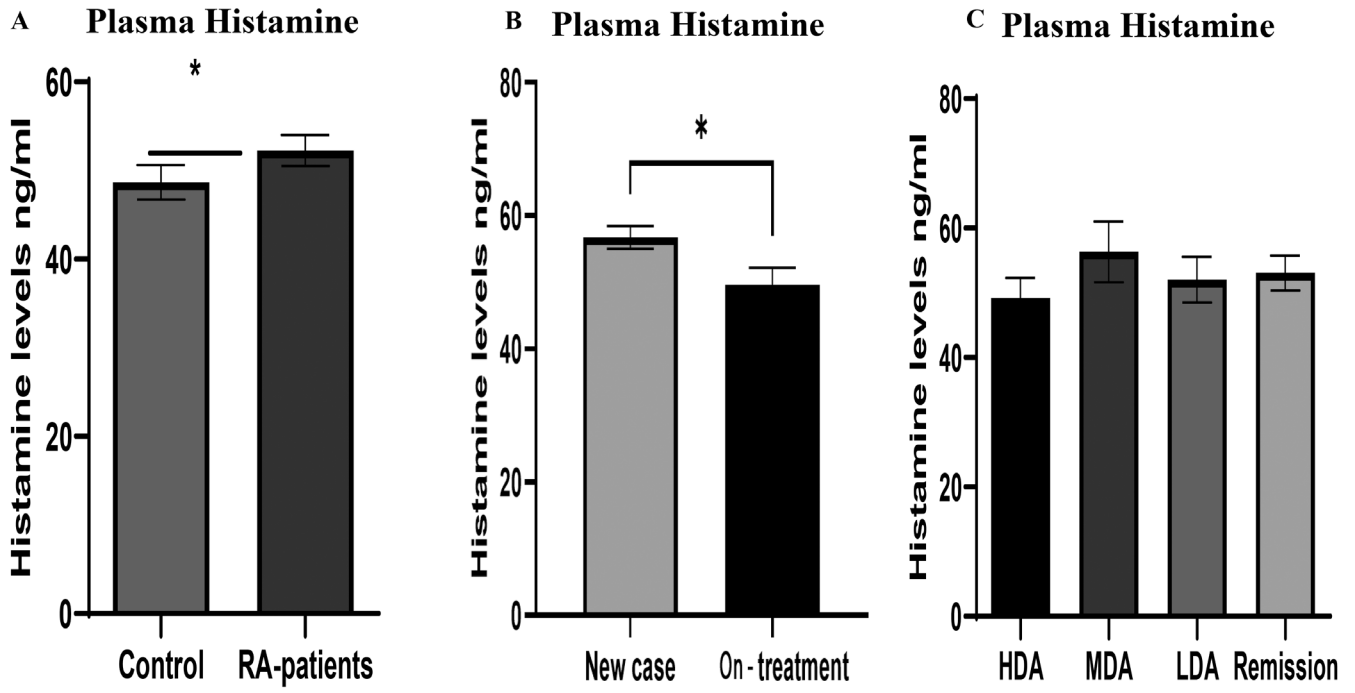

FIGURE 3 | The histamine plasma levels in RA patients. (A) The histamine plasma levels in RA patients were significantly higher than healthy controls ($p = 0.0497$). (B) The histamine levels are significantly decreased in on- treatment patients in comparison to new case patients ($p = 0.0277$). (C) Histamine levels do not show a significant relationship with each RA score. Significance was calculated using the unpaired t- test and one-way ANOVA/Kruskal–Wallis test. Data are displayed as Means ± SEM. (* $p \leq 0.05$).

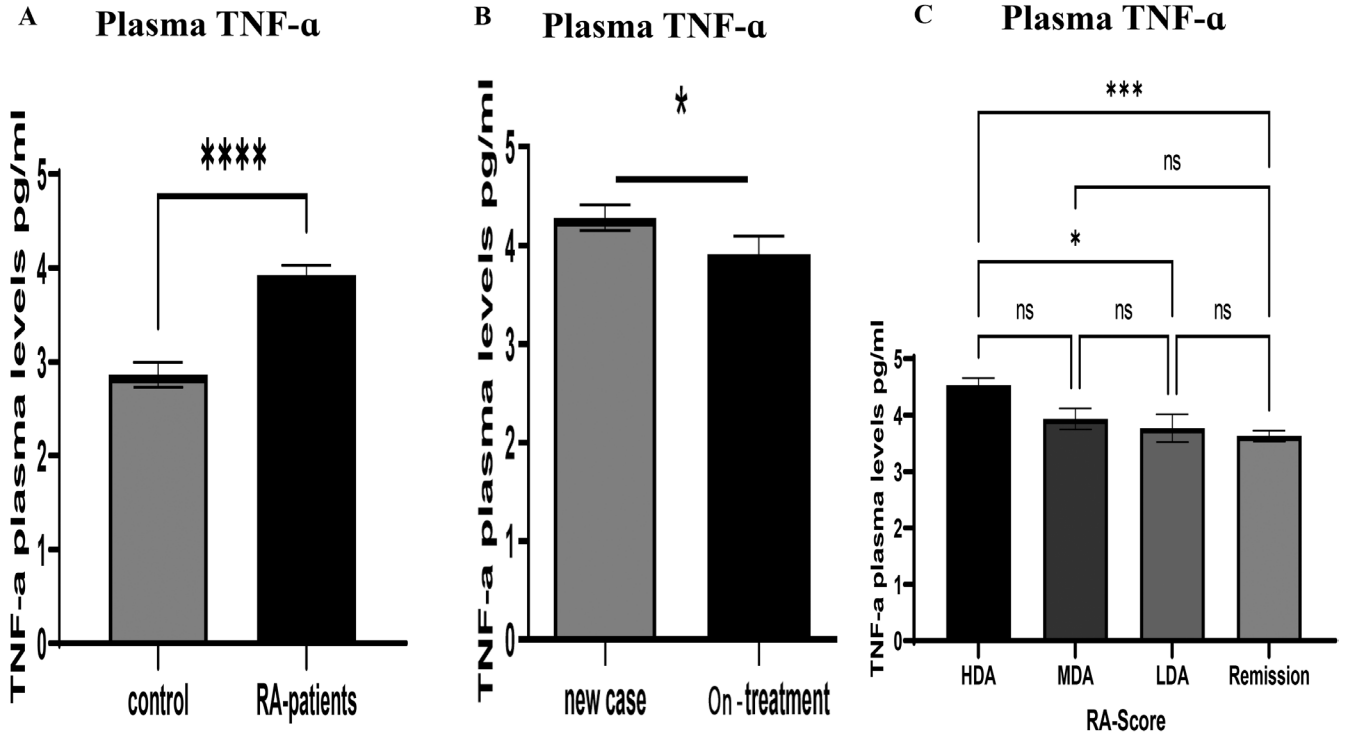

FIGURE 4 | The TNF- α plasma levels in RA patients. (A) The plasma levels of TNF-α are significantly increased in RA patients in comparison to healthy controls ($p < 0.0001$). (B) Within RA patients, plasma levels of TNF- α are significantly increased in on-treatment patients in comparison to new diagnostic patients ($p = 0.0151$). (C) TNF- α levels do not show a significant relationship with each RA score. Significance was calculated using the unpaired t- test and one-way ANOVA/Kruskal–Wallis test. Data are displayed as mean± SEM. (*$p \leq 0.05$. ****$p \leq 0.0001$, $p \leq 0.001$, ns: non-significant).

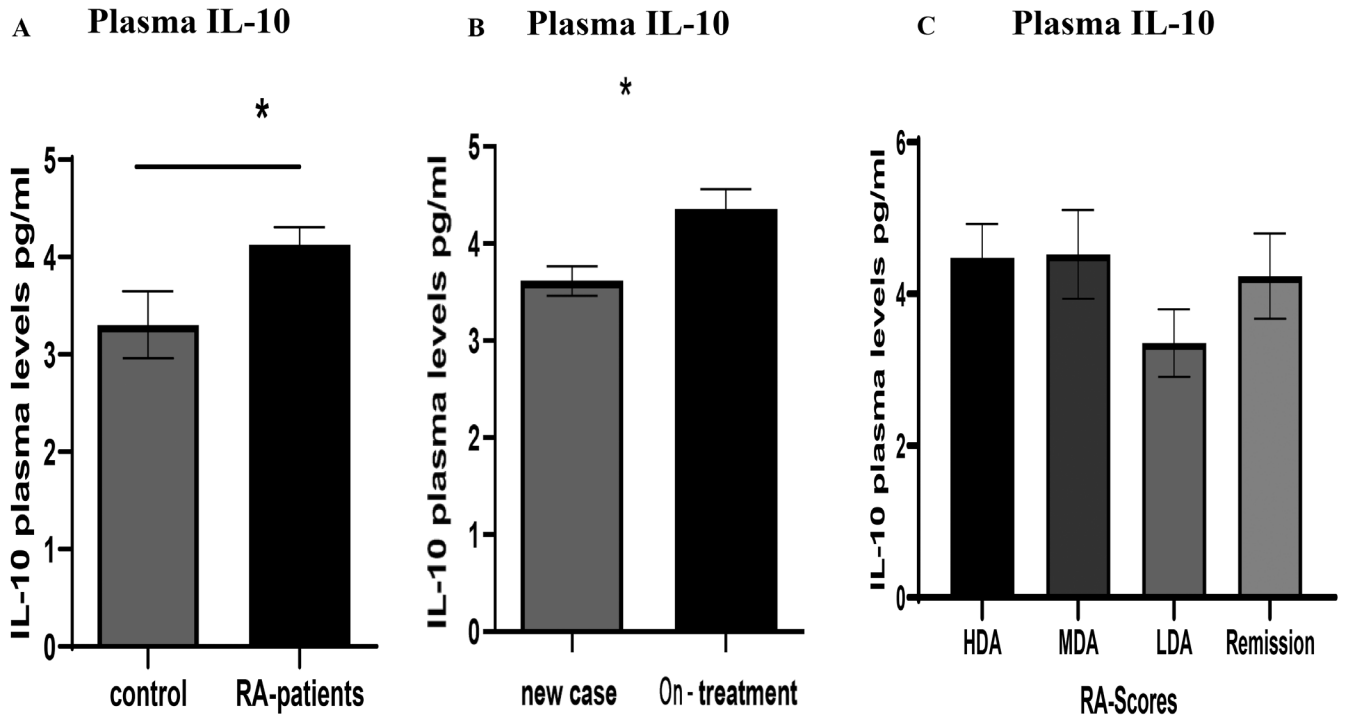

FIGURE 5 | The IL- 10 plasma levels in RA patients. (A) IL-10 levels in RA patients are significantly increased in comparison with healthy controls. (B) Within RA patients, IL- 10 plasma levels are significantly increased in under-treatment patients in comparison to new case patients. (C) Histamine levels do not show a significant relationship with each RA score. Significance was calculated using the unpaired *t*-test and one-way ANOVA/Kruskal–Wallis test. Data are displayed as mean ± SEM. (*$p \leq 0.05$).

role of H2R in RA remains less explored. Our data suggest that H2R expression may be modulated by treatment with Disease-modifying antirheumatic drugs (DMARDs), as we observed lower expression in patients under treatment compared to newly diagnosed individuals. By targeting various inflammatory pathways [17], DMARDs could indirectly influence H2R expression. Alternatively, they might directly interact with H2R regulatory pathways [5]. Further research is crucial to dissect the precise mechanisms by which DMARDs affect H2R expression. It is also important to acknowledge that PBMCs comprise various immune cell subsets (T cells, B cells, and monocytes), each potentially exhibiting different H2R expression patterns. Future studies should investigate the specific effects of altered H2R expression on these subsets and how these effects are modulated by treatment [5, 18]. Interestingly, our findings differ from earlier reports, such as the study by Tanaka et al. [19], which described downregulation of H2R in certain cell types, including synovial fibroblasts and lymphocytes. This discrepancy may be attributed to differences in cell populations analyzed, as our study focused on total PBMCs rather than isolated cell subsets. Additionally, advances in detection methods, as well as differences in disease stage and treatment status, may contribute to these contrasting observations, suggesting that H2R expression in RA is dynamic and context- dependent. These findings suggest that the role of H2R in RA may warrant further investigation, particularly given its relatively limited exploration compared to other histamine receptor subtypes in recent years.

Our observation of elevated TLR7 gene expression in RA PBMCs aligns with previous work demonstrating increased TLR3 and TLR7 expression in RA synovium [20]. TLR7, crucial for innate immune responses, recognizes ssRNA from viruses and self- RNA released from damaged cells [21]. The heightened TLR7 expression in RA PBMCs, particularly in newly diagnosed RA patients, could contribute to chronic inflammation by promoting pro- inflammatory cytokine production and immune cell activation. This finding is consistent with the notion that TLR signaling pathways are activated early in RA progression [22]. The observed decrease in TLR7 expression in treated patients may reflect the immunomodulatory effects of DMARDs and other therapies. TLR7 expression can be influenced by various factors, including IFN- α [23], epigenetic regulation [24] age, disease activity, and glucocorticoid treatment [25]. Our finding of significant differences in TLR7 expression across RA disease activity scores (HDA vs. Remission, MDA vs. Remission) further supports the link between TLR7 and disease status [26].

The elevated plasma histamine levels in RA patients corroborate some studies [5] but contradict other previous findings [27]. The discrepancy may be due to differences in patient populations, disease activity, or assay methodologies. Our data did not reveal significant differences in histamine levels across RA disease activity scores, consistent with some prior reports [28]. The increased TNF- α and IL-10 levels in RA patients are well-established [29]. The higher TNF- α in newly diagnosed RA patients and the higher IL- 10 in treated patients are interesting observations that warrant further investigation. The inverse relationship of TNF- α and IL-10 with treatment status could be related to the natural progression of the disease or could be influenced by the treatment itself. IL- 10 is generally considered an anti- inflammatory cytokine, and its increase with treatment could be a mechanism to control the inflammatory response [30]. Importantly, the assessment of TNF- α and IL-10 in this study was not intended to establish novel cytokine findings,

but rather to provide functional context for the observed alterations in H2R and TLR7 expression. These cytokines were included as representative markers of pro- and anti- inflammatory responses, allowing for a more integrated interpretation of the immunological environment associated with this receptor axis in RA.

The observed strong positive correlation between H2R and TLR7 gene expression in RA PBMCs represents a novel finding. However, this association should be interpreted with caution, as it does not imply a direct causal or functional interaction between these pathways. Rather, the concurrent upregulation of H2R and TLR7 may reflect a shared upstream regulatory mechanism or a common inflammatory milieu characteristic of RA. Although prior evidence suggests that histamine signaling can modulate immune responses and cytokine production, the potential interplay between H2R signaling and TLR7 activation has not been directly investigated in this study. Therefore, our findings primarily support the existence of an association rather than a mechanistic link [5]. Given the cross- sectional case–control design of this study, causal inference cannot be established. To better define the nature of this relationship, future studies employing functional approaches—such as receptor- specific inhibition, gene silencing, and pathway- level analyses in both in vitro and in vivo models—are required. These investigations would help clarify whether a direct regulatory interaction exists or whether both pathways are independently driven by the inflammatory environment in RA. The relatively small sample size, particularly in the healthy control group, represents another limitation of this study and may affect the generalizability of the findings. Therefore, the results should be interpreted with caution. Nevertheless, the statistically significant differences and correlations observed suggest that these findings may reflect underlying biological patterns. Future studies with larger and more balanced cohorts are required to confirm and extend these results.

5 | Conclusions

Our findings suggest a potential association between histamine signaling through H2R and TLR7 activation in RA. While this relationship may reflect a coordinated involvement of these pathways in the inflammatory processes underlying RA, no causal interaction can be inferred from the present study. The observed association highlights the possibility that H2R and TLR7 signaling may contribute to the complex immunological network involved in disease progression. Although previous evidence has suggested that modulation of these pathways may have therapeutic relevance, the current findings should be interpreted as hypothesis- generating rather than indicative of direct clinical application. Further mechanistic and longitudinal studies are required to elucidate the precise nature of this relationship and to determine whether targeting these pathways may offer potential therapeutic benefits in RA.

Author Contributions

H.D. contributed to conceptualization, methodology, and supervision of the study. A.K.A.- B. conducted the investigation, performed formal data analysis, and drafted the original manuscript. H.A. contributed to writing – review and editing of the manuscript. Y.B. and M.S.J. contributed to methodology and assisted with data analysis. S.S. contributed to the clinical aspects of the study. E.H. contributed to data acquisition and experimental procedures. All authors reviewed and approved the final version of the manuscript.

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The authors have nothing to report.

Ethics Statement

This study was funded by Golestan University of Medical Sciences (GOUMS) with Ethics No: (IR.GOUMS.REC.1401.396). This original research was extracted from an MSc thesis in medical immunology.

Consent

All authors have provided their consent for the publication of this study.

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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LETTER TO THE EDITOR

Case Report: Leflunomide-Induced Cutaneous Eruption in a Patient With Probable Chemotherapy-Associated Inflammatory Polyarthritis

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Dear Editor,

Rheumatologic manifestations emerging after cancer therapy are increasingly recognized in on Dcology practice and may mimic seronegative inflammatory arthritis [1–3]. Although postchemotherapy arthralgia has been previously described, inflammatory polyarthritis requiring disease-modifying antirheumatic drug (DMARD) therapy remains relatively uncommon, and severe cutaneous adverse reactions during treatment in this setting are rarely reported [1, 4]. We present a patient with probable chemotherapy-associated inflammatory polyarthritis who subsequently developed a generalized cutaneous drug eruption shortly after initiation of leflunomide therapy.

A 51-year-old woman presented with pain and swelling involving the small joints of the hands and feet approximately six weeks after completing adjuvant chemotherapy for hormone receptor–positive breast cancer. Her treatment regimen included cyclophosphamide- and anthracycline-based chemotherapy followed by tamoxifen therapy. She had no previous history of inflammatory rheumatic disease, psoriasis, or known drug allergy.

Physical examination demonstrated tenderness and swelling in the metacarpophalangeal and metatarsophalangeal joints without nodules, mucosal lesions, or other systemic manifestations. Laboratory investigations revealed normal inflammatory markers and negative rheumatoid factor, anti-cyclic citrullinated peptide (anti-CCP) antibody, and antinuclear antibody tests. During the cutaneous eruption, eosinophil count remained within normal limits ($0.03 \times 10^3/\mu L$), and liver function tests were unremarkable (ALT 19 U/L, AST 23 U/L, ALP 57 U/L, GGT 21 U/L, total bilirubin 0.32 mg/dL). Magnetic resonance imaging was performed on the symptomatic right hand and demonstrated joint effusions involving the second, third, and fourth metacarpophalangeal joints. Considering the temporal relationship with chemotherapy, imaging findings, seronegativity, and absence of prior rheumatologic disease, probable chemotherapy-associated inflammatory polyarthritis was considered [1, 2].

Treatment with oral prednisolone (15 mg/day) and leflunomide (10 mg/day) was initiated. Joint symptoms improved rapidly; however, on the fifth day of leflunomide therapy, the patient developed a widespread erythematous eruption involving the trunk and upper extremities (Figure 1). Leflunomide was immediately discontinued, and the patient was referred to the dermatology department for further evaluation. During hospitalization in the dermatology department, a skin biopsy was performed and demonstrated superficial perivascular dermatitis with focal lymphocytic exocytosis, spongiosis, focal vacuolar degeneration, scattered necrotic keratinocytes, and perivascular lymphocytic and eosinophilic infiltrates, findings supportive of a drug-induced cutaneous eruption (Figure 2). There was no mucosal involvement or clinical evidence suggestive of Stevens–Johnson syndrome, toxic epidermal necrolysis, or drug reaction with eosinophilia and systemic symptoms. Oral prednisolone was increased from 15 mg/day to 60 mg/day and cetirizine 10 mg twice daily was initiated. Because the patient demonstrated rapid clinical improvement after discontinuation of leflunomide and corticosteroid initiation, cholestyramine washout therapy was not considered necessary. Marked improvement occurred within 5 days, with complete resolution during follow-up after drug discontinuation and corticosteroid treatment (Figure 1).

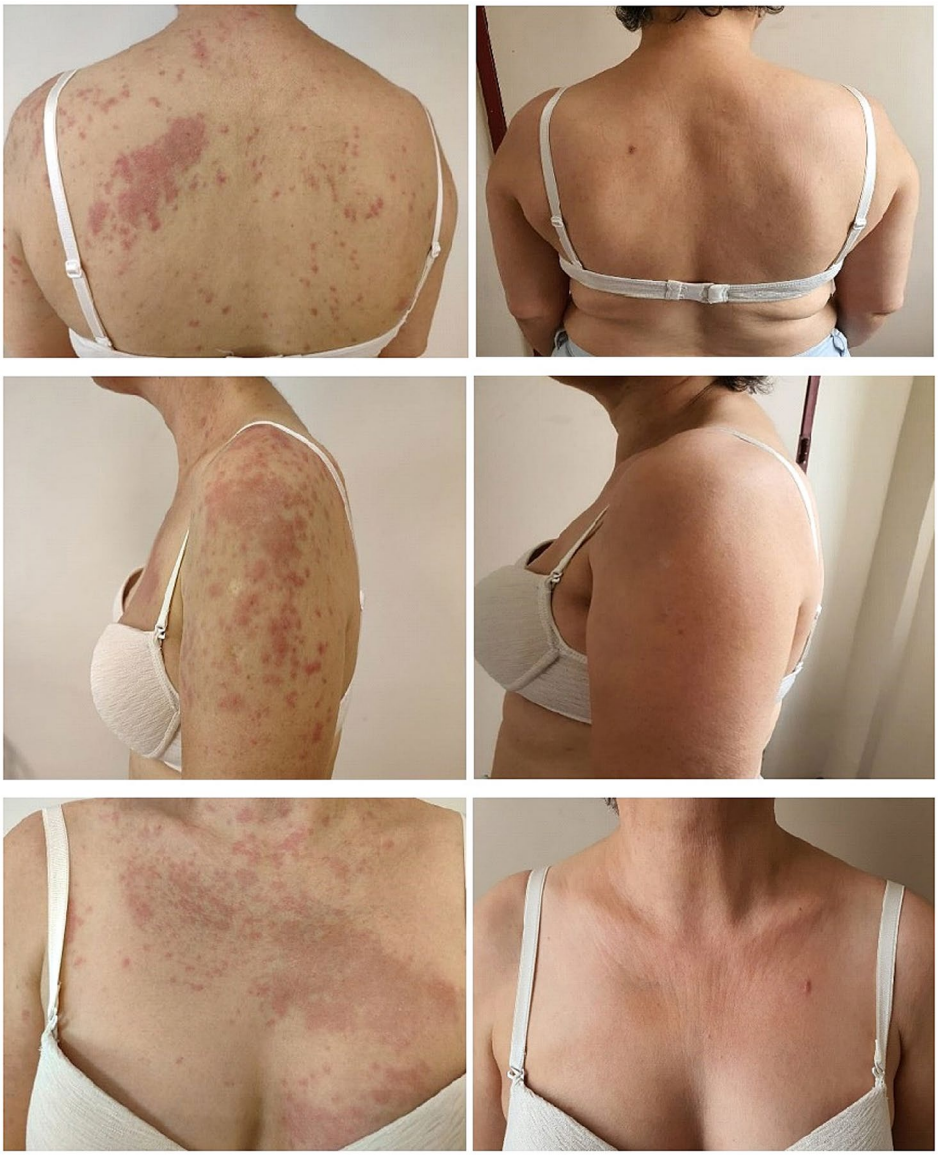

FIGURE 1 | Generalized erythematous drug eruption involving the trunk and upper extremities following initiation of leflunomide therapy (left panel), with complete clinical resolution after drug discontinuation and systemic corticosteroid treatment (right panel).

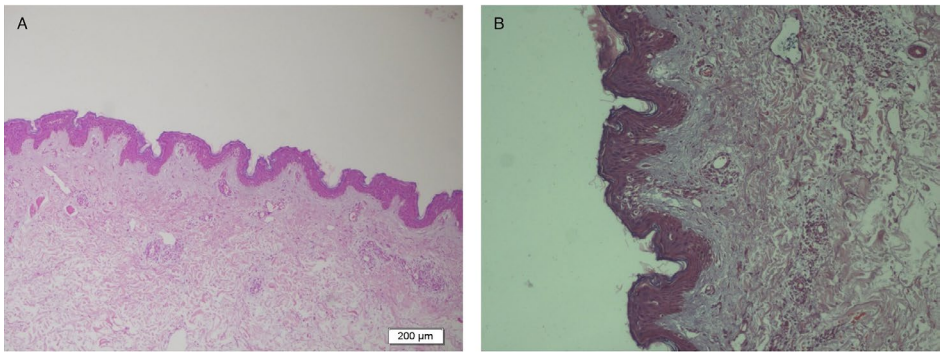

FIGURE 2 | Histopathological findings of the skin biopsy. (A) Low- power photomicrograph demonstrating superficial perivascular dermatitis with focal spongiosis and focal vacuolar degeneration. (B) Higher- power photomicrograph demonstrating superficial perivascular lymphocytic and eosinophilic infiltrates with scattered necrotic keratinocytes, findings supportive of a drug-induced cutaneous eruption.

After discharge, the patient was followed in the rheumatology clinic for several months with low- dose oral prednisolone and hydroxychloroquine, without recurrence of the rash. Hydroxychloroquine was subsequently discontinued, and she remained under clinical follow-up for possible recurrence of inflammatory joint symptoms.

Chemotherapy- associated inflammatory arthritis is an uncommon but increasingly recognized entity in cancer survivors [1–3]. Previous reports have described inflammatory arthritis after cytotoxic therapy in patients with breast and lung cancer, frequently with negative serologic markers and variable inflammatory findings [1, 4]. Proposed mechanisms include immune dysregulation, altered immune tolerance, and autoantigen exposure following chemotherapy- induced apoptosis [2, 3]. Nevertheless, diagnostic uncertainty remains common because alternative explanations such as tamoxifen- associated musculoskeletal symptoms, paraneoplastic arthritis, or coincidental seronegative inflammatory arthritis may coexist [5]. In the present case, the close temporal association with chemotherapy and absence of prior rheumatologic history supported a treatment-related inflammatory process, although alternative etiologies could not be completely excluded.

Leflunomide is generally well tolerated; however, reported cutaneous adverse reactions associated with leflunomide range from mild maculopapular eruptions and urticaria to more severe manifestations, including drug reaction with eosinophilia and systemic symptoms (DRESS) [6–8], Stevens–Johnson syndrome [9], toxic epidermal necrolysis [10–12], and exfoliative dermatitis [13].

The rapid onset of rash after treatment initiation, histopathologic findings, and complete recovery following drug discontinuation strongly supported leflunomide- induced cutaneous toxicity in our patient. This case highlights the importance of careful monitoring for DMARD- related adverse effects in patients developing inflammatory musculoskeletal manifestations after cancer therapy.

In conclusion, inflammatory arthritis may emerge after cytotoxic chemotherapy even in the absence of classical serologic findings. Clinicians should remain aware of potentially severe cutaneous reactions associated with DMARD therapy in this setting and adopt a multidisciplinary approach involving rheumatology, oncology, and dermatology specialists.

Author Contributions

Conceptualization: Kübra Neslihan Kurt Oktay. Investigation: Kübra Neslihan Kurt Oktay, Samet Aygun, Elem Yorulmaz, Duygu Geler Kulcu. Data curation: Kübra Neslihan Kurt Oktay, Samet Aygun, Elem Yorulmaz. Visualization: Kübra Neslihan Kurt Oktay, Samet Aygun. Writing – original draft: Kübra Neslihan Kurt Oktay. Writing – review and editing: All authors. Supervision: Duygu Geler Kulcu. Project administration: Kübra Neslihan Kurt Oktay. All authors read and approved the final version of the manuscript.

Consent

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

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

From Hands to Imaging: The Emerging Role of Musculoskeletal Ultrasonography in Juvenile Idiopathic Arthritis Disease Activity Assessment

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Dear Editor,

Disease activity assessment tools are widely and successfully used in clinical practice to evaluate disease activity and guide treatment accordingly. Juvenile idiopathic arthritis is no exception, and disease activity scores specific to JIA have been developed and validated in recent years [1–3]. Although minor differences exist between these scoring systems in terms of their components, all include clinical joint examination [1–3]. While the burden of JIA extends beyond articular involvement, arthritis remains the central feature of the disease. However, the assessment of arthritis may be more complex than initially appreciated.

Physical examination (PE) remains the cornerstone of joint assessment but has important limitations compared with advanced imaging modalities, such as contrast-enhanced magnetic resonance imaging (MRI). Hemke et al. have shown that PE of the knee joint, which is the most commonly involved and reliably accessed joint, failed to identify MRI-confirmed synovitis in 35.9% of patients [4]. Likewise, whole-body MRI (WB-MRI) has further highlighted the limitations of PE by revealing up to 49% subclinical joint inflammation in JIA patients [5]. However, despite its superior sensitivity, MRI remains impractical for routine disease assessment because of its high cost, limited availability, prolonged acquisition time, and the frequent need for sedation in young children. Accordingly, there remains a need for an imaging modality that combines the sensitivity of advanced imaging with the feasibility required for routine clinical assessment, a role that musculoskeletal ultrasonography (MSUS) is increasingly well positioned to fulfill.

The use of MSUS in the diagnosis and monitoring of JIA has grown considerably [6, 7]. The standardization of normal anatomical landmarks and pathological thresholds for pediatric ultrasonography by Outcome Measurement in Rheumatology (OMERACT) and Childhood Arthritis and Rheumatology Research Alliance (CARRA) has enabled more advanced research comparing MSUS with physical examination and MRI [8–10]. Although efforts toward standardization and validation are still ongoing, MSUS has shown superior sensitivity compared to PE; Magni-Manzoni et al. demonstrated that MSUS captured 5.5% subclinical synovitis in JIA patients [11]. Furthermore, this finding has been confirmed by various studies, with subclinical synovitis rates varying by joint and being particularly higher at joints other than the knee, consistent with adult rheumatoid arthritis (RA) studies [12–14]. Although not as sensitive as MRI, Sande et al. compared MSUS with WB-MRI and found a strong correlation between synovitis scores (Spearman's correlation coefficients = 0.74), compatible with the study by Vega-Fernandez et al., in which a strong correlation was demonstrated between MSUS and contrast enhanced MRI scores [15, 16].

Consequently, investigators further explored the clinical implications of MSUS-detected subclinical synovitis. Although initial reports by Magni-Manzoni et al. and Zhao et al. were unsupportive, De Lucia et al. have shown that MSUS-detected abnormalities in clinically inactive patients increased the risk of flare-up by approximately 4 times, compatible with Fingerhutová et al.'s and adult RA studies [14, 17–20]. Additionally, Miotto E Silva et al. have shown that Power Doppler positivity predicted joint damage along with risk of flare in clinically inactive patients

[21]. Although MSUS- detected subclinical synovitis may predict relapse, not all patients who exhibited subclinical synovitis relapsed, and it can be detected in up to 11.1% of healthy controls [22]. Notably, there is considerable heterogeneity across these studies regarding patient selection, treatment, follow-up duration, and MSUS operators and technique. Therefore, further evidence and validation are needed before evidence- based recommendations can be established for patients with MSUS-detected subclinical synovitis.

Another advantage of MSUS over PE is its ability to grade arthritis. OMERACT has developed scoring systems for B- mode and Color and Power Doppler imaging allowing clinicians to evaluate and grade effusion and inflammatory activity through visualization of synovial hypertrophy and vascularity [10]. The ability to grade inflammatory activity enhances disease activity assessment to capture changes in inflammatory activity more sensitively. In addition, MSUS is particularly valuable in detecting extra- articular involvement, such as enthesitis, an important inflammatory manifestation in enthesitis- related arthritis (ERA) and psoriatic arthritis [23]. Weiss et al. have demonstrated that physical examination and examination with a standardized dolorimeter have poor accuracy and reliability compared to MSUS in ERA patients [24]. However, with respect to structural damage, such as bone erosions, MSUS is only as sensitive as X- ray, which is lower than MRI [25].

The Juvenile Arthritis Disease Activity Score (JADAS) and Wallace criteria primarily focus on active joint count, whereas the American College of Rheumatology (ACR) Pediatric criteria additionally incorporate the number of joints with limited range of motion (ROM), highlighting chronic structural damage [1–3]. Yet chronic articular sequelae in JIA may present with a spectrum of findings, including chronic anatomical changes and persistent tenderness along with restricted ROM, making it difficult to distinguish acute inflammatory findings from chronic sequelae [26]. This becomes particularly evident in patients with longstanding disease, residual joint sequelae, and recurrent flares, which is a common scenario in JIA. As a result, discrepancies arise between clinicians when disease activity scoring relies solely on physical examination, with Guzman et al. reporting only moderate interobserver agreement ($\kappa = 0.47$) even for joint swelling [27]. This reflects a fundamental shortcoming of clinical assessment: limited interobserver agreement and the difficulty of distinguishing chronic changes from active inflammation, an area where musculoskeletal ultrasonography (MSUS) can be remarkably advantageous, especially with Power Doppler imaging [6]. Supporting this concept, Fingerhutová et al. demonstrated that MSUS can resolve uncertainty in clinically equivocal joints by identifying active synovitis and that joints with MSUS- confirmed synovitis carry a significantly higher risk of subsequent relapse than MSUS- inactive joints [20].

These advantages prompted investigators to evaluate MSUS's correlation with standardized composite disease activity measures [16]. Across two studies, Vega- Fernandez et al. demonstrated a moderate correlation of MSUS- detected synovitis with cJADAS10 ($rs = 0.45$) and patient global assessment ($rs = 0.44$, $p = 0.014$), whereas the correlation with active joint count was weaker ($rs = 0.37$, $p = 0.048$) [13, 28]. These findings were further supported by Snipaitiene et al. even after disease remission has been achieved [29]. Consistent with this, Licciardi et al. reported that the burden of subclinical synovitis per patient correlated with JADAS- 27 ($p = 0.03$) [30].

TABLE 1 | Comparison of imaging modalities used for the diagnosis and monitoring of juvenile idiopathic arthritis.

Feature	Conventional radiography (X-ray)	MSUS	MRI
Synovitis	−	++/+++[a]	+++
Joint effusion	−	++/+++[a]	+++
Active vascular inflammation	−	++	+++
Bone marrow edema	−	−	+++
Cartilage	+	++	+++
Bone erosions	++	++	+++
Dynamic assessment	−	+++	−
Examination time	Short	Short	Long
Radiation	Yes	No	No
Cost	Low	Low—Moderate	High
Accessibility	+++	++	+
Bedside availability	++	+++	−
Repeatability	++	+++	++
Need for sedation	−	−	Sometimes
Guidance for joint injection	−	+++	−

Note: The ratings represent the overall utility of each modality in JIA, taking into account diagnostic performance, anatomical coverage, technical limitations, and feasibility, rather than accuracy alone. Scoring: −, not applicable or not useful; +, limited; ++, moderate; +++, excellent.
[a]Performance may be limited in deep or poorly accessible joints.

Taken together, these findings suggest that MSUS reflects a different, and possibly more objective, domain of disease activity than traditional PE- based measures. This may be particularly relevant in patients with clinically inactive disease or clinically equivocal arthritis. However, it should be noted that ultrasound- guided treatment strategies did not yield clear superiority in the ARCTIC and TaSER trials in adult rheumatoid arthritis [31, 32]. While direct extrapolation of these studies to JIA is not feasible, further evidence is required before adopting purely MSUS- guided disease activity scoring and treatment strategies in JIA.

The search for optimal methods to diagnose and monitor joint inflammation has been ongoing since the earliest use of X- ray. Magnetic resonance imaging can address many unmet needs, and promising advances are being made in near- infrared optical imaging, photoacoustic techniques, and thermal imaging; however, MRI has not displaced traditional physical examination due to limited accessibility and cost. Likewise, MSUS will not end this quest, as challenges regarding operator dependency, technique heterogeneity, and validation persist. Nevertheless, MSUS offers higher sensitivity than PE, the ability to grade arthritis, and direct visualization of active inflammation and extra- articular structures, such as entheseal structures, along with substantial technical advantages, including wide availability, ease of use, absence of ionizing radiation, and a non-invasive, sedation- free nature (Table1). Therefore, moving toward MSUS- aided clinical assessment represents a logical and timely step in refining disease activity scoring in JIA and informing future research directions.

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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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REVIEW

Dual Roles of Free Fatty Acids in Gout Pathogenesis: Inflammatory Drivers and Metabolic Mediators

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ABSTRACT

Gout, a prototypical crystal-induced arthropathy, is characterized by a complex interaction between metabolic dysregulation and inflammatory activation. Although monosodium urate (MSU) crystal deposition remains the central pathognomonic feature, accumulating evidence suggests that free fatty acids (FFAs) play a significant role as co-modulators in the disease's pathogenesis. This review systematically examines the dual role of FFAs in gout pathophysiology: (1) their pro-inflammatory effects during gout flares through direct modulation of innate immune responses, and (2) their role as metabolic modulators contributing to disease comorbidities. We explore the molecular mechanisms by which saturated and n-6 polyunsaturated fatty acids (PUFAs) promote NLRP3 inflammasome activation, ultimately leading to cytokine storm formation and acute inflammatory responses. Paradoxically, short-chain fatty acids and n-3 polyunsaturated fatty acids exhibit anti-inflammatory properties and confer protection against gout flares via distinct immunomodulatory pathways. The review further examines FFA-mediated metabolic disturbances associated with gout, such as insulin resistance, renal fibrosis, and non-alcoholic fatty liver disease. Additionally, this review provides novel insights into targeted therapeutic strategies for the adjuvant treatment of gout, including lipid-targeted therapy, microbiome modulation, and dietary recommendations regarding polyunsaturated fatty acids.

1 | Introduction

Gout is a metabolic disorder resulting from impaired purine metabolism or diminished uric acid excretion, and it is also a prevalent inflammatory arthritis characterized by sudden flares of excruciating joint pain and swelling. It has evolved from a "disease of kings" to a global health concern, with an incidence rate ranging from 0.6 to 2.9 per 1 000 person-years worldwide, and the prevalence of gout ranges from 0.68% to 3.9% among the population [1, 2]. This metabolic-arthritis continuum progresses through distinct phases: Hyperuricemia (HUA), gout flares, intercritical gout, and chronic tophaceous disease [3]. While MSU crystal deposition is necessary for clinical manifestation, its occurrence in only 14% of asymptomatic hyperuricemics suggests a requirement for secondary triggers [4].

The incidence and prevalence of gout are increasing globally, closely linked to the rising epidemic of obesity and metabolic syndrome [5]. Obesity, particularly visceral adiposity (waist circumference > 102 cm), confers a 2.24-fold increased gout risk (95% CI 1.76–2.86). This association is mediated through hyperleptinemia, insulin resistance, and altered adipokine profiles. Mendelian randomization studies confirm causal links between glycemic traits ($\beta = 0.24$, $p = 3.2 \times 10^{-5}$) and urate levels, highlighting shared genetic architecture [6]. Zhang L et al. performed a genome-wide cross-over trait analysis, which clearly demonstrated a shared genetic basis and pleiotropic loci between obesity and gout. This genetic evidence underscores the significant role of obesity in the onset and progression of gout [7]. In a 2018 meta-analysis, obesity (BMI $\geq 30\,kg/m^2$) was associated with a more than two-fold increased risk of incident gout (adjusted relative

Highlights

1. Free fatty acids (FFAs) exhibit dual inflammatory effects in gout, as saturated and n- 6 polyunsaturated fatty acids promote NLRP3 inflammasome activation and cytokine storms while SCFAs and n- 3 polyunsaturated fatty acids exhibit anti- inflammatory properties and confer protection against gout flares via distinct immunomodulatory pathways.
2. FFAs also play a mediating role in metabolic disturbances associated with gout, including insulin resistance, renal fibrosis, and non- alcoholic fatty liver disease.
3. Plant- based polyunsaturated fatty acids show stronger gout protection than marine sources. Targeted lipid and microbiome modulation offer promising adjuvant management strategies for gout.

risk 2.24, 95% CI 1.76–2.86) compared with individuals with a BMI < 30 kg/m^2 [8].

In prolonged overnutrition, men with obesity have higher levels of circulating free fatty acids (FFAs). As the physiologically significant energy substrates released from adipose tissue through lipolysis in response to the body's energy demands, FFAs are now increasingly recognized as potent immunomodulators; excess FFAs are stored in different organs as ectopic fat and produce excess reactive oxygen species (ROS) and pro- inflammation [9]. A study evaluated the relationship between hyperuricemia and FFA. During gout flares, plasma FFA concentrations were significantly higher than those during intercritical gout (median: 0.60, IQR: 0.26 vs. median: 0.39, IQR: 0.12, $p < 0.001$), hyperuricemia (median: 0.45, IQR: 0.18, $p < 0.001$), and normal controls (median: 0.46, IQR: 0.20, $p < 0.001$) [10]. In a cross- sectional study, elevated levels of FFAs have also been identified as an independent risk factor for tophus formation, with a serum FFA level of 0.46 mmol/L found to be predictive for the onset of tophus [11]. It is suggested that obesity- induced elevation in FFAs is likely to play a significant role in the development of gout. This review synthesizes cutting-edge evidence on FFA- mediated mechanisms in gout, offering new perspectives on therapeutic targeting.

2 | FFA Classification and Metabolic Pathways

Fatty acids (FAs) are carboxylic acids with long saturated or unsaturated aliphatic chains and commonly present in organisms as three major forms: Triglycerides, phospholipids, and cholesterol esters. When FAs are not present in the plasma as esters, they are referred to as non- esterified FAs or FFAs. There are many kinds of FFAs in the blood, including saturated FAs (SFAs), monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), and others. These FFAs in blood can also be classified based on the carbon chain length of their aliphatic tail: Short-chain fatty acids (SCFAs) contain fewer than six carbon atoms, medium- chain fatty acids (MCFAs) comprise six to twelve carbon atoms, and long- chain fatty acids (LCFAs) consist of twelve or more carbon atoms (Table 1) [12, 13].

SCFAs (including acetate, propionate) are produced by gut microbial fermentation of indigestible dietary fiber and are also found in fermented foods. MCFAs (such as caprylate, laurate) primarily originate from dietary triglycerides. They are directly absorbed into the bloodstream through intestinal capillaries and transported through the portal vein alongside other absorbed nutrients. LCFAs, including both saturated LCFAs (e.g.,

TABLE 1 | Structural and functional diversity of FFAs.

Class	Chain length	Major species	Primary Sources	Pro-inflammatory potential	FFA profiles in Gout
SCFAs	C1–C6	Acetate, propionate	Gut microbiota fermentation	Anti-inflammatory	Reduced
MCFAs	C6–C12	Caprylate, laurate	Dietary triglycerides	Moderate	Unknown
Saturated LCFAs	≥C12	Palmitate (C16:0), stearate	Adipose lipolysis, de novo synthesis	High (NLRP3 activation)	Elevated
Monounsaturated LCFAs	≥C12	Oleate (C18:1), linoleate	Dietary absorption	Low/ Context-dependent	Elevated
Polyunsaturated LCFAs	≥C12	Omega-3 fatty acids (such as eicosapentaenoic acid, docosahexaenoic acid)	Dietary absorption	Anti-inflammatory	Reduced
		Omega-6 fatty acids (such as arachidonic acid)	Dietary absorption	High	Elevated

Abbreviations: LCFAs, long- chain fatty acids; MCFAs, medium-chain fatty acids; NLRP3, NOD-, LRR-and pyrin domain- containing protein 3; SCFAs, short-chain fatty acids.

palmitate (C16:0), stearate) derived from adipose lipolysis and de novo synthesis, as well as unsaturated LCFAs (e.g., oleate (C18:1), linoleate) absorbed from the diet, are taken up by the perivillus adipose tissue and subsequently reassembled into triglycerides [12]. The circulating pool of FFAs consists of up to 40 distinct molecular species, among which 9 LCFAs constitute over 96% of the total FFAs by molarity [14].

In adipose tissues, FFAs are re- esterified to form triglycerides (TAGs), which are stored in adipose droplets of adipocytes and subsequently mobilized through lipolysis, i.e., the hydrolysis of TAGs. Albumin in circulation acts as a binding agent for FFAs, which can be taken up by mitochondria- containing cells from circulating plasma and undergo β- oxidative metabolism in mitochondria, resulting in the production of carbon dioxide and water in the end. The energy released during β- oxidation and the citric acid cycle generates significant amounts of ATP, making FFAs an essential energy source for various tissues [13]. In addition to serving as an energy source, FFAs also play crucial roles in receptor signaling and inflammatory responses.

3 | FFA Profiles in Gout

In obese individuals, the levels of total SFAs, total MUFAs, and total PUFAs are significantly higher compared to those in normal- weight individuals. Furthermore, long-chain FFAs were found to consist of approximately 35% saturated fatty acids and 65% unsaturated fatty acids [15, 16]. Analysis of plasma free fatty acid profiles in gout patients revealed elevated levels of MUFAs (such as oleic acid) and reduced levels of PUFAs (such as arachidonic acids) across various lipid fractions in the plasma [16]. A previous study comparing synovial fluid and serum composition demonstrated significantly higher concentrations of free fatty acids in gout patients compared to those with rheumatoid arthritis (RA) or osteoarthritis (OA) [17]. This elevation was particularly pronounced for SFAs (such as palmitic acid and stearic acid) and MUFAs (such as oleic acid). Additionally, gene polymorphism analysis indicated that single nucleotide polymorphisms (SNPs) located in the elongation of long- chain-fatty-acid- like family member genes are associated with an increased susceptibility to gout [18]. For polyunsaturated fatty acids, plasma metabolic profiling of patients with acute gout revealed that arachidonic acid metabolites, such as leukotriene B4 (LTB4) and 5- oxo-eicosatetraenoic acid (5-oxo-ETE), were significantly elevated [19].

Notably, gout patients exhibited a significantly lower abundance of short- chain fatty acid-producing bacteria in the intestinal tract, such as butyrate- and propionate- producing bacteria, compared to hyperuricemic individuals [20]. A metagenomic analysis of the gut microbiome revealed that the Enterobacteriaceae family and butyrate- producing species were reduced in gout patients. Levels of SCFAs were remarkably increased during the intercritical gout compared to the gout flare [21]. Additionally, genes involved in urate degradation and short- chain fatty acid production were reduced in gout patients, indicating a lower level of short- chain fatty acids in this patient cohort.

LCFAs circulate while bound to albumin (with 99.97% protein-bound) and enter cells via CD36 or fatty acid transport proteins (FATPs). CD36, a cell membrane- associated protein that functions both as a signaling receptor and as a fatty acid transporter, is transcriptionally regulated by peroxisome proliferator-activated receptor γ (PPARγ), which is a ligand- activated nuclear receptor, a physiological sensor of FFA oxidation, and is recognized as a functional receptor that governs numerous physiological and pathophysiological processes [22]. During gout flare, it has been confirmed that the expression of CD36 is upregulated in monocytes stimulated by MSU crystals. This upregulation is dependent on PPARγ ligand activation and culminates in the production of proinflammatory cytokines in gout [23].

4 | Molecular Mechanisms of Saturated LCFAs-Induced NLRP3 Inflammasome Activation

Saturated LCFAs, such as palmitic acid, are predominantly involved in modulating key immune- inflammatory cell populations, including neutrophils, macrophages, and lymphocytes. Research indicates that palmitic acid and stearic acid primarily activate the Toll- like receptor signaling pathways, thereby initiating inflammatory responses in gout flare [24, 25]. The acute symptoms of arthritis are triggered by the inflammatory response to MSU crystals, primarily mediated by macrophages and neutrophils. It is currently recognized as a prototypical inflammatory disorder characterized by the activation of NOD- , LRR- , and pyrin domain-containing protein 3 (NLRP3) inflammasome and subsequent release of pro- inflammatory cytokines [26]. Inflammasome engagement can be dissected into two prerequisite steps: Priming and activation. Recent studies have demonstrated that pure MSU crystals do not elicit an inflammatory response and are incapable of activating the NLRP3 inflammasome. Co- stimulatory factors such as lipid mediators, pro- inflammatory cytokines, and complement activation are required for this process [2, 26]. The combination of saturated LCFAs, specifically C18.0 and C16:0 fatty acids, with MSU crystals exhibits a potent synergistic effect on the activation of the NLRP3 inflammasome in peripheral blood mononuclear cells (PBMCs) obtained from patients with gout. This synergistic effect is mediated by the recruitment of the adaptor protein apoptosis- associated speck-like protein containing CARD (ASC), followed by caspase 1 recruitment, which facilitates the assembly and activation of NLRP3 inflammasomes and initiates the conversion of pro- IL-1β to bioactive IL-1β [27, 28].

The mechanism through which long- chain fatty acids contribute to the activation of NLRP3 inflammasomes is complex. NLRP3 inflammasomes priming phase encompasses the transcriptional upregulation of all structural components necessary for inflammasome assembly, as well as the synthesis of precursor proteins that serve as substrates for inflammatory caspases. Palmitic acid is a toll- like receptor agonist that can induce the secretion of inflammatory cytokines by binding to toll- like receptor (TLR) family members. This, in turn, amplifies the phosphorylation of myeloid differentiation factor 88 (MyD88) and the subsequent phosphorylation of p38 and c- Jun N-terminal kinases (JNK) in macrophages, as demonstrated in numerous animal and cell experiments employing specific inhibitors and siRNAs [25, 29–31]. This phosphorylation event promotes nuclear factor kappa B (NF- κB) signaling and AP-1 (c-Jun) transcription factor activation, leading to the expression of functional

NLRP3 inflammasome components [29, 32–33]. Frommer KW et al. analyzed the effect of FFA on synovial fibroblasts, human chondrocytes, and endothelial cells. Their findings indicated that FFA (both saturated and unsaturated) dose—dependently enhanced the secretion of the pro—inflammatory cytokine in synovial fibroblasts via TLR4 and required extracellular and intracellular access to the TLR4 receptor complex [30].

The assembly of the NLRP3 inflammasome is initiated by MSU, a process that involves potassium efflux through ion channels and mitochondrial perturbations, which lead to the production and cytosolic release of mitochondrial ROS [33]. Subsequently, NLRP3- activating factors are recruited, facilitating NLRP3 oligomerization and inflammasome assembly. LCFAs transported into mitochondria via CD36 exert multiple regulatory effects on inflammasome activation. Firstly, palmitate and MSU crystals synergistically suppress the expression of Sirt3, a key regulatory enzyme involved in the deacetylation of mitochondrial proteins, thereby promoting mitochondrial oxidative stress and activating the NLRP3 inflammasome [34]. Meanwhile, LCFAs also inhibit the mitochondrial respiratory chain by interfering with electron transport at complexes I and III, thereby increasing electron transport chain (ETC) leakage and promoting superoxide generation. Finally, LCFAs facilitate NLRP3 structural rearrangement, as reactive oxygen species (ROS) can oxidize tyrosine residues of NLRP3 (e.g., Tyr30 and Tyr32), thereby enhancing its interaction with ASC. This process may be modulated by phosphatases such as PTEN [35] (Figure 1).

5 | The Role of Unsaturated Fatty Acids in Gout Flare

The associations between circulating unsaturated fatty acids and gout were found to be complex, according to a population- based cohort analysis. The study revealed that baseline levels of polyunsaturated fatty acids (PUFAs), n- 6 PUFAs, and linoleic acids (LAs) were inversely related to the incidence of gout. In contrast, baseline levels of monounsaturated fatty acids (MUFAs), n- 3 PUFAs, and docosahexaenoic acids (DHAs) showed positive associations with gout flare. Furthermore, longitudinal increases in n- 6 PUFAs and LAs were linked to a reduced risk of subsequent gout flare, while an increase in n- 3 PUFAs was associated with an elevated risk [36].

PUFAs, including omega- 3 fatty acids (n-3 PUFAs) and omega- 6 fatty acids, play significant roles in regulating lipid metabolism and inflammatory responses [37, 38]. Monounsaturated fatty acids, which mainly include oleic acid, demonstrate anti- inflammatory properties by inhibiting the expression of tumor necrosis factor (TNF) and interleukin- 1 (IL-1), showing promise as a therapeutic approach. In the pathogenesis of gout, arachidonic acid (ARA), an omega- 6 polyunsaturated fatty acid, serves as a precursor to several potent pro- inflammatory mediators, such as prostaglandins and leukotrienes. It has also been reported to contribute to MSU crystal- induced inflammation by promoting the production of TNF- α and IL-1β in macrophages. Omega-3 polyunsaturated

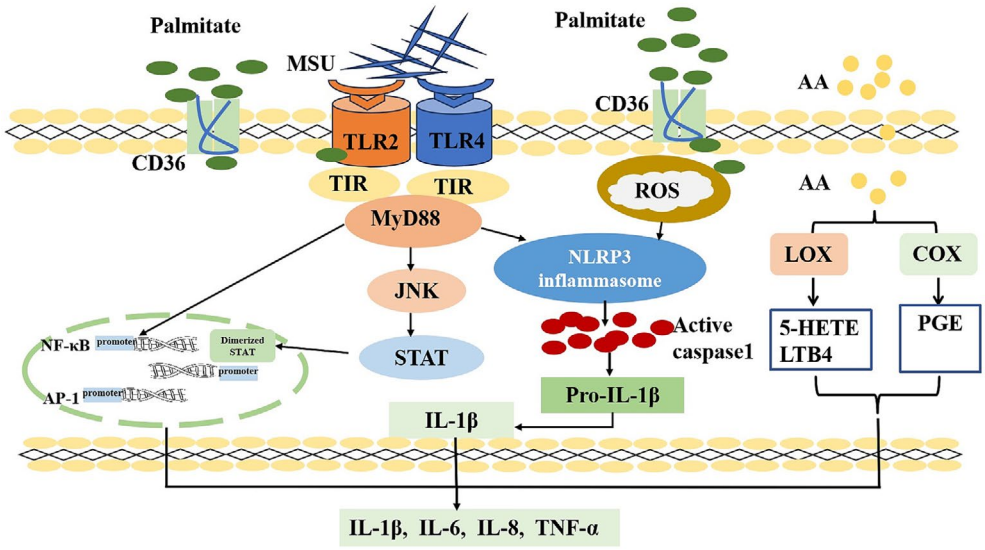

FIGURE 1 | Proposed pro-inflammatory mechanism of FFA- MSU synergy in gout flare. In the phase of gout flare, a robust synergistic effect on the inflammatory response is observed when LCFAs are combined with monosodium urate (MSU) crystals. (1) Saturated LCFAs, such as palmitate, enhance NLRP3 inflammasome priming by augmenting TLR2 or TLR4 signaling. Ligand binding recruits the adaptor protein MyD88, initiating phosphorylation cascades that activate JNK kinases. These events lead to the activation of the transcription factors NF- κB and AP-1, resulting in the upregulation of inflammasome components and precursor proteins. Regarding inflammasome activation, stimuli such as MSU crystals induce mitochondrial ROS production, which promotes NLRP3 oxidation and facilitates ASC interaction and inflammasome assembly. (2) n- 6 polyunsaturated fatty acids, such as arachidonic acid, activate the cyclooxygenase and lipoxygenase pathways, stimulating the production of prostaglandins and leukotrienes, which in turn enhance the synthesis of TNF- α and IL-1β. 5-HETE, 5-Hydroxyeicosatetraenoic Acid; AA, Arachidonic acid; AP-1, Activator protein- 1; CD36, Cluster of differentiation 36; COX, Cyclooxygenase; IL-1β, Interleukin-1β; JNK, C-Jun N-terminal kinase; LCFAs, Long-chain fatty acids; LOX, Lipoxygenase; LTB4, Leukotriene B4; MSU, Monosodium urate crystals; MyD88, Myeloid differentiation primary response 88; NF- κB, Nuclear factor kappa B; NLRP3, NOD-, LRR-and pyrin domain- containing protein 3; PGE, Prostaglandin E; ROS, Reactive oxygen species; STAT, Signal transducer and activator of transcription; TLR, Toll-like receptor; TNF, Tumor necrosis factor.

fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), demonstrate significant anti-inflammatory effects in models of crystal- mediated inflammation. Mechanistically, n- 3 PUFAs suppress the activation of the NLRP3 inflammasome induced by a high- fat diet in a type 2 diabetes model in vivo. Consequently, this inhibits the subsequent activation of caspase- 1 and the secretion of IL-1β. These processes involve the G- protein-coupled receptors 120 (GPR120) and 40 (GPR40), as well as their downstream scaffold protein β- arrestin-2 [39, 40].

6 | SCFAs Process Anti-Inflammatory Mediators in Gout Flare

Acetate, a major SCFA, has previously been shown to alleviate MSU- induced arthritis, reduce neutrophil infiltration, and mitigate inflammatory hyperalgesia. Butyrate, another type of SCFA, has been reported to decrease palmitic acid levels and suppress the inflammatory response induced by MSU [41]. In a gout mouse model, SCFAs have been found to facilitate the resolution of neutrophilic inflammation by inhibiting NF- κB activity and promoting the production of anti-inflammatory mediators including IL- 10, TGFβ, and annexin A1. This effect was also observed in mice fed a high- fiber diet, which enhances SCFA production [42]. Furthermore, SCFAs can inhibit the production of nitric oxide and proinflammatory cytokines, including tumor necrosis factor (TNF) and cytokine- induced neutrophil chemoattractant-2 (CINC-2), by neutrophils. Notably, microbiota depletion in MSU- induced mice leads to reduced SCFA production, which correlates with decreased neutrophil infiltration, hyperalgesia, and production of IL- 1β and CXCL1. Moreover, treatment of germ-free mice with propionate and butyrate inhibited both in vitro and ex vivo production of proinflammatory cytokines (TNF- α and CINC- 2αβ) and NO, indicating that SCFAs have anti-inflammatory effects by inhibiting HDAC activity and NF- κB activation [43].

7 | Role of Free Fatty Acid Receptors (FFARs) in SCFA-Mediated Anti-Inflammation

FFARs comprise a subfamily of G protein- coupled receptors (GPCRs) that are activated by FFAs and their metabolites. To the present date, four FFARs (FFAR1- 4) have been identified, with FFAR2 (GPR43) and FFAR3 (GPR41) receiving particular attention for their roles in inflammatory disease pathogenesis due to their specific activation by SCFAs [44]. It has been reported that FFAR2 signaling inhibits NF- κB nuclear translocation, consequently reducing the expression of inflammatory cytokines such as IL- 1β and IL-6 [12, 41]. In gout patients, FFAR2 expression exhibits dynamic regulation across disease states, showing significant upregulation compared to individuals with hyperuricemia. This upregulation is particularly pronounced in the presence of MSU crystal deposition and during intercritical gout periods. Strikingly, FFAR2 expression reaches peak levels during gout flare [45].

Functional studies in GPR43- deficient (FFAR2−/−) mice have revealed critical insights into SCFA- mediated inflammatory responses. These animals demonstrate attenuated neutrophil recruitment following MSU crystal challenge, accompanied by reduced IL- 1β production and diminished ROS generation. These findings suggest that while SCFAs can promote inflammasome assembly and IL- 1β production, this process is partially dependent on FFAR2 signaling (Figure 2) [46].

8 | FFAs In Gout Comorbidities

8.1 | Insulin Resistance

Gout is closely associated with insulin resistance syndrome. Previous studies suggest that uric acid generation and fat accumulation were associated with fructose metabolism. Excessive fructose intake has been implicated in the development of hyperuricemia. Fructose is primarily metabolized in the liver and phosphorylated by fructokinase C, leading to a reduction in ATP levels and intracellular phosphates. The decrease in intracellular phosphate activates adenosine monophosphate (AMP) deaminase, an enzyme that converts AMP to inosine monophosphate (IMP), resulting in purine nucleotide turnover and subsequent uric acid formation [47].

The metabolic pathway begins with fructose phosphorylation to fructose- 1-phosphate, which is then cleaved by aldolase B (ALDOB) into dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde. These intermediates subsequently enter glycolytic and gluconeogenic pathways, contributing to the synthesis of glucose, glycogen, and triglycerides [48]. This metabolic flux promotes the progression of metabolic diseases (such as glucose intolerance, dyslipidemias, and insulin resistance).

Insulin resistance is defined by impaired cellular responses to insulin's metabolic actions, while its mitogenic effects remain relatively preserved. This metabolic dysfunction leads to dysregulated lipid metabolism, characterized by increased free fatty acid release from adipose tissue and exacerbation of systemic inflammation [49, 50]. SFAs play a pivotal role in insulin resistance development through their interaction with pattern recognition receptors. Specifically, SFAs bind to TLR- 2 and 4, which are expressed on both adipocytes and macrophages. These receptors, classically involved in pathogen recognition and innate immunity, initiate pro- inflammatory signaling cascades upon SFA engagement.

The TLR4- SFA interaction triggers the activation of JNK, which subsequently phosphorylates insulin receptor substrate 1 (IRS- 1) at serine residues. This post- translational modification disrupts normal IRS- 1 tyrosine phosphorylation, impairing downstream insulin signaling through the PI3K- Akt pathway [51, 52]. Concurrently, TLR4 activation stimulates the NF- κB signaling cascade, leading to increased transcription of pro- inflammatory cytokines, including TNF- α and IL-6. These cytokines form a positive feedback loop: They further activate JNK signaling, exacerbate IRS- 1 impairment, amplify macrophage inflammatory responses, and contribute to the chronic inflammatory state characteristic of insulin resistance [52].

The inflammatory milieu created by TNF- α and IL-6 has particular relevance in gouty flares. These cytokines enhance

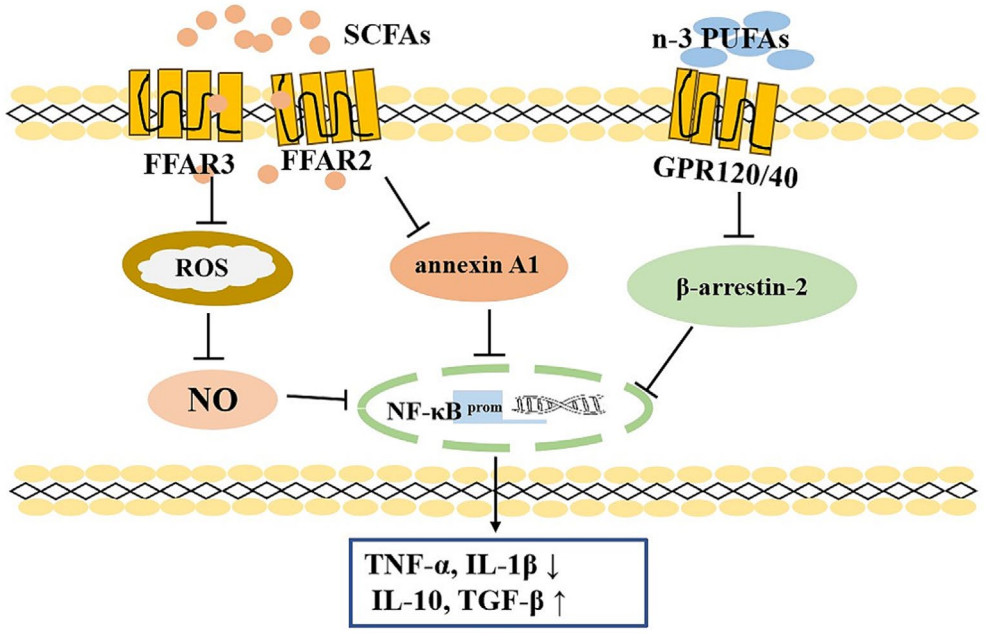

FIGURE 2 | Proposed anti-inflammatory mechanism of FFA in gout. SCFAs and n- 3 PUFAs exhibit anti-inflammatory effects in the context of gout. (1) SCFAs bind to FFAR2/3, thereby inhibiting the nuclear translocation of NF- κB, which leads to a reduction in the expression of pro-inflammatory cytokines such as IL- 1β and enhances the production of anti-inflammatory mediators, including IL- 10 and TGF-β. Additionally, SCFAs suppress nitric oxide production, further decreasing the expression of pro-inflammatory cytokines. (2) n- 3 PUFAs interact with the downstream scaffold protein β- arrestin-2, thereby inhibiting the activation of the NLRP3 inflammasome. This results in the suppression of caspase-1 activation and IL- 1β secretion, processes that are mediated through GPR120 and 40. FFAR, Free fatty acid receptor; GPR, G-protein-coupled receptors; IL- 10, Interleukin-10; IL-1β, Interleukin-1β; n-3 PUFAs, Omega-3 fatty acids; NF-κB, Nuclear factor kappa B; NO, Nitric oxide; ROS, Reactive oxygen species; SCFAs, Short- chain fatty acids; TGF-β, Transforming growth factor-β; TNF, Tumor necrosis factor.

macrophage activation and pro- inflammatory polarization, increase synovial inflammation and joint destruction, and potentiate the inflammatory response to MSU crystals [26, 33]. This interplay between metabolic dysfunction and chronic inflammation highlights the bidirectional relationship between insulin resistance and inflammatory diseases, where each condition exacerbates the other through shared molecular pathways [53, 54].

Recent evidence further supports this mechanistic link. McCormick et al. reported that hyperinsulinemia leads to hyperuricemia, while the reverse causality is not observed [55]. In a study combining biochemistry with population genetics, urate transporter 1 (URAT1) has been found to be regulated by hyperinsulinemia via AKT, which phosphorylates URAT1- Thr408 to reduce URAT1 cell- surface abundance and urate transport activity [56]. These findings suggest that interventions targeting insulin resistance may effectively lower serum urate acid (SUA) levels and mitigate gout risk, whereas reducing SUA levels is unlikely to alleviate insulin resistance or its cardiovascular-metabolic consequences.

8.2 | Renal Fibrosis in Gouty Nephropathy

Tubular injury, characterized by apoptosis and necrosis of renal tubular epithelial cells (TECs), is a hallmark of gouty nephropathy. This injury triggers a cascade of pathological events, including interstitial inflammation and fibrosis; the underlying mechanism involves the activation of the NLRP3 inflammasome and disturbances in lipid metabolism [57].

FFAs, particularly palmitic acid, activate TECs through lipid overload, inducing mitochondrial reactive oxygen species (ROS) generation and endoplasmic reticulum (ER) stress. These stressors promote NLRP3 inflammasome oligomerization, facilitating caspase- 1 cleavage and subsequent maturation of pro- inflammatory cytokines IL-1β and IL-18 [58, 59]. Elevated IL- 1β levels recruit macrophages via chemokine (C- C motif) ligand 2 (CCL2) secretion, perpetuating a pro-inflammatory microenvironment. Chronic inflammation further stimulates fibroblast activation, marked by α- smooth muscle actin (α- SMA) upregulation and extracellular matrix (ECM) deposition (e.g., collagen I/III), driving tubulointerstitial fibrosis [60, 61].

8.3 | Non- Alcoholic Fatty Liver Disease

Recent research findings have underscored a critical role for elevated serum uric acid levels in the pathogenesis of metabolic disorders, including metabolic dysfunction- associated steatotic liver disease (MASLD). Recent investigations have revealed that hyperuricemia not only promotes adipogenesis and accelerates obesity onset but also directly exacerbates hepatic steatosis through interorgan crosstalk.

Elevated SUA levels can augment lipogenesis and fat accumulation, thereby driving the development of NAFLD. Su M et al. reported that a high level of UA could induce hypertrophy of adipocytes, inhibit their hyperplasia effects potentially attributed to dysregulated leptin signaling, a hormone critically involved in

fatty acid metabolism and energy homeostasis. Transcriptomic analysis further linked this phenotype to modulation of the AMPK signaling pathway, a key regulator of lipid metabolism [62]. It was also reported that uric acid influenced adipogenesis in MSC- derived adipocytes by upregulating adipogenesis markers C/EBPα, PPARγ, and Mest while downregulating small lipid droplet formation and Wnt10b expression, a Wnt signaling molecule that inhibits adipogenesis [63]. These findings collectively highlight UA's dual role in promoting adipocyte maturation and impairing adipose tissue plasticity.

Beyond adipose remodeling, UA induces adipocyte dysfunction through oxidative stress. UA activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [64], resulting in an elevation in O2− production and subsequent ROS accumulation. Excess ROS promotes monocyte chemotactic protein- 1 (MCP- 1) production, a proinflammatory adipokine recruiting macrophages to adipose tissue, fostering a chronic low- grade inflammatory state—a hallmark of obese adipocytes [65, 66]. This inflammatory milieu further exacerbates adipose dysfunction and systemic insulin resistance.

Furthermore, UA- induced oxidative stress activation is potentiated by augmentation of xanthine oxidoreductase (XOR), the rate- limiting enzyme in UA biosynthesis. XOR catalyzes the oxidation of hypoxanthine and xanthine to UA, and its activity is tightly linked to NADPH oxidase- mediated ROS production [67]. Genetic studies in murine models demonstrate that downregulation of XOR expression exacerbates adipocyte lipid accumulation and oxidative stress, ultimately leading to age- related obesity accompanied by insulin resistance [68]. Meanwhile, adipose tissue itself serves as a significant source of UA production through XOR activity, with this process being potently upregulated in obesity—a key driver of NAFLD development [69].

In the liver, UA disrupts metabolic homeostasis through multiple interrelated mechanisms. First, UA induces hepatic mitochondrial dysfunction, characterized by impaired oxidative phosphorylation, reduced adenosine triphosphate (ATP) production, and mitochondrial DNA damage. These structural and functional abnormalities in mitochondria exacerbate ROS generation, as evidenced by elevated levels of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) [70]. The resulting oxidative stress activates the pro- inflammatory JNK/AP-1 pathway. JNK/AP- 1 hyperactivation, in turn, upregulates transcription of pro- lipogenic genes (e.g., sterol regulatory element-binding protein 1c [SREBP- 1c], fatty acid synthase [FASN]) while suppressing genes involved in fatty acid oxidation (e.g., peroxisome proliferator- activated receptor γ coactivator 1α [PGC-1α]), culminating in dysregulated hepatic lipid accumulation- manifested as steatosis, triglyceride (TG) deposition, and lobular inflammation [70]. Moreover, it modulates the expression of enzymes involved in fatty acid synthesis pathways or chemokines within the liver, disrupting lipid homeostasis [71].

9 | Therapeutic Implications

Treating gout involves two key approaches: One focused on lowering uric acid levels and the other on reducing inflammation. Nonsteroidal anti- inflammatory drugs (NSAIDs), colchicine, and glucocorticoids are commonly used and effectively alleviate pain and inflammation during acute attacks. Allopurinol, febuxostat, and benzbromarone are medications designed to lower uric acid levels [2]. However, abnormal lipid profile distribution in the body can partially contribute to the pathogenesis of gout and the development of its associated complications. Therapeutic strategies targeting FFAs indicate that this approach represents a promising novel supplementary therapeutic option.

9.1 | Lipid- Targeted Strategies Are an Important Supplementary Treatment for the Long- Term Management of Gout

A post hoc analysis of the randomized, controlled Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial demonstrated that fenofibrate, a peroxisome proliferator-activated receptor- α (PPAR-α) agonist lipid-lowering agent, decreased serum uric acid concentrations by 20% and nearly halved the incidence of first on- study gout events over a 5-year treatment period [72]. Several studies also provided strong evidence that fenofibrate intervention exerted a significant reduction in serum uric acid and decreased the uric acid level by approximately 0.73 mg/dL compared to placebo [73, 74]. Notably, fenofibrate's urate- lowering action synergizes with its lipid- modulating properties, as evidenced by concurrent reductions in triglycerides (−35%) and LDL cholesterol (−18%) [75]. Therefore, the administration of a lipid- lowering agent in patients with gout who also present with hyperlipidemia or obesity may contribute to the effective long- term management of the disease.

9.2 | Microbiome Modulation Serves as a Promising Supplementary Treatment Approach

Dietary fiber metabolism by gut microbiota generates bioactive SCFAs (acetate, propionate, butyrate), which exert pleiotropic effects on uric acid homeostasis. Acetate- derived butyrate enhances intestinal barrier integrity via upregulation of tight junction proteins (e.g., occludin, ZO- 1), reducing systemic endotoxin leakage and subsequent NLRP3 inflammasome activation [75, 76]. It could ameliorate hepatic triglyceride accumulation in fructose- insulin-resistant rats to suppress uric acid and lactate production. An in vivo animal study demonstrates that oral administration of the Lactobacillus strain MJM60390 significantly reduces blood uric acid levels. Furthermore, supplementation with this strain in hyperuricemic model mice increases the relative abundance of the Rikenellaceae family, a known producer of the short- chain fatty acid butyrate [77]. This contributes to the maintenance of optimal intestinal health and may serve as a potential preventive strategy against hyperuricemia. Enterobacter faecalis W5 ameliorates HUA by restoring the impaired intestinal barrier, enhancing intestinal UA secretion through the regulation of ABCG2 expression, and decreasing intestinal UA synthesis via modulation of purine metabolism [78]. These findings may provide a promising basis for the development of novel therapeutic strategies targeting HUA via intestinal intervention.

9.3 | Recommendations for Diet

The dietary consumption of fish rich in omega- 3 polyunsaturated fatty acids (n- 3 PUFA) was associated with a reduced risk of recurrent gout attacks after adjusting for total purine intake [79]. A cohort study on dietary polyunsaturated fatty acids and risk of gout revealed that the intake of PUFAs is significantly and negatively associated with the risk of gout incidence, whereas arachidonic acid intake demonstrates a positive correlation with gout risk. Among the various types of PUFAs, plant- derived ones, including alpha- linolenic acid and linoleic acid, exhibit the strongest protective effects and are inversely related to the risk of elevated uric acid levels. In contrast, marine- derived n-3 PUFAs did not demonstrate a statistically significant protective association against gout [37].

Therefore, dietary recommendations for individuals with gout suggest increasing the intake of fish that are rich in n- 3 PUFAs, incorporating more alpha- linolenic acid-rich foods such as flaxseeds, walnuts, and rapeseed oil, and moderately increasing consumption of linoleic acid sources such as sunflower seeds, corn oil, and soybean oil. Fatty fish, which contain a higher ratio of n- 3 to n-6 fatty acids, are also encouraged in the diet. However, clinical trials involving self- directed supplementation with n- 3 PUFAs have produced inconsistent outcomes, and meta- analyses have not demonstrated a significant reduction in serum uric acid (UA) levels [79, 80]. Further clinical studies are needed to evaluate the efficacy of n- 3 PUFAs from specific sources and at sufficient dosages in preventing gout flares. In clinical practice, recommendations regarding n- 3 PUFA supplementation should be made with caution.

10 | Conclusion

This review establishes FFAs as molecular bridges between metabolic dysfunction and inflammatory arthritis in gout. FFAs act as metabolic- immune rheostats in gout, with LCFAs driving inflammasome- mediated pathology and SCFAs resolving inflammation via the microbiota- gut axis. Future research should explore tissue- specific FFA metabolism, temporal lipidomic changes during flares, and microbiome- derived lipid mediators.

Author Contributions

N.L. performed the conceptualization and wrote the original draft; C.S. performed the review, editing, and supervision.

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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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ORIGINAL ARTICLE

Effectiveness of Platelet-Rich Plasma in Knee Osteoarthritis: A Systematic Review and Meta-Analysis

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ABSTRACT

Introduction: Knee osteoarthritis (OA) causes chronic pain and disability, with limited benefit from conventional treatments. Platelet-rich plasma (PRP) has been proposed as a biologic therapy with potential tissue-repair properties; however, its clinical efficacy remains uncertain.

Objective: To assess the effectiveness of intra-articular PRP injections in reducing pain and improving function in patients with knee OA.

Methodology: This systematic review and meta-analysis included randomized controlled trials comparing intra-articular PRP with placebo or no treatment. Pain outcomes were measured using the Visual Analog Scale (VAS), while functional outcomes were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) or Knee injury and Osteoarthritis Outcome Score (KOOS).

Results: PRP significantly reduced pain and improved function compared with placebo across short-, mid-, and long-term follow-up. The magnitude of benefit varied but remained clinically relevant.

Conclusion: PRP provides significant improvements in pain relief and functional outcomes compared with placebo or no treatment in patients with knee OA, supporting its potential as a therapeutic option. Standardized protocols and larger RCTs are warranted to further confirm its clinical effectiveness.

1 | Introduction

Osteoarthritis (OA) is a joint disease characterized by the slow degradation of the cartilage that covers the joints. Various symptoms of this illness, such as pain, stiffness, and edema, have a major influence on functional ability and quality of life [1, 2]. The knee joint is the most impacted by osteoarthritis due to its regular use and increased strain throughout life. Knee osteoarthritis (OA) primarily affects people over the age of aged people, particularly those with a higher BMI. In response to population aging and the increasing prevalence of obesity, the risk and epidemiological trend of knee osteoarthritis are anticipated to rise significantly [3].

The fundamental characteristics of knee osteoarthritis, such as and functional impairment are routinely assessed with validated methods such as the Western Ontario and McMaster Universities Arthritis Index and the Visual Analog Scale [4–7]. Treatment aims to relieve symptoms and improve functional ability safely and effectively. Physical therapy and oral medication are usually the first choices of treatment. Nevertheless, should the symptoms continue to manifest, the administration of intra-articular injections may be considered as a potential intervention [7].

Corticosteroids, hyaluronic acid, and Platelet-rich plasma (PRP) are among the options utilized in these injections, and

continuing research is being conducted to assess their safety and effectiveness [8]. PRP is derived from the patient's blood and is processed to extract a high concentration of platelets. These platelets are rich in growth factors and bioactive molecules, which are believed to play a crucial role in promoting wound healing and modulating the repair process [9, 10]. However, the findings regarding PRP's effectiveness vary across different studies, indicating a need for further research to assess its role in treating knee osteoarthritis [11–13].

Given the rising prevalence of knee osteoarthritis, it is critical to assess the safety and efficacy of various treatments, especially PRP. The purpose of this systematic review and meta- analysis is to determine the therapeutic efficacy of intra- articular platelet-rich plasma injections in knee osteoarthritis.

2 | Methods

2.1 | Search Strategy

This review followed the PRISMA guidelines and was registered with PROSPERO (CRD420251019846) [14]. A comprehensive literature search was performed on March 30, 2025, utilizing four electronic databases: PubMed, the Virtual Health Library (VHL), the Cochrane Library, and Open SIGLE. Only published articles were included, with no language restrictions applied. Eligible studies were randomized clinical trials (RCTs) involving participants aged 18 years and older.

In each database, advanced search options were utilized with specific keywords such as "clinical trial AND platelet- rich plasma OR PRP AND knee osteoarthritis OR OA".

2.2 | Inclusion and Exclusion Criteria

Titles and abstracts were used to screen the search results from each database (Table 1). The inclusion criteria were as follows:

1. Studies published until 2025.
2. Clinical trials evaluating the safety and efficacy of intra- articular PRP injections in patients with knee osteoarthritis.
3. Participants aged ≥ 18 years and diagnosed with knee OA (no further restrictions on patient demographics).
4. In order to assess the efficacy of PRP injections for the treatment of knee osteoarthritis, the analysis was restricted to studies that employed a true placebo- controlled design.

The criteria for exclusion were as follows:

1. Studies with only abstracts, no full- text articles, or case reports.
2. Studies using non- human subjects.
3. Studies involving participants under 18 years of age were excluded.

Articles meeting the eligibility criteria were included, and duplicate records were removed (Table 1).

2.3 | Data Extraction

Statistical analysis was performed using Review Manager 5.4. Two independent reviewers conducted the literature screening and data extraction for studies published up to 2025. Any disagreements between the reviewers were addressed through discussion with a third reviewer to ensure consistency and accuracy in the selection process.

2.4 | Data Extraction Process

The systematic review was performed using Rayyan, an online platform specifically developed to facilitate the organization and screening of studies in systematic reviews [20]. To avoid observer bias, reviewers informed each other during the screening process whether they were including or excluding items. Rayyan also allowed reviewers to specify the reasons

TABLE 1 | Description of the five included randomized, placebo- controlled clinical trials.

Author (Year)	Country	Average age (years)	Average BMI (kg/m^2)	Injections received	Participants	Study assessment tool
Ghai et al. (2019) [15]	India	Average—49.8	Average weight—67 kg	PRP—1 P—1	PRP – 10 P—10	VAS & WOMAC
Murillo et al. (2021) [16]	Brazil	PRP—66.4 P—62.5	PRP – 28.3 P—27.6	PRP—2 P—2	PRP—20 P—21	VAS & WOMAC
Chu et al. (2022) [17]	China	PRP—53.91 P—54.51	PRP—27.5 P—27.9	PRP – 3 P—3	PRP—308 P—302	VAS & WOMAC
Mardani-Kivi et al. (2023) [18]	Iran	PRP – 62.6 P—60.6	PRP—29.7 P—29.1	PRP – 1 P—1	PRP—30 P—30	VAS & WOMAC
Yoshioka et al. (2024) [19]	Japan	PRP – 65.9 P—67.9	PRP—28.7 P—28.1	PRP – 1 P—1	PRP—15 P—15	VAS & WOMAC

Abbreviations: P, Placebo; PRP, platelet- rich plasma; VAS, Visual Analog Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

for their decisions to include or exclude studies. Once the initial screening was completed, the results were unblinded, and the reviewers discussed and resolved any discrepancies regarding article selection.

For data extraction, all eligible articles were independently reviewed using Google Forms, and reviewers were blinded to each other's inputs. The extracted data included the extraction date, journal, publication year, study location, design, objectives, outcomes, treatment details, demographics, effect sizes, and potential conflicts of interest. Statistical analyzes were then performed by the first reviewer based on this data.

2.5 | Selective Studies and Sample Characteristics

A comprehensive search identified 6566 prospective studies from four databases: PubMed ($n = 3642$), Cochrane ($n = 2666$), VHL ($n = 158$), and Open Sigle ($n = 100$) (Figure 1). An automated screening procedure was implemented, utilizing publication date and study type as criteria for eligibility assessment. The process led to the exclusion of 3344 papers that didn't meet the criteria. Subsequent screening led to the removal of an additional 3200 papers due to issues of duplication or non- compliance with qualification requirements. Ultimately, 22 studies remained for final screening and data extraction. During the screening process, the remaining eligible study data were collected in a blinded manner by 2 authors.

This criterion, however, led to the exclusion of 17 studies, leaving 5 studies for the meta- analysis.

2.6 | Selected Studies and Sample Characteristics

Individual research data were presented utilizing several techniques in this study. Detailed tables were prepared to arrange each study's main data, including the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Visual Analog Scale (VAS) scores. These tables provide the mean values, standard deviations (SD), and sample sizes (n) for both the PRP and placebo groups at baseline and 6 months post- treatment. To assess the therapeutic efficacy of PRP, this study assessed the effect size (Cohen's d) and reported the corresponding standard errors and p-values to quantify and compare treatment outcomes across studies.

2.7 | Meta- Analysis

Due to the diversity of our data, we employed meta- analysis methods to aggregate the findings from numerous investigations. A random- effect model was used to analyze each comparison, such as baseline PRP vs. 6 months, baseline placebo vs. 6 months, or 6- month PRP vs. placebo for WOMAC and VAS, to calculate the combined effect size. The model takes into account both between and within- study variances, as well as prospective variations. We quantified total heterogeneity in the study using the limited maximum likelihood (REML) approach and τ^2 estimator [21]. Cochran's Q statistic was employed to assess statistical heterogeneity. This statistic utilizes the chi- square distribution to calculate variability between studies, rather than within individual study participants, and indicates high heterogeneity when the p- value is <0.0001 [22]. Furthermore, the I^2 index was utilized to evaluate heterogeneity. This index

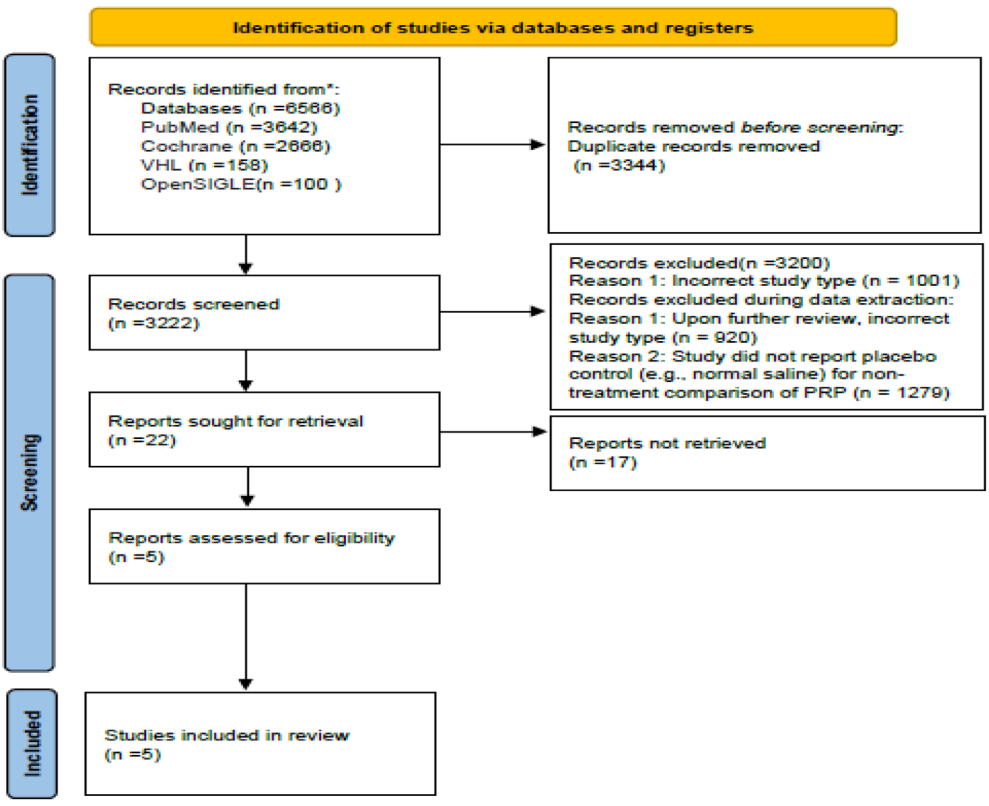

FIGURE 1 | PRISMA flow diagram.

determines heterogeneity by computing the ratio of the Cochran Q test result to the degrees of freedom, as well as the Q value [23]. The I^2 index enhances the Cochran Q test by revealing greater heterogeneity [24].

2.8 | Cochrane Risk of Bias Tool

The Cochrane Risk of Bias Tool was used in this systematic review and meta- analysis to thoroughly analyze the methodological quality, credibility of the evidence, and potential sources of bias in the included studies. The analysis included only randomized controlled trials (RCTs), which are widely regarded as the gold standard for assessing the efficacy and safety of therapeutic interventions. To ensure objectivity and consistency, a blinded assessment was conducted based on the six domains specified in the Cochrane Risk of Bias Tool, which include: (1) random sequence creation, (2) allocation concealment, (3) participant and personnel blinding, (4) outcome assessment blinding, (5) missing outcome data, (6) selective reporting, and (7) other potential sources of bias.

The two authors found no domains with a "high" risk of bias among the five research studies included in the meta- analysis. Additionally, one study raised "some concerns" about bias, as one author marked at least one area as "unclear." However, we approached the evaluation of bias risk with great care, and these studies received a maximum of one or two "unclear". No study was evaluated by both authors as exhibiting a "low" risk of bias in any domain. Although one author rated all five studies as "low" in each domain, the other author still provided an "unclear" rating in at least one domain [15–19]. Therefore, it was concluded that these studies raised "some concerns" regarding overall bias risk.

A post hoc sensitivity analysis was performed for each outcome by excluding studies with a high risk of bias and including only the five studies assessed as having a low risk of bias to determine the robustness of the pooled estimates. This method aimed to evaluate the robustness of the meta- analysis findings by excluding the two studies that presented bias concerns. This action was taken before finalizing the meta- analysis, and the authors were unaware of the outcomes of the bias risk evaluations (Figure 2A,B).

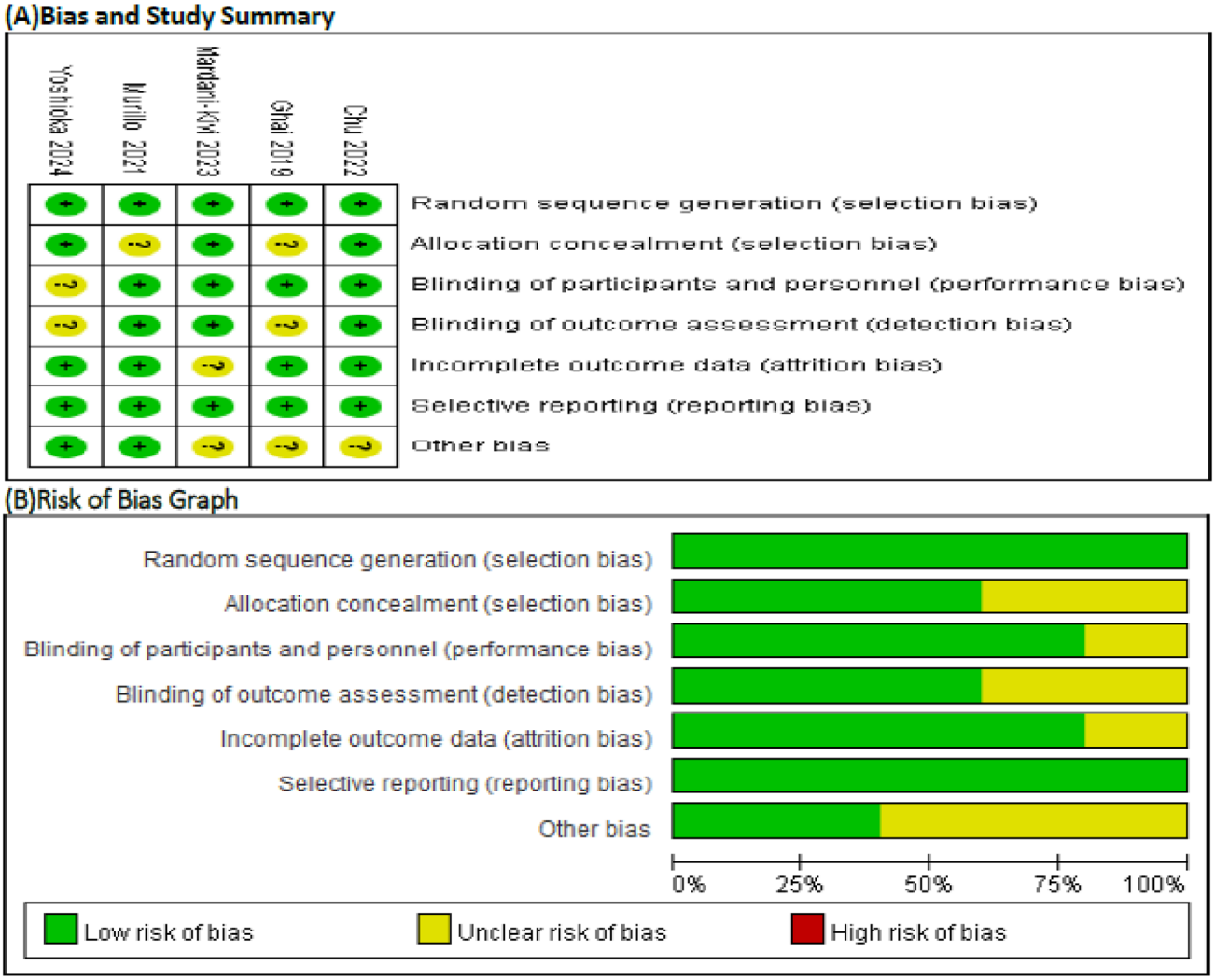

FIGURE 2 | (A) Bias and study summary. (B) Risk of bias graph.

This approach facilitated a comprehensive evaluation of the internal validity of the included trials [25, 26].

3 | Results

All five studies in this systematic review were randomized, double- blinded, placebo-controlled clinical trials, indicating a high level of scientific rigor [15–19]. The frequency of interventions exhibited variability among the studies reviewed. In the five included studies, participants were administered three injections of either platelet- rich plasma or saline, typically on a weekly basis. Five studies administered a single intra- articular injection of either PRP or normal saline. In two of the included studies, ultrasound- guided intra-articular injections were administered into the knee joint to deliver either platelet- rich plasma or normal saline as a placebo, with injection intervals set at 2 weeks. To evaluate treatment efficacy, five studies used the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Visual Analog Scale (VAS) as the primary outcome measures for assessing functional outcomes and pain intensity, respectively. Additionally, five studies incorporated both VAS and WOMAC scores at the six- month follow-up to evaluate sustained treatment effects (Table 1).

3.1 | Visual Analog Scale (VAS)

The objective of this meta- analysis was to evaluate the effectiveness of platelet- rich plasma injections in comparison to placebo in alleviating pain related to knee osteoarthritis (OA), with a particular focus on alterations in the Visual Analog Scale. We analyzed data from five studies to determine the impact of PRP treatment over 6 months.

The results revealed a significant reduction in pain intensity following PRP treatment at 6 months, indicating a notable therapeutic benefit. At the six- month follow-up, the aggregated mean difference (MD) between platelet- rich plasma and placebo was found to be 0.53, with a 95% confidence interval (CI) ranging from 0.39 to 0.68 and an I^2 statistic of 2% (Figure 3). This statistically significant difference suggests PRP's potential efficacy in treating knee osteoarthritis- related pain.

3.2 | Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

In addition to pain intensity, our meta- analysis also utilized the WOMAC index as a primary evaluation tool to assess the functional outcomes of PRP treatment for knee osteoarthritis. This review included five trials that compared WOMAC scores at baseline and 6 months after treatment, assessing changes in pain, stiffness, and physical function in patients with knee osteoarthritis.

The aggregated data demonstrated a significant improvement in WOMAC ratings in the PRP group after 6 months, indicating improved joint function and fewer symptoms.

At 6 months post- treatment, there was a significant difference in WOMAC ratings between the PRP and placebo groups (MD = 0.14, 95% CI: −0.00 to 0.28, $I^2 = 0\%$) (Figure 4). This study indicates that PRP injections had a consistent and beneficial effect on patients' reported functional outcomes.

4 | Discussion

This comprehensive review and meta- analysis examined five randomized controlled trials with 761 patients in total. Platelet-rich plasma therapy appears to considerably reduce pain in patients with knee osteoarthritis. Pain outcomes were predominantly assessed using the Visual Analogue Scale and the

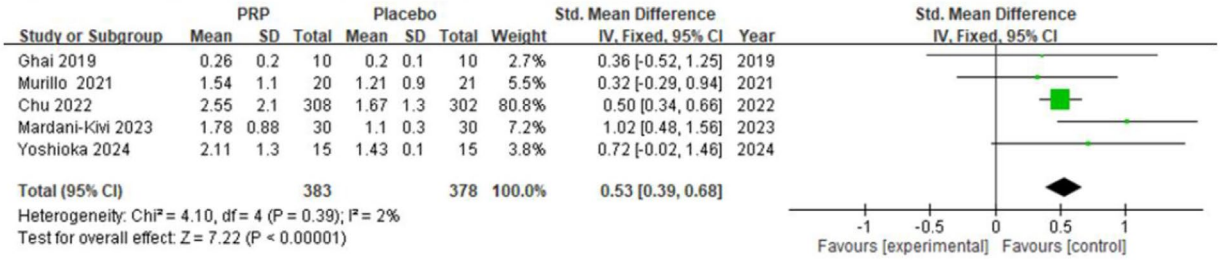

FIGURE 3 | The comparison of VAS scores between PRP and placebo.

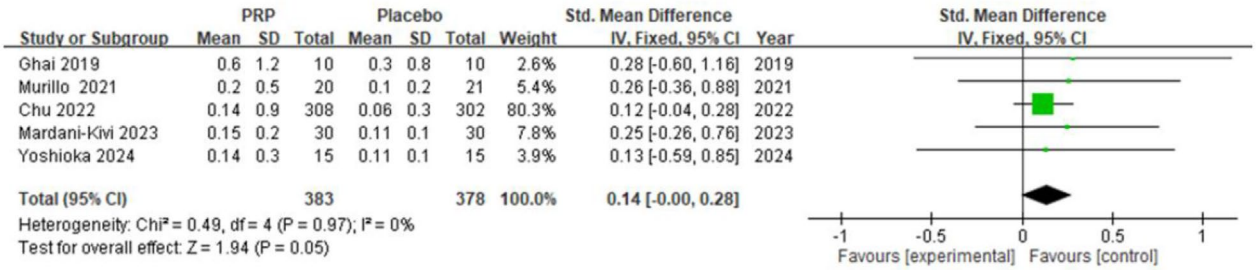

FIGURE 4 | The comparison of WOMAC scores between PRP and placebo.

Western Ontario and McMaster Universities Osteoarthritis Index, with both methods consistently demonstrating greater pain alleviation following PRP injection. The results of this study suggest that PRP therapy has potential clinical benefits for controlling knee OA symptoms; however, more research with bigger sample numbers and longer follow-up periods is required to establish its long- term efficacy and stability.

The findings of this study suggest that PRP therapy may offer potential clinical benefits for improving knee osteoarthritis symptoms. However, further large- scale studies with longer fol low-up periods are needed to confirm its long- term efficacy and sustained clinical effects.

The pooled analysis of VAS scores revealed a significant effect of PRP treatment at 6 months, with an approximate mean reduction of 9 mm in pain intensity compared to placebo. Analysis of WOMAC scores also showed a significant overall effect with an estimated mean reduction of 6.15 points in the PRP group compared to placebo, indicating improved joint function and symptom relief. The heterogeneity observed across included studies suggests the need for further investigation into factors that may influence patient outcomes, such as injection protocols, treatment regimens, and population characteristics.

Intra- articular PRP injections demonstrated superior efficacy over placebo in relieving pain and improving knee function within 6 months; however, the long- term effectiveness remains uncertain. While this study focused on the therapeutic efficacy of PRP alone, a 2022 meta- analysis reported that the incidence of adverse events was lower when PRP was combined with hyaluronic acid (HA) compared to PRP alone, suggesting that combination therapy may offer enhanced safety [27]. Though the combination of PRP and HA did not show superior efficacy over PRP alone, future research may explore the potential benefits of combining PRP with other agents, such as corticosteroids, to enhance clinical outcomes [27].

A meta- analysis compared leukocyte-rich and leukocyte-poor PRP to determine which is more effective for treatment [27–31]; however, this issue was not further explored in the present study.

In our investigation, all control groups demonstrated Q- statistics with *p*- values less than 0.0001, signifying a substantial level of heterogeneity. These analyzes were carried out utilizing Review Manager version 5.4 software in conjunction with the "meta" package [32].

4.1 | Limitations

This study has several limitations. First, there were no specific guidelines regarding the type of PRP or the injection protocols used. It is currently unknown whether a defined number of injections can provide the best therapeutic benefit [27, 28].

Furthermore, the data utilized in this systematic review and meta- analysis were determined by the availability of infor mation and the comparison methods employed, resulting in a restricted number of studies after screening. This limitation could impact the generalizability and strength of the findings.

4.2 | Strengths

This analysis only examined randomized clinical studies (RCTs) that compared PRP to placebo or control groups, not other potential treatments. This design lowers variability when comparing findings. We also assessed the outcomes at 6 months, which is a key time point for evaluating the effect of PRP injections. Furthermore, we included the most recent articles (published from 2019 to 2025) with no restrictions on study location.

This study followed the PRISMA standards and used tools like the Cochrane Intervention Systematic Review Handbook to ensure that systematic measures were taken throughout the research process. Blinding was implemented at several phases, including data retrieval, screening, and extraction. All effect sizes were determined using the same approach (Cohen's *d*), allowing more exact comparisons between trials.

Several tests revealed high heterogeneity in the study, with no evidence of publication bias. Finally, we assessed two outcomes to gain a better understanding of the efficacy of PRP injections for treating knee osteoarthritis.

5 | Conclusion

Overall, most studies discovered that PRP treatment reduced Visual Analogue Scale and Western Ontario and McMaster University Osteoarthritis Index scores at 6 months, indicating improved pain and function.

Author Contributions

Hsuan-Wei Liu: writing – original draft, writing – review and editing.

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Disclosure

The author confirm that no paper mills and artificial intelligence were used.

Consent

The author has nothing to report.

Conflicts of Interest

The author declares 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

Palindromic Rheumatism: Revisiting the Approach to an Enigmatic Condition

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1 | Introduction

Palindromic rheumatism (PR) is a rheumatic condition defined by intermittent, self-resolving flares of inflammatory arthritis/periarthritis [1]. Currently, it remains debatable whether it falls under the spectrum of rheumatoid arthritis (RA) manifestations, or is considered a "pre-RA" condition [1]. In fact, it has been reported that 10% of RA cases start off with episodic arthritis considered to be "palindromic" [2]. Nonetheless, PR is distinctive from classical RA in that there must be absence of joint inflammation in between episodes [1]. From epidemiological studies, it is found that 50% of PR cases go on to develop persistent arthritis typically in the form of RA, 40%–50% continue its palindromic form, and 15% spontaneously remit [3]. While this condition was first described in 1944 by Hench and Rosenburg [4], there has been little progress in terms of management guidance [3].

In this article, we suggest a practical 5 "S" (separate between diagnostic mimics, shared treatment decision-making, based on symptom burden, sonographic assessment of subclinical synovitis, and seropositivity and other patient-specific risk factors for RA progression) framework for managing patients presenting with chronic, episodic inflammatory arthritis that is suggestive of PR.

2 | Separate Between Diagnostic Mimics

From most descriptions, PR is diagnosed by recurrent episodes of sudden-onset monoarthritis (or less commonly, oligo/polyarthritis), one of which should be physician-witnessed, and ≥ 3 joints involved in the different attacks [5]. Importantly, other forms of episodic arthritis must be excluded, and radiographic bony erosions that suggest established inflammatory arthritis should not be present [5].

The main diagnostic differential for PR is crystal arthritis, typically in the form of either gout or calcium pyrophosphate deposition disorder (CPPD). Rarely, for older patients with predominant hands and feet swelling, remitting seronegative symmetrical synovitis with pitting edema (RS3PE) should be considered. Distinguishing between these conditions requires careful clinical assessment of symptom presentation, patient-specific risk factors, and adjunctive investigations (Table 1). In general, PR flares often present as acute inflammatory monoarthritis (and less commonly, oligo- or polyarthritis) [5], lasting from days to 2 weeks [1]. It most commonly affects metacarpophalangeal (MCP), proximal interphalangeal, wrist, and knee joints [1]. Unlike rheumatoid synovitis, patients with PR frequently have peri-articular tenosynovitis [1]. Gout is characterized by abrupt joint flares, often with podagra and lower limb predominance initially, with rapid-peaking pain within 1 day and improvement by 3–5 days [6, 7]. CPPD flares tend to have a more insidious onset with slower resolution over several weeks [6]. While the wrist and knee joints are most commonly implicated, other areas such as shoulders, elbows, MCP joints, hips, and ankles can also be affected [8–10]. RS3PE tends to be characterized by abrupt development of symmetrical, marked swelling of the hands and feet, with associated synovitis and flexor/extensor tenosynovitis [11].

In terms of disease epidemiology, the usual age of onset in PR is 30–50 years, with a female preponderance (2 times more common than in males) [12]. While gout occurs within a similar age

TABLE 1 | Differentiating between diagnostic mimics of Palindromic rheumatism.

	Palindromic Rheumatism	Gout	Calcium pyrophosphate deposition disease	RS3PE
Nature of joint pains	Acute onset; usually monoarthritis (less commonly, oligo/poly-arthritis), often with significant periarthritis (tenosynovitis)	Acute onset (often early morning/at night), rapidly peaking within 12–24 h; usually monoarthritis (can also manifest as oligo/poly- arthritis)	Less abrupt onset peaking over days; usually monoarthritis (can also manifest as oligoarthritis)	Acute onset; typically symmetrical synovitis and/or tenosynovitis
Location of joints involved	Metacarpophalangeal, proximal interphalangeal, wrist, and knee joints	Lower limb predominant—metatarsophalangeal joints (especially 1st), feet, ankle, and knee joints; can affect upper limb joints in long- standing disease	Most commonly, wrists and knees, but can also affect shoulders, elbows, metacarpophalangeal, hip, and ankle joints	Predominant feature of pitting edema of hands and feet; with synovitis of small joints of hands/ft, and may also affect wrists, elbows, ankles, and knees
Duration of symptoms	Days to 2 weeks; faster resolution with use of NSAIDs, corticosteroids	Usually 3–5 days, up to 2 weeks; faster resolution with use of colchicine, NSAIDs, corticosteroids	Can last up to several weeks; faster resolution with use of colchicine, NSAIDs, corticosteroids	May spontaneously remit over weeks to months, but is typically characterized by exquisite corticosteroid responsiveness (however, does not respond as well to NSAIDs)
Epidemiology	30–50 years More common in females	40–60 years More common in males; very rare in pre- menopausal females	> 60 years More common in females	> 60 years More common in males
Risk factors	Family history of rheumatoid arthritis	Dietary triggers (e.g., alcohol, seafood/shellfish, red meat, animal innards, fructose- containing beverages), renal impairment, drugs (e.g., pyrazinamide, ethambutol, thiazide diuretics, calcineurin inhibitors)	Underlying osteoarthritis, trauma/meniscal injury, metabolic risk factors (e.g., hyperparathyroidism, hemochromatosis, hypomagnesemia, hypophosphatasia, hypothyroidism)	Associated with malignancy (i.e., paraneoplastic phenomenon), underlying rheumatic condition
Laboratory tests	May have elevated ESR/CRP during flares; RF, anti- CCP may be positive	Elevated ESR/CRP during flares; elevated uric acid (although may be spuriously low/normal during an acute flare)	Elevated ESR/CRP during flares; consider secondary workup (e.g., calcium, parathyroid levels, iron panel, magnesium levels, alkaline phosphatase, thyroid function test) in young patients	May have elevated ESR/CRP; usually seronegative (RF, anti- CCP, ANA)

(Continues)

TABLE 1 | (Continued)

	Palindromic Rheumatism	Gout	Calcium pyrophosphate deposition disease	RS3PE
Imaging	Radiograph of affected joints should be normal	Dual energy CT scan may show uric acid crystal deposition in joints	Radiograph of wrists and knees may show radiographic chondrocalcinosis	Ultrasound or MRI typically reveals tenosynovitis of affected areas; rarely associated with radiographic joint erosions
Joint fluid analysis	Inflammatory yield with synovial white blood cell (WBC) > 2000/mm^3	Inflammatory yield with synovial WBC > 2000/mm^3; compensated polarized light microscopy may reveal negatively birefringent needle- shaped crystals in gout or positively birefringent rhomboid crystals in pseudogout		Not usually done

Abbreviations: ANA, antinuclear antibody; Anti- CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; CT, computed tomography; ESR, erythrocyte sedimentation rate; MRI, magnetic resonance imaging; NSAIDs, non-steroidal anti- inflammatory drugs; RF, rheumatoid factor; RS3PE, remitting seronegative symmetrical synovitis with peripheral edema.

range, it is more common in males (2–4 times more common than females in Europe and North America, 8 times more common than females in Asia), and is particularly unlikely to occur in pre- menopausal females (due to the uricosuric effect of es trogen) [7]. On the contrary, CPPD usually affects older adults above 60 years of age [8], with prevalence continuing to rise with increasing age, a phenomenon driven by its close association with degenerative osteoarthritis [9]. It is marginally more common in females [10]. RS3PE is typically seen in older adults (> 60 years of age) with significant male preponderance (2–5 times more common than in females) [11, 13].

The main risk factor for PR is family history of rheumatic diseases such as RA [2]. For gout, risk factors include classic dietary triggers (e.g., alcohol, seafood/shellfish, red meat), renal impairment, and culprit drugs (e.g., anti- tuberculous medica tions, thiazide diuretics) [6]. Risk factors for pseudogout include underlying osteoarthritis, prior joint trauma, and various metabolic conditions (e.g., hyperparathyroidism, hemochromatosis) [9, 10]. RS3PE is often a paraneoplastic phenomenon, associated with both hematological and solid malignancies in 31%–54% of cases [11, 13].

Arthrocentesis of an acutely inflamed joint with effusion is the most definitive way to diagnose crystal arthritis (i.e., demonstrating negatively birefringent, needle- shaped crystals in gout, and positively birefringent, rhomboid- shaped crystals in pseudogout). A review of radiographs of wrists/knees would be useful to look for radiological chondrocalcinosis at the triangular fibrocartilage complex or knee joints. Dual- energy computed tomography scan may be helpful to identify uric acid crystal deposition when a diagnostic joint tap is not possible. Of note, radiographs of affected joints in PR and RS3PE do not typically reveal bony erosions, while established chronic CPPD arthritis or gouty arthropathies may manifest with abnormal radiographs with erosive changes. Ultrasound scan may reveal double contour sign in gout [14], intracartilage calcifications in pseudogout, and flexor/extensor tenosynovitis in RS3PE [15].

3 | Shared Treatment Decision-Making Based on Symptom Burden, Sonographic Assessment of Subclinical Synovitis, and Seropositivity and Other Patient- Specific Risk Factors for Progression to RA

As treatment is not universally indicated in all patients with PR, given the heterogeneity of clinical outcomes, shared decision-making on clinical management of diagnosed cases of PR is necessary, based on three principles: assessment of symptom burden, presence of subclinical synovitis, and determining and managing the risk of progression to RA.

3.1 | Symptom Burden

It is known that severity and frequency of PR flares vary among patients [16]. For patients deemed to have low symptom burden (i.e., they do not find the symptoms to be bothersome), with minimal risk factors for progression to RA, it may be a reasonable to adopt a watchful waiting approach without pre- emptive

initiation of immunomodulatory treatments, provided they are adequately counseled on the risks of RA development.

3.2 | Sonographic Assessment for Subclinical Synovitis

Ultrasound is more sensitive than clinical examination in detecting inflammatory arthritis [17]. Sonographic abnormalities reported in rheumatoid synovitis and tenosynovitis include hypoechoic synovial hypertrophy and hypoechoic thickened tendon sheath respectively, with positive power doppler signals in areas of inflammation [17]. In 2022, Matteo and colleagues studied a cohort of anti- cyclic citrullinated peptide (anti-CCP) positive at- risk patients who initially had musculoskeletal symptoms with no overt clinical synovitis and eventually developed inflammatory arthritis [18]. The authors found that 77.3% of patients had US detected subclinical synovitis that preceded development of clinically overt inflammatory arthritis, which represents a window of opportunity to initiate early treatment to avert progressive joint disease [18]. Therefore, in places where ultrasound scans are readily available, it should be routinely performed during interval pain- free periods to determine whether there is an underlying persistent inflammatory arthritis that suggests established RA which would warrant methotrexate as part of standard first- line treatment strategy.

3.3 | Seropositivity and Other Risk Factors for Progression to RA

Assess the risk of progression based on disease and patient profile. In general, seropositive (RF and/or anti- CCP positivity) patients with arthralgias or PR are at increased risk of progression to RA [3, 16]. In fact, seropositive RA patients have a more aggressive disease course with higher risk of erosive disease and extra- articular manifestations. Given that approximately 50% of PR cases are seropositive [16], it may be reasonable to offer pre- emptive disease modifying anti-rheumatic drugs for this potentially high- risk group. Other than disease factors, there are other patient- specific risk factors that may portend higher susceptibility to the development of RA. This includes smoking and periodontal disease which are associated with citrullination and autoantibody production [19], as well as a positive family history (especially first- degree relatives) of RA that predisposes to genetic susceptibility to RA [20]. Clinical manifestations that could portend a higher risk of RA development include MCP joint involvement, early morning symptom predilection, or morning stiffness lasting > 60 min [20].

3.4 | Shared Decision-Making Regarding Immunomodulatory Treatment Initiation

Ultimately, the decision to initiate immunomodulatory treatment in PR remains a shared decision- making between patients and their physicians based on risk–benefit assessment, weighing the projected benefit of initiation of DMARDs (in reducing/preventing PR flares and/or delaying progression to RA) and the side effect profile for the given patient. For acute joint flares, non- steroidal anti-inflammatory drugs and corticosteroids are usual treatments, similar to RA [3]. Among the DMARD options, hydroxychloroquine (200–400 mg OD; avoid exceeding 5 mg/kg/day), an antimalarial and immunomodulatory agent, has the most robust evidence supporting its efficacy in reducing/preventing PR flares and potentially delaying progression to RA [16]. Other csDMARDs (methotrexate, sulfasalazine, leflunomide) and biological DMARDs (anti- CD20 (rituximab), IL-1 receptor inhibitor (anakinra, canakinumab)) [3, 21–23] have also been described in small cohort studies or case reports/series to be helpful in preventing joint flares. Most recently, a randomized, open- label trial showed promising results with the use of abatacept treatment for PR [24]. The study authors reported that 2- year treatment course with abatacept, as compared to hydroxychloroquine, led to significantly longer time to progression to RA, reduced intensity of joint flares, and higher rate of symptom remission [24].

Nevertheless, current evidence for the use of pre- emptive DMARDs in PR remains limited. Further studies, particularly randomized controlled trials, are needed to interrogate its efficacy in both symptom control and averting progression to RA. For all patients with diagnosed PR, regular clinic follow-up is required and modifiable risk factors for RA development should be addressed, including smoking cessation and dental treatment of periodontal disease.

4 | Conclusion

PR is a chronic, episodic inflammatory arthritis that can be functionally debilitating and portends significant risks of progression to RA. However, unlike RA, which has an established treatment window of opportunity for early DMARD initiation, there is no universally accepted management algorithm for PR due to its uncertain natural history. We herein describe a practical framework for clinicians managing suspected cases of PR based on excluding diagnostic mimics and basing immunomodulatory treatment decisions on the symptom, sonographic, and risk factor profile of patients.

Author Contributions

I.K.S.N. wrote the manuscript. J.T., A.J.L., and M.M. critically reviewed and edited 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 analysed during the current study.

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LETTER TO THE EDITOR

Pregnancy Outcome in a Patient With DADA2 Syndrome on Continued Adalimumab Therapy

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Dear Editor,

Deficiency of adenosine deaminase 2 (DADA2) is a recently described autoinflammatory disease, with limited published data on pregnancy outcomes. Herein, we report the pregnancy course and outcome of a patient with DADA2 syndrome on adalimumab.

A 25-year-old female was diagnosed with DADA2 syndrome when she presented to our center with fever, nodular skin lesions, polyarthritis, hearing impairment, elevated inflammatory markers, and an anterior inferior cerebellar artery pseudoaneurysm, which was treated with glue embolization. Her plasma ADA2 activity was low (2.4 U/L), and Sanger sequencing of the ADA2 gene revealed heterozygous mutations in exon 2 (p. Gly47Arg) and exon 4 (p. Leu188Val). Treatment with adalimumab resulted in significant improvement, except for residual hearing impairment. She was also diagnosed with systemic hypertension (a few months after disease onset), which was well controlled with telmisartan.

After a year of quiescent disease and normalization of inflammatory markers, she conceived without any assisted reproductive technique. Upon conception, aligning with the updated European Alliance of Associations for Rheumatology (EULAR) guidelines [1], adalimumab was continued. Telmisartan was discontinued, and she was transitioned to labetalol 100 mg twice daily, which initially led to adequate blood pressure control. However, at 31 + 6 weeks of gestation, rising blood pressure prompted escalation of labetalol to 200 mg three times daily. Despite dose escalation, she developed severe hypertension at 32 + 6 weeks of gestation, with blood pressure recordings ranging from 160–165/110–112 mmHg. Urine examination demonstrated proteinuria (urine protein 513 mg; urine creatinine 924 mg), and blood investigations revealed thrombocytopenia (platelet count 109×10^9/L) and elevated liver enzymes (AST 78 U/L, ALT 60 U/L), leading to the diagnosis of preeclampsia with severe features superimposed upon chronic hypertension. Fetal growth scan showed an estimated fetal weight of 1.5 kg, and antenatal doppler ultrasonography at 33 weeks demonstrated an umbilical artery S/D ratio of 3.3 (90th centile). However, absent or reversed end-diastolic flow was not observed.

Hypertension was controlled with intravenous anti-hypertensives, and dexamethasone injections were administered for fetal lung maturity. Adalimumab (40 mg subcutaneously every 2 weeks) was continued throughout pregnancy, with the last dose administered 13 days prior to delivery. A lower segment cesarean section was performed at 34 + 6 weeks of gestation in view of severe preeclampsia and history of aneurysmal embolization, and a live baby weighing 1.73 kg was delivered. No peripartum complications were observed. As the mother was receiving adalimumab, Bacille Calmette Guérin (BCG) and oral poliovirus vaccine (OPV) were not administered to the neonate at birth. Adalimumab therapy was continued in the mother during the postpartum period. The newborn's ADA2 activity, measured by spectrophotometric assay, was 8.0 U/L (reference range: 7.9–28 U/L). At follow-up, both the mother and infant remained clinically well, and the infant weighed 2.43 kg at 30 days of age.

Pregnancy in systemic vasculitis is generally considered high risk, with outcomes influenced by disease activity, severity of organ damage, and medications. Literature regarding pregnancy

outcomes in medium vessel vasculitis is scarce. While disease relapses are rare when pregnancy occurs during remission, preterm deliveries have been frequently reported, with rare instances of intrauterine deaths and pregnancy losses [2, 3]. Additionally, the development of preeclampsia in a patient with pre- existing vasculitis can severely impact the developing fetus, resulting in preterm delivery, intrauterine growth restriction, and stillbirths. In a single- center case series by Sara Beça et al. [3] evaluating maternal and fetal outcomes in 20 patients with primary systemic vasculitis, one patient with DADA2 syndrome was described. Notably, the patient did not receive anti- tumor necrosis factor therapy during either of her two pregnancies. She experienced an uneventful first pregnancy but developed pre-eclampsia at 40.5 weeks' gestation during her second pregnancy despite being in remission.

Our patient maintained good disease control with continued adalimumab throughout pregnancy. However, the fetus developed growth restriction, which could be attributable to pre-eclampsia superimposed upon chronic hypertension, although contributions from disease- related vascular or placental fac tors cannot be definitively excluded. Unfortunately, a placental histopathological examination was not performed in this case. Further case reporting and targeted research, including placental histopathological analyses, are needed to better delineate the effects of DADA2 on pregnancy and the associated maternal and fetal outcomes.

Author Contributions

Bhargavi Pridhivi: conceptualization, data curation, patient management, writing – original draft; GSRSNK Naidu: conceptualization, patient management, supervision; Prateek Bhatia: investigation (genetic analysis), writing – review and editing; Pui Y. Lee: investigation (ADA2 testing), writing – review and editing; Minakshi Rohilla: patient management, writing – review and editing, supervision; Aman Sharma: conceptualization, patient management, supervision, writing – review and editing. All authors reviewed and approved the final manuscript.

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Consent

Patient consented for publication. Written informed consent has been taken.

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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ORIGINAL ARTICLE

Association of Plasma miRNA-7-5p and miRNA-214-5p With Rheumatoid Arthritis Associated Interstitial Lung Disease

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ABSTRACT

Objective: Rheumatoid arthritis–associated interstitial lung disease (RA-ILD) is a major extra-articular complication contributing to increased morbidity and mortality. Circulating microRNAs (miRNAs) have emerged as potential non-invasive biomarkers in autoimmune diseases. This study aimed to evaluate whether plasma miRNA-7-5p and miRNA-214-5p are associated with the presence of RA-ILD and with radiological and functional indicators of pulmonary involvement.

Methods: In this cross-sectional study, 58 RA patients (29 RA-ILD, 29 without ILD) and 30 matched healthy controls were included. RA-ILD was diagnosed by multidisciplinary assessment (HRCT and pulmonary function tests). Plasma miRNA levels were measured by qRT-PCR and analyzed using the 2^−ΔΔCT method. Diagnostic performance was evaluated by ROC analysis.

Results: Plasma miRNA-7-5p and miRNA-214-5p levels were significantly higher in RA patients compared with healthy controls ($p < 0.05$ for both). Among RA patients, both miRNAs were significantly lower in those with ILD ($p < 0.05$). miRNA-7-5p demonstrated promising discriminatory performance for identifying RA-ILD (AUC = 0.87, 95% CI: 0.760–0.947; $p < 0.001$), whereas miRNA-214-5p showed modest accuracy (AUC = 0.676, 95% CI: 0.539–0.794; $p = 0.01$). In multivariable analysis, ILD presence was independently associated with decreased levels of both miRNAs. Neither miRNA correlated significantly with DAS28-CRP, disease duration, nor with radiological or functional severity parameters.

Conclusion: Circulating miRNA-7-5p and miRNA-214-5p are associated with the presence of RA-ILD but do not reflect disease severity in this cohort. Larger prospective studies are required to validate their potential role as adjunctive biomarkers for RA-ILD detection and risk stratification.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease primarily characterized by symmetric polyarthritis; however, extra-articular manifestations are common, and pulmonary involvement represents one of the most clinically significant complications. Among these, interstitial lung disease (ILD) substantially contributes to morbidity and mortality [1]. The reported prevalence of RA-associated ILD (RA-ILD) varies widely, ranging from 1.7% to 71.6%, largely reflecting differences in study populations and diagnostic methods [2]. Importantly, pulmonary involvement may remain clinically silent until advanced

Significance and Innovations

- Circulating miRNA-7-5p and miRNA-214-5p are downregulated in RA patients with ILD.
- miRNA- 7-5p shows good discriminatory ability for RA-ILD.
- These miRNAs are associated with disease presence but not pulmonary severity.

stages, limiting early diagnosis and timely intervention. Once clinically evident, RA-ILD is associated with functional decline and increased mortality [3, 4].

The pathogenesis of RA-ILD is incompletely understood and likely involves a complex interplay of autoimmune mechanisms, dysregulated inflammatory responses, fibrotic pathways, and genetic susceptibility [5, 6]. Diagnosis relies on multidisciplinary evaluation integrating clinical assessment, high-resolution computed tomography (HRCT), and pulmonary function tests (PFTs) [7]. Despite advances in imaging and functional evaluation, reliable non-invasive biomarkers for early detection and risk stratification remain limited. Identifying circulating molecular markers that reflect pulmonary involvement in RA is therefore an unmet clinical need [8].

In recent years, microRNAs (miRNAs), small non-coding RNA molecules that regulate gene expression at the post-transcriptional level, have emerged as important epigenetic regulators and potential biomarkers in autoimmune diseases. Aberrant miRNA expression has been shown to contribute to immune dysregulation by promoting excessive autoantibody production and increased inflammatory cytokine release [9–11]. Through their ability to fine-tune immune and fibrotic signaling pathways, miRNAs are involved in key biological processes such as T-cell activation, fibroblast proliferation, and cytokine regulation, mechanisms that are central to the pathogenesis of both RA and ILD [12].

Over the past decade, increasing evidence has highlighted the role of epigenetic mechanisms, including miRNAs, in the development and progression of autoimmune disorders [13–15]. In addition to circulating miRNAs, exosomes have been recognized as an important reservoir of miRNAs, protecting them from RNase-mediated degradation and thereby enhancing their stability and detectability in biological fluids [16]. Consequently, exosomal miRNAs have attracted considerable interest as potential biomarkers for the early detection of various diseases [17, 18].

Several studies have demonstrated altered miRNA expression profiles in RA, linking specific miRNAs to disease activity and immune dysregulation [19–21]. However, data regarding circulating miRNA profiles in RA-ILD remain limited. Oka et al. first reported elevated plasma levels of miRNA-7-5p and miRNA-214-5p in RA patients with ILD compared to those without ILD [22]. Nevertheless, the discriminatory performance of these miRNAs and their relationship with radiological and functional severity indices has not been comprehensively evaluated.

We hypothesized that circulating miRNA-7-5p and miRNA-214-5p levels would be related to RA-ILD and might correlate with validated radiological and functional markers of pulmonary involvement. Therefore, the present study aimed to investigate the association between plasma miRNA-7-5p and miRNA-214-5p expression levels and both the presence and severity of RA-ILD. By integrating molecular data with HRCT findings and pulmonary function parameters, we sought to determine whether these circulating miRNAs may serve as clinically relevant biomarkers for RA-ILD identification and risk stratification.

2 | Materials and Methods

2.1 | Patient Selection

A cross-sectional study design was employed from February to November 2024 at a tertiary-level rheumatology referral center. The cohort comprised 58 patients with RA and 30 age- and sex-matched healthy volunteers. RA was classified based on the 2010 ACR/EULAR criteria [23]. Patients were excluded if they had a history of malignancy, active infection, other autoimmune diseases, chronic pulmonary diseases unrelated to RA, or significant metabolic disorders. RA patients were classified into two groups based on the presence of ILD: RA-ILD ($n = 29$) and RA without ILD ($n = 29$). The diagnosis of RA-ILD was established through multidisciplinary evaluation involving a rheumatologist and a pulmonologist, based on clinical findings, HRCT, and PFTs. Clinical and demographic information was systematically recorded using a standardized data collection template. Variables of interest included age, sex, disease duration, articular involvement, and extra-articular manifestations. Disease activity was assessed using the Disease Activity Score in 28 joints with C-reactive protein (DAS28-CRP) [24], and laboratory markers including erythrocyte sedimentation rate (ESR) and CRP were recorded as indicators of systemic inflammation.

2.2 | Clinical and Radiological Evaluation of ILD

HRCT scans were evaluated using the Warrick scoring system by a pulmonologist who was blinded to clinical and laboratory data. The total Warrick score ranges from 0 to 30, with higher scores indicating more extensive radiological involvement [25]. PFTs were performed according to American Thoracic Society (ATS) and European Respiratory Society (ERS) standards [26]. Forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DLCO) were used for functional assessment. Functional submaximal exercise capacity was evaluated using the 6-min walk test (6MWT), conducted according to ATS guidelines [27].

2.3 | RNA Extraction

Plasma samples were collected from 58 patients with RA and from 30 healthy controls. Peripheral venous blood was drawn into ethylenediaminetetraacetic acid (EDTA)-containing tubes, and plasma was separated by centrifugation and stored at −80°C until analysis. Expression levels of miRNA-7-5p and

miRNA-214-5p were subsequently analyzed in individual plasma samples. Total RNA was extracted using a conventional guanidine isothiocyanate/phenol–chloroform isolation method with minor modifications [28]. To enhance the recovery of small RNAs, the TRIzol-based extraction protocol was optimized by incorporating additional ethanol precipitation and extended isopropanol incubation, in accordance with standard approaches for miRNA isolation [29]. Briefly, EDTA-anticoagulated samples were mixed with TRIzol reagent (Invitrogen, USA), vortexed, and incubated on ice for 15 min. Chloroform–isoamyl alcohol (24:1) was then added, followed by centrifugation at 14 000 rpm for 10 min at 4°C to achieve phase separation. The aqueous phase containing RNA was transferred to a new tube, and RNA was precipitated with isopropanol, washed with 80% ethanol, air-dried, and dissolved in nuclease-free water. Purified RNA samples were stored at −20°C until further analysis.

2.4 | miRNA Quantification and cDNA Synthesis

RNA concentration and purity were assessed spectrophotometrically using a NanoDrop instrument (Thermo Fisher Scientific, USA). Samples with an OD260/280 ratio between 1.8 and 2.0 were considered acceptable and used for subsequent analyses. Reverse transcription was performed in a reaction mixture containing 5 μL of extracted total RNA, 0.2 μL of stem-loop RT primer (Table 1), 3 μL of 5× RT buffer (Fermentas, Lithuania), 1.5 μL of dNTP mix (10 mM; BioBasic, USA), 0.25 μL of RevertAid Reverse Transcriptase (200 U/μL; Fermentas, Lithuania), 0.1 μL of Ribolock RNase Inhibitor (40 U/μL; Fermentas, Lithuania), and 5 μL of nuclease-free water. Reverse transcription reactions were carried out on an ABI Prism 7500 Real-Time PCR System (Applied Biosystems, USA) using the following thermal protocol: 16°C for 30 min, 42°C for 30 min, 85°C for 5 min, and a final hold at 4°C.

2.5 | Quantitative Real-Time PCR

Relative miRNA expression levels were quantified using the comparative CT ($2^{-\Delta\Delta CT}$) method with SDS Software v2.0.6 on an ABI Prism 7500 Real-Time PCR System (Applied Biosystems, USA). The endogenous control was hsa-miR-26b-5p, and TaqMan Control Human RNA (50 ng/μL; Applied Biosystems, USA) was used as a reference RNA sample. Specific primers and TaqMan probes for hsa-miR-7-5p, hsa-miR-214-5p, and hsa-miR-26b-5p were obtained from Metabion International AG (Martinsried, Germany). Each PCR reaction was performed in a final volume of 25 μL containing 5 μL of cDNA, 12.5 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems, Cat. No. 4369016), 1 μL of each primer (forward and universal reverse), 0.5 μL of the TaqMan probe, and nuclease-free water to volume. Thermal cycling conditions consisted of an initial incubation at 50°C for 2 min and 95°C for 10 min, followed by 50 amplification cycles at 95°C for 15 s and 60°C for 90 s. All reactions were performed in triplicate.

2.6 | Ethical Statement

The study protocol was reviewed and approved by the Local Ethics Committee of Manisa Celal Bayar University (approval date: 14 February 2024; approval number: 20.478.486/2263). All participants provided written informed consent prior to

TABLE 1 | Sequences of MIRNA primers and probes utilized in the study.

miRNAs	Gene ID[a]		Primer/probe sequence[b]
hsa-miR-214-5p	406 996	RT	5′-GTCGTATGCAGTGCAGGGTCCGAGGTATT CGCACTGCATACGACGCACAGCA-3′
		F	5′-GCCGCTGCCTGTCTACACT-3′
		PR	5′-FAM-TG(pdC)ATA(pdC)GA(pdC) GCACAGCA-BHQ-1-3′
hsa-miR-26b-5p[a]		RT	5′-GTCGTATGCAGTGCAGGGTCCGAGGTATT CGCACTGCATACGACACCTATCC-3′
		F	5′-GCCGCTTCAAGTAATTCAGG-3′
		PR	PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)ACCTATCC-ZNA4-BHQ-1-3′
hsa-miR-7-5p		RT	5′-GTCGTATGCAGTGCAGGGTCCGAGGTATT CGCACTGCATACGACAACAACAA-3′
		F	5′-GCCGCTGGAAGACTAGTGATT-3′
		PR	5′-FAM-TGCATACGACAACAACAA-MGB-NFQ-3′
hsa-miR-26b-5p[a]		RT	5′-GTCGTATGCAGTGCAGGGTCCGAGGTATT CGCACTGCATACGACACCTATCC-3′
		F	5′-GCCGCTTCAAGTAATTCAGG-3′
		PR	5′-FAM-TGCATACGACACCTATCC-MGB-NFQ-3′
		Universal R	5′-GTGCAGGGTCCGAGGTAT-3′

Note: Universal R (900 nmol), HPLC 1 μmol; Primers, HPLC 0.2 μmol; Probes (RP- HPLC purification and ESI-ToF analysis were done.) 05–10nmol. F:Forward (900 nmol), PR:Probe (200 nmol).

[a]RT: RT Primers (http://www.ncbi.nlm.nih.gov/gene); Primers and probes for miRNAs were designed by Prof. Dr. M.E.E. (https://orcid.org/0000-0002-6191-2930) using the Primer Express 3.0 (Applied Biosystems) program. hsa- miR-26b-5p was evaluated using two different probe designs (ZNA4-BHQ-1 and MGB-NFQ); therefore, separate entries are shown.

[b]hsa-miR-26b-5p was evaluated using two different probe designs (ZNA4-BHQ-1 and MGB-NFQ).

participation. The study was carried out in compliance with the ethical principles outlined in the Declaration of Helsinki and its later revisions, as well as applicable national and institutional research ethics regulations.

2.7 | Statistical Analysis

Statistical analyses were performed using Jamovi software (version 2.6.25). The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Continuous variables were expressed as mean ± standard deviation or median with interquartile range (IQR), as appropriate, while categorical variables were presented as frequencies and percentages. For univariate analyses, logarithmic transformation of raw miRNA expression values was explored because of the wide range and marked right-skewness of the data. Descriptive statistics, however, were presented on the original scale for clinical interpretability. Continuous variables were analyzed according to distribution characteristics, using either the Student's *t*-test or the Mann–Whitney *U* test for comparisons between independent groups. Relationships between categorical variables were examined with Pearson's chi-square test. Correlation analyses for continuous parameters were performed using Spearman's rank correlation coefficient. miRNA expression level was treated as a continuous dependent variable. Since miRNA expression values ($2^{-\Delta\Delta Ct}$) were positive continuous variables with a markedly right-skewed distribution that persisted despite logarithmic transformation, multivariable associations were evaluated using generalized linear models (GLM) with gamma distribution and log link. This approach was preferred over standard linear regression because it is more appropriate for positively skewed continuous outcomes, preserves the original measurement scale, ensures positive fitted values, and allows interpretation of effects on a relative scale through exponentiated coefficients. Age, sex, pulmonary involvement, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) status, smoking and disease duration were included as covariates. GLM results were expressed as regression coefficients and its standard errors (SE), exponentiated coefficients Exp(β), 95% confidence intervals (CI), and *p* values. The discriminative ability of miRNA expression levels for identifying RA-ILD was assessed through receiver operating characteristic (ROC) curve analysis. Diagnostic accuracy was quantified by calculating the area under the curve (AUC) along with 95% CI, sensitivity, specificity, optimal threshold values, and the corresponding Youden index. Comparisons between AUCs were carried out using the DeLong method. Statistical significance was defined as a two-tailed p value < 0.05. For univariate analyses, *p* values were additionally adjusted for multiple testing within each table using the Benjamini–Hochberg false discovery rate procedure.

3 | Results

3.1 | Baseline Characteristics

The study population consisted of 58 individuals with RA and 30 healthy controls. The two groups were comparable with respect to age and sex distribution. The mean age was 60.2 ± 9.88 years in the RA group and 58.1 ± 8.05 years among controls ($p = 0.317$). Women represented 77.6% of patients with RA and 76.7% of controls ($p = 0.922$). Within the RA cohort, no significant differences were observed between patients with and without ILD in terms of age, body mass index, disease duration, DAS28-CRP, sex distribution, smoking status, RF positivity, or ACPA positivity after adjustment for multiple comparisons (all adjusted $p > 0.05$) (Table 2).

3.2 | miRNA Expression Levels in RA Patients and Healthy Controls

Plasma miRNA-7-5p expression could not be quantified in one healthy control and in one RA patient without ILD, while miRNA-214-5p expression could not be quantified in one RA-ILD patient; all remaining samples were successfully analyzed. Plasma miRNA-7-5p and miRNA-214-5p expression levels were significantly higher in RA patients compared with healthy controls (both $p < 0.05$) (Table 3). Furthermore, expression levels of both miRNA-7-5p and miRNA-214-5p were significantly higher in RA patients without ILD compared to those with ILD ($p < 0.05$ for both) (Table 3). The distribution and overlap of circulating miRNA-7-5p and miRNA-214-5p levels across study groups are additionally illustrated in Figure 1 using boxplots with individual data points. ROC curve analysis was performed to evaluate the discriminatory ability of miRNA expression levels in distinguishing RA patients with and without ILD. For miRNA-7-5p, the AUC was 0.87 (95% CI: 0.760–0.947; sensitivity 0.69; specificity 1.00; Youden index 0.69; cut-off ≤ 0.58), suggesting good discrimination ability ($p < 0.001$). For miRNA-214-5p, the AUC was 0.676 (95% CI: 0.539–0.794; sensitivity 1.00; specificity 0.37; Youden index 0.379; cut-off ≤ 0.017), indicating lower discriminatory ability ($p = 0.01$) (Figure 2). Comparison of AUC values using the DeLong test demonstrated that miRNA-7-5p had significantly better discriminatory performance than miRNA-214-5p ($p = 0.01$).

3.3 | Relationship Between Clinical Characteristics and miRNA Expression Levels

No significant correlations were observed between miRNA-7-5p or miRNA-214-5p expression levels and DAS28-CRP or disease duration ($p > 0.05$). Likewise, miRNA expression levels did not differ significantly according to RF or ACPA positivity ($p > 0.05$). In patients with RA-ILD, neither miRNA-7-5p nor miRNA-214-5p expression levels were significantly correlated with Warrick total score, DLCO%, FVC%, or 6MWT results ($p > 0.05$ for all) (Table 4).

Multivariable GLM analysis with gamma distribution and log link was performed using miRNA expression values as dependent variables. ILD presence was independently associated with lower levels of both miRNA-7-5p ($\beta = -1.669$, exp(β) = 0.189, 95% CI: 0.099–0.360; $p < 0.001$) and miRNA-214-5p ($\beta = -2.125$, exp(β) = 1.055, 95% CI: 1.009–1.103; $p < 0.001$) after adjustment for age, sex, disease activity, serological status, smoking, and disease duration. In addition, disease duration was inversely associated with miRNA-7-5p ($\beta = -0{,}004$; exp(β) = 0.996, 95% CI: 0.992–0.999; $p = 0.005$), whereas age showed a positive association with miRNA-214-5p (exp(β) = 1.055, 95% CI: 1.009–1.103; $p = 0.019$) (Table 5).

TABLE 2 | Clinical characteristics of RA patients.

Variable	RA with ILD ($n = 29$)	RA without ILD ($n = 29$)	Adjusted p
Continuous variables			
Age (years)	62.6 ± 8.5	57.8 ± 10.7	0.248
BMI (kg/m^2)	27.6 ± 4.8	27.4 ± 4.5	0.889
DAS28-CRP	3.1 ± 1.5	4.2 ± 1.5	0.064
Disease duration (months), median (IQR)	84.0 (162.0)	120.0 (126.0)	0.760
Warrick total score, median (IQR)	10.0 (11.5)	—	—
FVC (% predicted)	84.6 ± 17.3	—	—
DLCO (% predicted)	51.3 ± 16.9	—	—
6- min walk test (m)	340.7 ± 107.1	—	—
Categorical variables			
Sex, *n* (%)			
Male	7 (24.1)	6 (20.7)	0.860
Female	22 (75.9)	23 (79.3)	
Cigarette use, *n* (%)			
No	20 (69.0)	22 (75.9)	0.760
Yes	9 (31.0)	7 (24.1)	
Rheumatoid factor (RF), *n* (%)			
Negative	10 (34.5)	8 (27.6)	0.760
Positive	19 (65.5)	21 (72.4)	
ACPA, *n* (%)			
Negative	13 (44.8)	10 (34.5)	0.760
Positive	16 (55.2)	19 (65.5)	
Medication use, n (%)			
Methotrexate			
No	23 (79.3)	15 (51.7)	0.186
Yes	6 (20.7)	14 (48.3)	
Leflunomide			
No	19 (65.5)	23 (79.3)	0.631
Yes	10 (34.5)	6 (20.7)	
Hydroxychloroquine			
No	22 (75.9)	19 (65.5)	0.684
Yes	7 (24.1)	10 (34.5)	
Sulfasalazine			
No	23 (79.3)	25 (86.2)	0.798
Yes	6 (20.7)	4 (13.8)	
Corticosteroids			
No	7 (24.1)	16 (55.2)	0.186
Yes	22 (75.9)	13 (44.8)	

(Continues)

TABLE 2 | (Continued)

Variable	RA with ILD ($n=29$)	RA without ILD ($n=29$)	Adjusted p
TNF inhibitor			
No	28 (96.6)	26 (89.7)	0.687
Yes	1 (3.4)	3 (10.3)	
JAK inhibitor			
No	29 (100.0)	24 (82.8)	0.186
Yes	0 (0.0)	5 (17.2)	
Rituximab			
No	25 (86.2)	29 (100.0)	0.336
Yes	4 (13.8)	0 (0.0)	
Mycophenolate mofetil			
No	25 (86.2)	29 (100.0)	0.336
Yes	4 (13.8)	0 (0.0)	
Azathioprine			
No	26 (89.7)	29 (100.0)	0.533
Yes	3 (10.3)	0 (0.0)	

Note: Age, BMI and DAS28- CRP values were given as mean± standard deviation, and disease duration value was given as median (IQR). Medication use is presented as n (%). p values were adjusted for multiple comparisons using the Benjamini- Hochberg false discovery rate procedure.
Abbreviations: 6MWT, six- minute walk test; ACPA, anticitrullinated protein antibody; BMI, body mass index; DAS-28-CRP, disease activity score-28-C reactive protein; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; JAK, Janus kinase; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumor necrosis factor.

TABLE 3 | Comparison of circulating miRNA levels across study groups.

	Healthy controls	RA patients		RA without ILD	RA with ILD	
	$n=30$	$n=57$	p*	$n=28$	$n=29$	p**
miRNA-214-5p	0.00268 (0.00434)	0.00410 (0.01187)	0.0115	0.0051 (0.0578)	0.003 (0.0061)	0.022
miRNA-7-5p	0.26082 (0.40418)	1.31938 (3.94006)	<0.001	4.0282 (5.671)	0.4113 (1.0757)	<0.001

Note: Data are presented as median (IQR).
Abbreviations: ILD, interstitial lung disease; RA, rheumatoid arthritis.
*Healthy controls versus overall RA.
**RA without ILD versus RA with ILD.

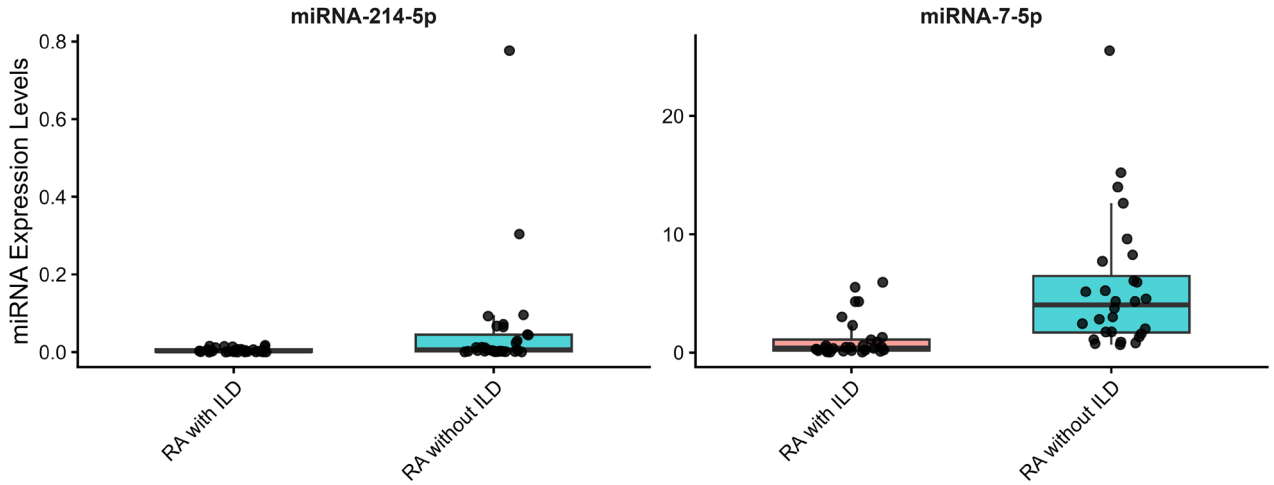

FIGURE 1 | Distribution of circulating miRNA- 7-5p and miRNA-214-5p levels in healthy controls, RA patients without ILD, and RA-ILD pa tients. Boxplots represent median and interquartile ranges, and individual data points are displayed as jittered scatter plots.

4 | Discussion

In this study, we investigated the association of circulating miRNA-7-5p and miRNA-214-5p with RA-ILD. Plasma levels of both miRNAs were significantly higher in patients with RA compared with healthy controls and were downregulated in those with ILD. These findings suggest a relationship between these circulating miRNAs and pulmonary involvement in RA.

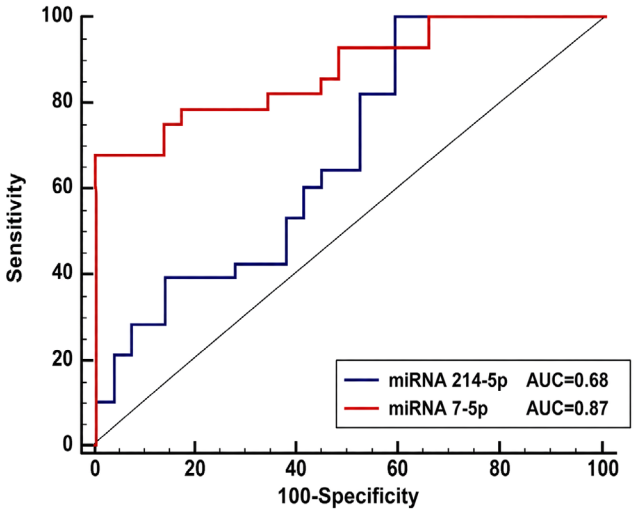

FIGURE 2 | Evaluation of plasma microRNAs for the diagnosis of RA- ILD patients. ROC was used to analyze the diagnostic value of plasma miRNA 7- 5p and plasma miRNA 214-5p in RA patients with ILD from RA patients without ILD.

Moreover, the observed downregulation may be related to underlying immune and inflammatory processes implicated in RA-ILD pathogenesis.

Despite their association with ILD presence, neither miRNA correlated with radiological severity (Warrick score) nor with functional indices such as FVC%, DLCO%, or 6MWT performance. This suggests that circulating miRNA expression may be associated with the presence of RA-ILD rather than the extent of radiological or functional impairment. An alternative interpretation of our findings is that circulating miRNA levels may be elevated in RA itself and subsequently decline in patients who develop ILD. In our cohort, both miRNAs were markedly increased in RA patients without ILD compared with healthy controls, whereas RA-ILD patients exhibited lower circulating miRNA levels than RA patients without ILD and levels that were numerically closer to those observed in healthy controls. Although the biological basis of this pattern remains unclear, it may reflect distinct regulatory mechanisms associated with pulmonary involvement. Further longitudinal studies are needed to clarify the temporal dynamics of these miRNAs during RA-ILD development.

Recent evidence increasingly supports the role of circulating miRNAs as clinically relevant biomarkers in RA. Exosomal miRNA profiling has demonstrated excellent diagnostic performance for RA, particularly in ACPA-negative individuals, and has shown dynamic modulation during methotrexate therapy, underscoring their value in both diagnosis and therapeutic monitoring [30]. Similarly, circulating miRNAs such as miR-146a, miR-155, miR-21, and miR-223 have been consistently correlated with DAS28, ESR, and CRP, indicating that certain miRNAs primarily reflect systemic inflammatory burden and synovial disease activity.

TABLE 4 | Association between clinical and pulmonary variables and circulating miRNA levels in RA patients.

	n	miRNA-214-5p	*p*	Adj-*p*	*n*	miRNA-7-5p	*p*	Adj-*p*
Clinical variable								
RF								
Positive	40	0.004 (0.011)	0.422	0.628	39	1.319 (3.910)	0.945	0.945
Negative	17	0.004 (0.014)			18	1.418 (3.779)		
ACPA								
Positive	34	0.005 (0.012)	0.454	0.628	34	1.441 (3.875)	0.515	0.628
Negative	23	0.003 (0.012)			23	1.319 (4.670)		
DAS28-CRP	57	rho = 0.120	0.373	0.628	57	rho = 0.203	0.126	0.504
Disease duration	57	rho = 0.228	0.088	0.504	57	rho = −0.080	0.550	0.628
Pulmonary variables (RA- ILD patients only)								
FVC	28	rho = 0.141	0.473	0.621	29	rho = 0.313	0.098	0.621
DLCO	28	rho = 0.124	0.530	0.621	29	rho = 0.151	0.433	0.621
6MWT	28	rho = 0.064	0.747	0.747	29	rho = −0.118	0.543	0.621
Warrick total score	28	rho = −0.139	0.482	0.621	29	rho = −0.125	0.517	0.621

Note: Data are presented as median (IQR) for categorical variables. Correlations with continuous variables were assessed using Spearman's rank correlation analysis. rho; Spearman's correlation coefficient. Adj-*p*: Adjusted *p* value based on the Benjamini–Hochberg false discovery rate.
Abbreviations: 6MWT, six- minute walk test; ACPA, anti-citrullinated protein antibody; DAS28-CRP, Disease Activity Score-28 using C-reactive protein; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; RF, rheumatoid factor.

TABLE 5 | Clinical factors associated with miRNA- 7-5p and miRNA-214-5p levels in RA patients.

	β (SE)	p	Exp(β)	%95 GA (exp(β))
miRNA-7-5p				
Age	0.004 (0.016)	0.788	1.004	0.973–1.037
Sex (Male)	−0.239 (0.478)	0.618	0.788	0.308–2.012
ILD presence	−1.669 (0.330)	<0.001	0.189	0.099–0.360
DAS28-CRP	−0.099 (0.098)	0.316	0.906	0.746–1.099
RF positivity	0.091 (0.343)	0.791	1.095	0.562–2.134
ACPA positivity	0.091 (0.340)	0.790	1.095	0.559–2.146
Smoking	0.042 (0.429)	0.921	1.043	0.450–2.419
Disease duration	−0.004 (0.001)	0.005	0.996	0.992–0.999
Intercept	2.370 (1.239)	0.056		
miRNA-214-5p				
Age	0.053 (0.022)	0.019	1.055	1.009–1.103
Sex (Male)	−1.112 (0.572)	0.052	0.329	0.107–1.009
ILD presence	−2.125 (0.388)	<0.001	0.119	0.056–0.256
DAS28-CRP	−0.013 (0.123)	0.917	0.987	0.775–1.257
RF positivity	−0.765 (0.484)	0.115	0.466	0.180–1.203
ACPA positivity	0.469 (0.519)	0.366	1.598	0.578–4.421
Smoking	−0.042 (0.521)	0.936	0.959	0.345–2.662
Disease duration	0.001 (0.002)	0.604	1.001	0.997–1.006
Intercept	−5.451 (1.610)	<0.001		

Note: β: regression coefficient on the log scale; se: standard error; Exp(β), exponential regression coefficient, indicating the proportional change in miRNA level associated with a one- unit increase in the relevant variable. Exp(β) > 1 indicates an increase, whereas Exp(β) < 1 indicates a decrease.
Abbreviation: 95% CI, 95% confidence interval.

In contrast, the present findings suggest a distinct molecular signature associated specifically with pulmonary involvement. Unlike inflammation-linked miRNAs, miRNA-7-5p and miRNA-214-5p were not correlated with DAS28-CRP or traditional inflammatory markers but were significantly associated with ILD. This divergence supports the concept that different circulating miRNA profiles may correspond to distinct clinical phenotypes of RA, separating articular inflammatory activity from extra-articular organ involvement.

Consistent with the findings reported by Oka et al. [22] we did not observe significant associations between circulating miRNA levels and conventional RA-related clinical variables such as DAS28-CRP, serological status, or sex. This consistency suggests that miRNA-7-5p and miRNA-214-5p may be independent of standard measures of systemic inflammatory activity in RA. In contrast to the same study where miR-7-5p and miR-214-5p showed a tendency toward increased expression in RA-ILD patients, we observed significantly lower levels of both miRNAs in the RA-ILD group compared to RA patients without ILD. It should be noted that the previously reported increase was not strong and was accompanied by modest diagnostic performance (AUC values of 0.63 and 0.65), suggesting a relatively weak discriminatory signal. Several factors may contribute to these divergent findings, including differences in cohort characteristics, treatment exposure, and disease phenotypes. In addition, circulating miRNA expression may be influenced by underlying disease heterogeneity and clinical context. Therefore, the opposite direction of expression observed between studies may not necessarily represent a true contradiction but rather reflect differences between patient populations. Further studies in larger independent cohorts are needed to clarify the biological significance of these findings.

Interestingly, Yu et al. [31] demonstrated a similar pattern in patients with idiopathic inflammatory myopathies (IIM). In that study, circulating miR-7 levels were significantly lower in patients with ILD compared to those without ILD and demonstrated strong discriminatory performance, with an AUC of 0.8978 and high sensitivity and specificity for distinguishing IIM patients with and without ILD. Taken together, the consistency between our findings and the IIM study, particularly regarding decreased miR-7 levels in ILD, supports the hypothesis that reduced circulating miR-7 expression may be associated with pulmonary involvement across different autoimmune disease contexts. However, the differences in diagnostic performance across studies further suggest that the magnitude and clinical utility of this signal may vary depending on disease type, cohort characteristics, and methodological factors. Therefore, circulating miR-7 alterations should be interpreted as context dependent rather than universally applicable biomarkers.

Seropositivity for RF and ACPA has been reported as a risk factor for RA-ILD [32]. A recent systematic review and meta-analysis further reported a pooled RA-ILD prevalence of approximately 18%–19% and identified several established risk factors, including male sex, older age, smoking history, RF and ACPA positivity, elevated ESR, and higher DAS28 scores. These findings underscore the importance of clinical and serological determinants in RA-ILD risk stratification and reflect the current reliance on conventional epidemiological approaches [2]. However, in the present study, no significant differences in RF or ACPA titers were observed between patients with and without ILD. This finding may be partly explained by the relatively small sample size. Furthermore, no significant associations were found between miRNA-7-5p or miRNA-214-5p expression levels and RF or ACPA, suggesting that altered expression of these miRNAs may occur independently of autoantibody production during the course of RA.

Although DAS28-CRP values were numerically lower in RA-ILD patients, this difference did not remain statistically significant after adjustment for multiple comparisons. The small sample size may also have reduced the power to detect subtle associations between disease activity and ILD. While established risk factors remain essential for clinical screening, molecular biomarkers capable of capturing disease-specific pathobiology remain limited. In this context, the present study contributes to an unmet need by evaluating circulating miRNA-7-5p and miRNA-214-5p as potential molecular indicators of pulmonary involvement in RA, potentially complementing established clinical risk factors.

In multivariable analysis, ILD presence remained independently associated with decreased levels of both miRNA-7-5p and miRNA-214-5p after adjustment for age, sex, disease activity, serological status, smoking, and disease duration. This finding suggests that the relationship between these miRNAs and RA-ILD is not fully explained by demographic or clinical factors.

Recent systematic reviews focusing on RA-ILD biomarkers have demonstrated elevated levels of inflammatory and fibrotic protein markers—including CRP, ESR, RF, ACPA, KL-6, SP-D, and MMP-7—in RA-ILD patients, with KL-6 and MMP-7 showing significant associations with pulmonary function impairment and adverse prognosis [33]. While these protein-based markers largely reflect downstream inflammatory or fibrotic processes, circulating miRNAs may act at an upstream regulatory level, modulating key signaling pathways such as NF-κB, MAPK, mTOR, and TGF-β–mediated fibrosis. Transcriptomic analyses comparing RA-ILD and RA without ILD have further identified enrichment of immune and inflammatory pathways, including NF-κB signaling and chemokine networks such as CXCL8, suggesting that RA-ILD represents a molecularly distinct phenotype rather than merely a complication of systemic inflammation [34]. Within this framework, miRNA-7-5p and miRNA-214-5p may function as regulatory nodes influencing transcriptional networks that link immune activation with pulmonary tissue remodeling.

Experimental studies provide a potential biological framework for the observed reduction of miRNA-7-5p in RA-ILD. miR-7-5p has been shown to directly target RelA, a key component of the NF-κB signaling pathway, thereby suppressing NF-κB activity and downstream inflammatory mediators such as IL-1β, IL-6, and IL-8 [35]. In addition, Oka et al. [22] identified several putative target genes for miRNA-7-5p and miRNA-214-5p through bioinformatic analyses, including SMAD4, a central mediator of TGF-β signaling implicated in pulmonary fibrosis. These observations provide a potential biological context for the altered miRNA expression observed in RA-ILD. However, the present study did not evaluate downstream target genes or signaling pathways, and these potential mechanisms remain speculative.

Prospective cytokine-based studies have also demonstrated elevated IL-18, MCP-1/CCL2, and SDF-1α levels in RA-ILD, with IL-18 independently associated with disease progression [36]. In addition, large multicenter biomarker clustering analyses have shown that peripheral biomarker signatures significantly improve RA-ILD discrimination beyond traditional clinical risk factors alone [37]. Together, these findings underscore the multifactorial and multilayered nature of RA-ILD pathobiology and support the hypothesis that circulating miRNAs may represent an additional regulatory layer within this complex biomarker network.

MicroRNAs have emerged as key post-transcriptional regulators implicated in fibrotic and inflammatory pathways underlying ILD. Accordingly, growing evidence has focused on circulating miRNA profiles in connective tissue disease–related ILD (CTD-ILD) [38]. In this context, Jiang et al. [39] examined miR-200c expression across different CTD-ILD subsets, including systemic sclerosis, IIM, primary Sjögren's syndrome, and RA. Their study showed that miR-200c levels were significantly elevated in patients with CTD-ILD compared to CTD patients without pulmonary involvement, and higher expression was associated with more severe lung disease. Nevertheless, miR-200c did not demonstrate disease-specific discriminatory capacity, as its circulating levels overlapped among different CTD-ILD entities, including RA-ILD.

Most previous studies in RA have focused on the diagnostic and treatment-response predictive value of miRNAs [10, 40–43]. In contrast, the present study evaluated circulating miRNAs in relation not only to the presence of RA and RA-ILD but also to radiological and functional measures of lung involvement, which represent a key strength of our study.

Certain limitations inherent to the study design warrant careful consideration. First, the relatively small sample size and single-center, cross-sectional design may limit the generalizability and robustness of the findings. Second, longitudinal changes in miRNA expression were not evaluated; therefore, no conclusions can be drawn regarding their prognostic value or their relationship with the progression of pulmonary involvement over time. In addition, the ROC analysis was performed in a relatively small cohort and without an independent validation cohort. Therefore, the observed discriminatory performance and high specificity of miRNA-7-5p should be interpreted with caution and considered exploratory until confirmed in larger independent studies.

5 | Conclusion

Despite these limitations, our findings suggest that plasma hsa-miR-214-5p and hsa-miR-7-5p may serve as potential biomarkers for identifying RA-ILD. However, neither miRNA reliably reflected radiological or functional severity of pulmonary involvement in the present cohort. Larger, prospective studies with

longitudinal follow-up are needed to validate these results and to clarify the potential role of these circulating miRNAs in early detection, risk stratification, and disease monitoring.

Author Contributions

All authors contributed to the study conception and design, data collection, analysis, and manuscript preparation. All authors read and approved the final manuscript.

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The authors have nothing to report.

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Ethics Statement

The study protocol was approved by the Local Ethics Committee of Manisa Celal Bayar University (approval date: 14 February 2024; approval number: 20.478.486/2263).

Consent

All participants provided written informed consent prior to participation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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ORIGINAL ARTICLE

Genetic Association Between BTN3A1 Polymorphisms and the Risk of Rheumatoid Arthritis in a Chinese Population

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ABSTRACT

Objectives: BTN3A1 has been associated with systemic lupus erythematosus (SLE) and psoriasis; however, its relationship with rheumatoid arthritis (RA) risk, particularly regarding BTN3A1 polymorphisms and RA susceptibility remains unclear.

Methods: A total of 390 age- and sex-matched participants, including RA patients and healthy controls, were enrolled. Demographic, laboratory, and clinical data were collected. Five BTN3A1 polymorphisms (rs1796520, rs3857550, rs3208733, rs6912853, rs10456045) were genotyped. Allele and genotype associations between the groups were analyzed, as well as their associations with clinical and laboratory features in RA patients.

Results: Among RA patients, 79.74% were female and 20.26% were male; among controls, 84.36% were female and 15.64% were male. For rs3208733, the frequencies of the CC genotype and C allele differed significantly between RA patients and controls. For rs6912853, the frequency of the TC genotype differed significantly. For rs10456045, the frequencies of the GG, AG, and GG + AG genotypes differed significantly. Regarding clinical and laboratory associations: for rs1796520, antinuclear antibody (ANA)-positive patients showed higher frequencies of the CC + TC genotype and C allele; anti-Rib-positive patients showed a higher frequency of the C allele. RA patients carrying the CC + TC genotype had fewer swollen joints and higher levels of IL-12p70 and IFN-α than TT carriers. For rs6912853, CC + TC carriers had fewer tender joints and higher levels of C-reactive protein (CRP) and IFN-γ than TT carriers.

Conclusion: BTN3A1 gene polymorphisms are associated with RA risk, suggesting future therapeutic exploration of BTN3A1 in RA.

1 | Introduction

Rheumatoid arthritis (RA) affects approximately 0.5%–1% of the global population, with higher prevalence in industrialized nations [1, 2]. In China, it estimates range from 0.2%–0.4%, affecting over 5 million adults. The global burden of RA is substantial: it causes chronic pain, joint destruction, and disability, reducing quality of life and increasing cardiovascular risk. RA leads to high direct medical costs and indirect losses from work disability. In China, rapid urbanization and aging populations exacerbate this burden, with many patients lacking timely access to biologics. Current RA treatment follows a "treat-to-target" strategy: first-line methotrexate, followed by other conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), biological DMARDs (for instance, TNF inhibitors, IL-6 receptor blockers), and targeted synthetic DMARDs (JAK inhibitors). It is accepted that pathogenesis of RA involves genetic (HLA-DRB1 shared epitope) and environmental (smoking, infection) triggers, which lead to loss of immune tolerance, autoantibodies production (ACPA, RF), and synovial inflammation. There are many key pathogenic cytokines including TNF-α, IL-6, IL-1β, and GM-CSF as well as chemokines such as CXCL8 (IL-8), CXCL10, CCL2 (MCP-1), and CCL5 (RANTES) involving in RA development. The inflammatory chemokines may recruit

neutrophils, monocytes, and T cells into joints, amplifying inflammation and pannus formation.

Genetic polymorphisms, particularly single nucleotide polymorphisms (SNPs) [3], significantly influence RA susceptibility and severity [4]. The strongest association lies within the HLA-DRB1 gene, where the "shared epitope" (a conserved amino acid sequence) increases risk by promoting presentation of arthritogenic peptides to T cells, leading to chronic synovial inflammation [5]. Beyond HLA, PTPN22 gene polymorphisms impair T- cell receptor signaling and central tolerance, fostering autoimmunity [6]. STAT4 variants enhance Th1/Th17 differentiation and interferon signaling, amplifying inflammatory cytokines production [7]. TRAF1/C5 polymorphisms affect TNF receptor signaling and complement activation, modulating cell survival and inflammation [8]. Less common but impactful mutations include loss- of-function variants in PADI4, which dysregulates protein citrullination, generating anti- citrullinated protein antibodies (ACPA)- a hallmark of RA [9]. Similarly, CTLA4 gene polymorphisms reduce regulatory T- cell inhibitory signals, breaking self- tolerance. These genetic variations are not deterministic alone but interact with environmental triggers (such as smoking) to initiate autoimmunity. Understanding these polymorphisms has enabled risk prediction models and targeted therapies (e.g., abatacept targeting T- cell co-stimulation), moving RA management toward precision medicine.

In our previous studies, we have evaluated association of five polymorphisms (rs1796520, rs3857550, rs3208733, rs6912853, and rs10456045) of BTN3A1 gene with Chinese Han SLE patients, showing that these gene polymorphisms may partly relate to SLE risk [10]. Allele A and genotype AA were negatively correlated with genetic susceptibility of SLE for BTN3A1 rs742090 polymorphism [11]. Moreover, BTN3A1 contributes to SLE pathogenesis by regulating $CD4^+$ T cells function [12]. These findings demonstrated that BTN3A1 associated with SLE pathogenesis, and targeting BTN3A1 may be potential for SLE management in the future. To date, no study has discussed BTN3A1 gene polymorphisms and RA risk, especially in Chinese Han population. Therefore, in the current study, we evaluated association of BTN3A1 gene polymorphisms (rs1796520, rs3857550, rs3208733, rs6912853, and rs10456045) with RA in Chinese Han population. We found that CC genotype, C allele of rs3208733 polymorphism, TC genotype of rs6912853 polymorphism, GG, AG, GG + AG genotypes of rs10456045 polymorphism were significantly related to RA susceptibility. Interestingly, the five polymorphisms were partly correlated with different clinical, laboratory features in RA patients. These data suggested that BTN3A1 gene polymorphisms also relate to RA risk in Chinese Han population.

2 | Methods

2.1 | RA Patients and Controls

RA patients were recruited from the department of Rheumatology and Immunology, affiliated hospital of Southwest Medical University. RA patients were diagnosed according to 1987 American College of Rheumatology revised criteria for RA [13]. A total of 390 RA patients were collected in this study. All the RA patients were treatment naive. For healthy controls, the participants were collected from the health examination center of the affiliated hospital of Southwest Medical University. Healthy controls did not have autoimmune diseases or had received immunosuppressive treatment recently. A total of 390 controls were collected in this study as well. Demographic information, clinical, and laboratory data of the RA patients and controls were collected when they admitted to the hospital. This study was permitted by the Ethics Committee of the affiliated hospital of Southwest Medical University.

2.2 | Blood Collection

Elbow vein blood of each RA patient and healthy control was collected. Blood samples were centrifuged and plasma, blood residues were separately saved. Plasma of all the participants was stored in −80°C refrigerator until usage. DNA was extracted from the blood residues according to manufacturer's instructions (TIANGEN, Beijing, China). In brief, first, an equal amount of cell lysis buffer was added to the blood residues, centrifuged and added 7.5 mL of cell lysis buffer for further lysis. Second, the supernatant was discarded and a mixture of 3.3 mL buffer and protease K was added, at 65°C for 10 min. Third, 3.3 mL isopropanol was added, centrifuged, and the supernatant. Fourth, 70% ethanol was added, centrifuged, and the supernatant was discarded. Finally, DNA was dissolved. DNA specimens with qualified concentration and purity were stored in −80°C refrigerator as well.

2.3 | Data Collection

For demographic information of patients and controls, including age, sex, is collected when the participants were on- the-spot inquired. Information about tender joints, swollen joints, HAQ score, and self- evaluation score for RA patients was collected when the patients were admitted in the hospital.

For laboratory features, erythrocyte sedimentation rate (ESR) was measured for each patient. Levels of rheumatoid factor (RF), C- reactive protein (CRP), and anti-cyclic citrullinated peptide (anti- CCP) antibodies were determined using scattering turbidimetry (Siemens, Germany). Antinuclear antibody (ANA) levels were assessed by indirect immunofluorescence (Omon Medical, Hangzhou, China). Anti- double-stranded DNA (anti- dsDNA) expression was evaluated using a colloidal gold spot filtration assay (Xitang Biology, Shanghai, China). Other autoantibodies, including anti- Sm, anti-SSA, anti-SSB, and anti-RNP, were examined via immunoblotting (YHLO, Shenzhen, China). Furthermore, the levels of immunoglobulins IgG, IgM, IgA, as well as complement components C3 and C4 were measured using DiaSys Diagnostic Systems (Shanghai, China).

Determination of inflammatory cytokines (IL- 2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, TNF-α, IFN-γ, IFN-α) was conducted according to manufacturer's instructions (Raisecare, Qingdao, China). In brief, first, matrix B and standard substance were added to the standard reaction tube, and buffer and samples were added to the sample reaction tube. Then, capture microsphere antibody and detection antibody

were added to each reaction tube, and incubated at room temperature for 2 h. Second, each reaction tube was added with SA-PE, and incubated at room temperature for 0.5 h. Third, each sample was washed, and washing buffer was added to immediately determine the expression profile of the cytokines. All the above markers were detected following the manufacturers' instructions.

2.4 | Polymorphisms Selection and Determination

To validate the association of the five polymorphisms of BTN3A1 gene and RA risk, we determined the alleles, genotypes frequencies of BTN3A1 gene by KASP method that was the same to the method in SLE patients [14]. Genotyping was performed as follows. DNA was loaded into a 96- well plate, then transferred to a 1536- well plate, and pre-denatured at 65°C for 30min. Each well received 0.5 μL 2× KASP Master Mix, 0.014 μL 72× Assay Mix, and 0.486 μL ddH_2O. After sealing, PCR cycling was: 94°C for 15 min (1 cycle); 94°C for 20s and 61°C–55°C for 60s (10 cycles); 94°C for 20s and 55°C for 60s (26 cycles). Following PCR, products were sequenced, and FAM/HEX fluorescence signals were collected for analysis [15].

2.5 | Statistics

For quantitative data, normality tests should be conducted. If it conforms to a normal distribution and has equal variance, mean ± standard deviation was used to perform statistical description. Otherwise, median and interquartile range should be used for description. Qualitative data were using percentage for description. The comparison between quantitative data is performed using two independent sample *t*-test or *U* test, and comparison between qualitative data is performed using chi- square test or fisher's exact probability method. A *p*- value less than 0.05 is considered statistically significant. SPSS software 16.0 was used for analysis.

3 | Results

3.1 | Hardy–Weinberg's Expectation Test in Healthy Controls and Power Analysis

The Hardy–Weinberg equilibrium (HWE) test was used to assess whether selection bias was present for the five BTN3A1 gene polymorphisms in healthy controls. The results indicated that all five polymorphisms were in accordance with HWE, with all *p* values greater than 0.05 (data not shown). We further evaluated the statistical power of the BTN3A1 gene polymorphisms to enhance the reliability of the study. The calculated power values were 0.911 for rs1796520, 0.867 for rs3857550, 0.899 for rs3208733, 0.912 for rs6912853, and 0.913 for rs10456045 to detect a 1.7- fold increased risk assuming an α value of 0.05.

3.2 | Features of RA Patients and Healthy Controls

There were 390 RA patients and 390 healthy controls in the current study. For RA patients, 79.74% were female, and 20.26% were male. The age for RA patients was 57.00 (51.00–66.00) years. For healthy controls, 84.36% were female, and 15.64% were male. The age for healthy controls was 56.00 (46.00–65.00) years. Comparison of the sex and age between RA patients and healthy controls did not show significant differences (for sex, $\chi^2 = 2.821$, $p = 0.093$; for age, $t = 1.263$, $p = 0.207$). For the spectrum of autoantibodies, 51.03% were positive for ANA, 22.31% were positive for anti- SSA, and 10.77% were positive for anti-SSB. For expression of ESR, the median was 52.50 mm/H, and for the CRP, the median was 19.05 mg/L. In this study, we examined the expression of 12 inflammatory cytokines in RA patients and controls. Expression of IL- 1 was 8.18 (2.85–20.90) pg/mL, IL-2 was 3.19 (2.22–5.66) pg/mL, IL- 4 was 2.23 (1.64–3.28) pg/mL. Other clinical, laboratory features were listed in the Table 1.

3.3 | Comparison of Frequencies of Alleles, Genotypes of the BTN3A1 Gene Polymorphisms Between Patients and Controls

We performed statistical analysis comparing the allele and genotype frequencies of BTN3A1 gene polymorphisms between RA patients and healthy controls (Table 2). For rs1796520, the frequencies of the CC, TC, and CC + TC genotypes, as well as the C allele, did not differ significantly between the two groups (all $p > 0.05$). Similarly, for rs3857550, no significant differences were observed for the GG, AG, and GG + AG genotypes, or the G allele (all $p > 0.05$). For rs3208733, the frequencies of the CC genotype and the C allele were significantly different between RA patients and controls ($p = 0.040$ and $p = 0.012$, respectively). For rs6912853, the frequency of the TC genotype differed significantly between the two groups ($p = 0.030$). For rs10456045, the frequencies of the GG, AG, and GG + AG genotypes were significantly different between RA patients and controls ($p = 0.019$, $p = 0.026$, and $p = 0.017$, respectively). No significant differences were found for other genotypes or alleles (Table 2).

3.4 | Association of BTN3A1 Gene Polymorphisms With RA Clinical, Laboratory Features

To further explore the association between BTN3A1 gene polymorphisms and RA risk, we analyzed their relationship with clinical and laboratory features of RA (Tables 3 and 4; Tables S1 and S2). For rs1796520, RA patients with positive ANA had a higher frequency of the CC + TC genotype and the C allele compared to ANA- negative patients ($p < 0.001$ and $p = 0.037$, respectively). Additionally, RA patients with positive anti- Rib antibody showed a higher frequency of the C allele than those negative for anti- Rib ($p = 0.010$). For rs3857550, RA patients positive for anti- Jo-1 had a lower frequency of the GG + AG genotype compared to anti- Jo-1-negative patients ($p = 0.027$). For rs3208733, RA patients with positive anti- Sm antibody exhibited a lower frequency of the C allele than those negative for anti- Sm ($p = 0.033$). Regarding quantitative features, for rs1796520, RA patients carrying the CC + TC genotype had a lower number of swollen joints and higher expression of IL- 12p70 and IFN-α compared to those with the TT genotype ($p = 0.037$, $p = 0.021$, and $p = 0.037$, respectively). For rs6912853, RA patients with the CC + TC genotype had a lower number of tender joints and higher expression of CRP

TABLE 1 | Main demographic and clinical characteristics in patients with rheumatoid arthritis and healthy controls.

Characteristics	Rheumatoid arthritis	Healthy controls
Female (%)/male (%)	79.74/20.26	84.36/15.64
Age (years)	57.00 (51.00–66.00)	56.00 (46.00–65.00)
Tender joints (*n*)	8.00 (3.00–12.00)	—
Swollen joints (*n*)	4.00 (1.00–9.00)	—
ESR (mm/H)	52.50 (26.75–80.25)	—
CRP (mg/L)	19.05 (5.80–53.93)	—
RF (IU/mL)	111.95 (32.25–163.05)	—
Anti-CCP (U/mL)	331.00 (140.40–500.00)	—
C3 (g/L)	1.29 (1.09–1.45)	—
C4 (g/L)	0.30 (0.23–0.39)	—
IgG (g/L)	12.51 (10.74–15.29)	—
IgA (mg/L)	3.05 (2.10–3.74)	—
IgM (mg/L)	1.48 (0.99–1.89)	—
HAQ score	11.00 (5.00–22.00)	—
Self-evaluation score	70.00 (55.00–83.00)	—
DAS28-ESR	5.68 (4.80–6.57)	—
DAS28-CRP	6.46 (5.25–7.86)	—
ANA (+) (*n*, %)	199 (51.03)	—
Anti- Sm (+) (*n*, %)	35 (8.97)	—
Anti- dsDNA (+) (*n*, %)	36 (9.23)	—
Anti- Rib (+) (*n*, %)	27 (6.92)	—
Anti- Scl70 (+) (*n*, %)	41 (10.51)	—
Anti- SSA (+) (*n*, %)	87 (22.31)	—
Anti- SSB (+) (*n*, %)	42 (10.77)	—
Anti- RNP (+) (*n*, %)	36 (9.23)	—
Anti-Jo-1 (+) (*n*, %)	29 (7.44)	—
IL-1 (pg/mL)	8.18 (2.85–20.90)	—
IL-2 (pg/mL)	3.19 (2.22–5.66)	—
IL-4 (pg/mL)	2.23 (1.64–3.28)	—
IL-5 (pg/mL)	3.71 (2.42–6.83)	—
IL-6 (pg/mL)	11.30 (4.57–51.19)	—
IL-8 (pg/mL)	19.33 (6.20–54.41)	—
IL-10 (pg/mL)	2.80 (1.91–4.20)	—
IL-12p70 (pg/mL)	2.71 (1.40–4.30)	—
IL-17 (pg/mL)	11.92 (5.67–28.27)	—
TNF-α (pg/mL)	4.59 (2.29–14.28)	—
IFN-γ (pg/mL)	8.00 (4.20–17.85)	—
IFN-α (pg/mL)	4.19 (2.78–6.28)	—

Abbreviations: CRP, C- reactive protein; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; RF, rheumatoid factor.

TABLE 2 | Allele and genotype frequencies of five SNPs in the BTN3A1 gene in RA patients and healthy controls.

Polymorphism	RA (*n*, %)	Ctl (*n*, %)	OR (95% CI)	*p*
rs1796520				
CC	178 (45.64)	192 (49.23)	1.360 (0.878–2.109)	0.168
TC	154 (39.49)	152 (38.97)	1.244 (0.796–1.946)	0.338
CC + TC	332 (85.13)	344 (88.20)	1.306 (0.862–1.979)	0.207
TT	58 (14.87)	46 (11.80)	—	
C	510 (65.38)	536 (68.72)	1.163 (0.941–1.437)	0.161
T	270 (34.62)	244 (31.28)	—	
rs3857550				
GG	227 (58.21)	236 (60.51)	1.204 (0.635–2.284)	0.570
AG	141 (36.15)	135 (34.61)	1.109 (0.574–2.140)	0.759
GG + AG	368 (94.36)	371 (95.12)	1.167 (0.621–2.193)	0.631
AA	22 (5.64)	19 (4.88)	—	
G	595 (76.28)	607 (77.82)	1.091 (0.861–1.381)	0.470
A	185 (23.72)	173 (22.18)	—	
rs3208733				
CC	217 (55.64)	243 (62.31)	1.848 (1.030–3.316)	0.040
TC	140 (35.89)	127 (32.56)	1.497 (0.817–2.814)	0.191
CC + TC	357 (91.53)	370 (94.87)	1.710 (0.963–3.036)	0.067
TT	33 (8.47)	20 (5.13)	—	
C	574 (73.59)	613 (78.59)	1.350 (1.067–1.707)	0.012
T	206 (26.41)	167 (21.41)	—	
rs6912853				
CC	305 (78.21)	300 (76.92)	1.456 (0.855–2.478)	0.166
TC	48 (12.30)	65 (16.67)	2.004 (1.068–3.762)	0.030
CC + TC	353 (90.51)	365 (93.59)	1.530 (0.902–2.595)	0.114
TT	37 (9.49)	25 (6.41)	—	
C	658 (84.36)	665 (85.26)	1.072 (0.813–1.414)	0.622
T	122 (15.64)	115 (14.74)	—	
rs10456045				
GG	235 (60.26)	247 (63.33)	2.044 (1.126–3.709)	0.019
AG	120 (30.77)	125 (32.05)	2.025 (1.088–3.770)	0.026
GG + AG	355 (91.03)	372 (95.38)	2.038 (1.133–3.664)	0.017
AA	35 (8.97)	18 (4.62)	—	
G	590 (75.64)	619 (79.36)	1.238 (0.976–1.571)	0.079
A	190 (24.36)	161 (20.64)	—	

and IFN- γ compared to those with the TT genotype ($p = 0.002$, $p = 0.011$, and $p = 0.042$, respectively). Other clinical and laboratory features of RA were not significantly associated with BTN3A1 gene polymorphisms.

4 | Discussion

In our previous study, we analyzed the association between five BTN3A1 gene polymorphisms (rs1796520, rs3857550,

TABLE 3 | Analysis of BTN3A1 gene polymorphisms (rs1796520, rs3857550 and rs3208733) in RA by clinical, laboratory features.

	rs1796520							rs3857550							rs3208733						
	Genotype (*n*)				Allele (*n*)			Genotype (*n*)				Allele (*n*)			Genotype (*n*)				Allele (*n*)		
Characteristics	**CC**	**TC**	**TT**	***p***	**C**	**T**	***p***	**GG**	**AG**	**AA**	***p***	**G**	**A**	***p***	**CC**	**TC**	**TT**	***p***	**C**	**T**	***p***
ANA																					
Positive	90	109	0	<0.001[a]	289	109	0.037[b]	112	78	9	0.333[a]	302	96	0.787[b]	107	78	14	0.292[a]	292	106	0.885[b]
Negative	88	75	28		251	131		115	63	13		293	89		110	62	19		282	100	
Anti-Sm																					
Positive	17	16	2	0.270[a]	50	20	0.265[b]	22	10	3	0.512[a]	54	16	0.859[b]	15	14	6	0.094[a]	44	26	0.033[b]
Negative	161	138	56		460	250		205	131	19		541	169		202	126	27		530	180	
Anti-dsDNA																					
Positive	18	14	4	0.761[a]	50	22	0.220[b]	22	11	3	0.634[a]	55	17	0.982[b]	17	14	5	0.371[a]	48	24	0.162[b]
Negative	160	140	54		460	248		205	130	19		540	168		200	126	28		526	182	
Anti-Rib																					
Positive	18	8	1	0.051[a]	44	10	0.010[b]	16	8	3	0.390[a]	40	14	0.693[b]	12	12	3	0.476[a]	36	18	0.232[b]
Negative	160	146	57		466	260		211	133	19		555	171		205	128	30		538	188	
Anti-Scl70																					
Positive	15	21	5	0.267[a]	51	31	0.521[b]	25	13	3	0.764[a]	63	19	0.902[b]	24	15	2	0.680[a]	63	19	0.482[b]
Negative	163	133	53		459	239		202	128	19		532	166		193	125	31		511	187	
Anti-SSA																					
Positive	42	38	7	0.137[a]	122	52	0.137[b]	47	33	6	0.703[a]	127	45	0.405[b]	50	31	6	0.821[a]	131	43	0.564[b]
Negative	136	116	51		388	218		179	108	16		466	140		167	109	27		443	163	
Anti-SSB																					
Positive	20	17	5	0.848[a]	57	27	0.614[b]	28	12	2	0.499[a]	68	16	0.287[b]	20	18	4	0.537[a]	58	26	0.317[b]
Negative	158	137	53		453	243		199	129	20		527	169		197	122	29		516	180	
Anti-RNP																					
Positive	15	16	5	0.815[a]	46	26	0.779[b]	23	11	2	0.754[a]	57	15	0.546[b]	19	13	4	0.824[a]	51	21	0.578[b]
Negative	163	138	53		464	244		204	130	20		538	170		198	127	29		523	185	

(Continues)

TABLE 3 | (Continued)

Characteristics	rs1796520						rs3857550						rs3208733					
	Genotype (*n*)				Allele (*n*)		Genotype (*n*)				Allele (*n*)		Genotype (*n*)				Allele (*n*)	
	CC	TC	TT	*p*	C	T	GG	AG	AA	*p*	G	A	CC	TC	TT	*p*	C	T	*p*
Anti-Jo-1																			
Positive	14	10	5	0.833[a]	38	20	0.982[b]	22	4	3	0.027[a]	48	10	0.228[b]	13	11	5	0.170[a]	37	21	0.079[b]
Negative	164	144	53		472	250		205	137	19		547	175		204	129	28		537	185	

[a]Patients positive versus patients negative using 3×2 contingency table.
[b]Patients positive versus patients negative using 2×2 contingency table, or Fisher's exact test.

rs3208733, rs6912853, and rs10456045) and SLE risk in a Chinese Han population, and found that these polymorphisms were partly associated with SLE susceptibility. To further investigate whether the same polymorphisms are associated with RA risk in a Chinese Han population, the present study analyzed their relationship with RA susceptibility. The Hardy–Weinberg equilibrium test indicated no selection bias for any of the five polymorphisms in the control population. Additionally, power analysis demonstrated that all polymorphisms had power values exceeding 0.75. Therefore, this age- and sex- matched case–control study provides reliable findings regarding the association between BTN3A1 gene polymorphisms and RA patients compared to healthy controls.

In our previous study, the rs3857550 polymorphism was not significantly associated with SLE risk. Consistent with those findings, the current study showed no significant differences in the frequencies of the GG, AG, and GG + AG genotypes or the G allele between RA patients and healthy controls. Similarly, the results for rs6912853 and rs10456045 were comparable between RA and SLE patients. Specifically, the current study found that the frequencies of the GG, AG, and GG + AG genotypes differed significantly between RA patients and controls, and similar significant associations were also observed in SLE patients. However, for rs1796520, although the frequencies of the CC, CC + TC genotypes, and the C allele did not differ significantly between RA patients and controls, significant differences were observed between SLE patients and controls. For rs3208733, the frequencies of the CC genotype and the C allele were significantly different between RA patients and controls, which aligned with findings in SLE patients. Nevertheless, no significant association was found for the TC + CC genotype in RA patients, whereas this comparison was significant in SLE patients. We propose that these similarities and differences may be attributed to several factors. First, RA and SLE are distinct rheumatic diseases. SLE often involves systemic dysregulation and damage to multiple organs and systems, whereas RA typically presents with symmetric small joint involvement throughout the body; thus, their pathogenic mechanisms may partially differ. Second, the sample sizes of recruited RA and SLE patients differ. Although the statistical power for each polymorphism is sufficient within each disease, the power values vary between the two diseases. Third, BTN3A1 may play partially different roles in the pathogenesis of RA and SLE.

It is of interest to explore the association between BTN3A1 gene polymorphisms and the clinical and laboratory features of RA. In our study, for rs1796520, RA patients with positive ANA had a higher frequency of the CC + TC genotype and the C allele, and those with positive anti- Rib antibody showed a higher frequency of the C allele. Conversely, for rs3857550, RA patients positive for anti- Jo-1 had a lower frequency of the GG+AG genotype. For rs3208733, RA patients with positive anti- Sm antibody exhibited a lower frequency of the C allele. For rs1796520, RA patients carrying the CC + TC genotype had a lower number of swollen joints and higher expression of IL- 12p70 and IFN-α. For rs6912853, RA patients with the CC + TC genotype had a lower number of tender joints and higher expression of CRP and IFN- γ. In SLE patients, for rs1796520, those with positive proteinuria had a lower frequency of the CC + TC genotype and the C allele. For rs3857550, SLE patients with alopecia had a higher frequency of

TABLE 4 | The disease activity parameters in relation to BTN3A1 gene genotypes in RA patients.

	rs1796520 [Parameter (M (P_{25}, P_{75}))]				rs3857550 [Parameter (M (P_{25}, P_{75}))]				rs3208733 [Parameter (M (P_{25}, P_{75}))]			
Features	**CC**	**TC**	**TT**	***p****	**GG**	**AG**	**AA**	***p****	**CC**	**TC**	**TT**	***p****
Tender joints (*n*)	8.00 (3.00–12.00)	8.00 (3.00–12.00)	9.00 (4.75–14.00)	0.103	8.00 (3.00–12.00)	9.00 (4.00–12.00)	8.00 (4.00–20.00)	0.292	8.00 (4.00–13.50)	8.00 (2.25–12.20)	5.00 (3.00–10.11)	0.289
Swollen joints (*n*)	3.00 (1.00–8.25)	4.00 (0.00–10.00)	6.00 (3.00–10.00)	0.037	4.00 (1.00–8.00)	4.00 (0.00–10.00)	2.00 (0.00–8.00)	0.425	4.00 (0.00–10.00)	3.00 (1.00–8.00)	6.00 (2.00–9.00)	0.521
ESR (mm/H)	51.50 (27.75–80.50)	53.50 (26.00–87.25)	56.50 (26.00–78.25)	0.642	57.00 (29.00–83.00)	50.00 (26.50–79.00)	39.50 (20.00–75.75)	0.256	52.00 (26.00–82.00)	52.50 (27.75–79.00)	58.00 (24.50–81.00)	0.711
CRP (mg/L)	15.65 (4.98–45.58)	20.20 (5.37–61.53)	24.35 (5.75–47.35)	0.804	19.10 (5.00–52.40)	17.20 (4.90–56.60)	15.90 (8.90–59.63)	0.993	19.00 (4.95–56.25)	16.00 (5.15–43.85)	30.30 (5.26–54.35)	0.994
Self-evaluation score	70.00 (57.50–83.50)	74.00 (54.50–80.75)	70.00 (50.00–80.00)	0.760	71.00 (50.00–85.00)	70.00 (57.00–80.00)	75.00 (55.75–81.25)	0.672	70.00 (54.00–84.00)	70.00 (55.25–80.00)	70.00 (57.50–85.00)	0.836
DAS28-ESR	5.62 (4.75–6.59)	5.70 (4.76–6.51)	5.95 (5.08–6.69)	0.247	5.59 (4.71–6.59)	5.72 (4.94–6.55)	5.94 (4.59–6.45)	0.861	5.79 (4.73–6.65)	5.53 (4.87–6.49)	5.59 (4.74–6.28)	0.458
DAS28-CRP	6.50 (5.10–7.74)	6.46 (5.28–8.02)	6.49 (5.57–8.16)	0.345	6.47 (5.11–7.85)	6.45 (5.38–7.96)	6.62 (5.56–7.37)	0.826	6.56 (5.09–8.02)	6.36 (5.40–7.82)	6.49 (5.18–7.83)	0.713
RF (IU/mL)	117.20 (34.28–163.63)	108.00 (29.60–162.90)	114.20 (31.60–167.15)	0.985	116.10 (35.00–162.90)	104.00 (31.80–166.00)	80.25 (9.20–167.30)	0.343	120.00 (32.80–162.90)	105.00 (27.10–161.60)	95.50 (41.65–186.45)	0.559
IL-1β (pg/mL)	11.16 (5.44–24.38)	11.64 (4.79–21.48)	8.83 (5.78–40.38)	0.920	10.12 (4.82–23.60)	11.38 (5.12–26.37)	10.30 (7.84–18.89)	0.968	9.19 (4.79–20.94)	13.59 (5.91–31.79)	11.64 (6.04–32.75)	0.085
IL-2 (pg/mL)	3.35 (2.47–6.20)	3.45 (2.50–5.48)	2.89 (1.95–4.71)	0.052	3.29 (2.47–5.94)	3.33 (2.27–5.76)	3.17 (2.25–5.93)	0.802	3.31 (2.31–5.93)	3.29 (2.43–6.09)	3.28 (2.49–4.98)	0.665
IL-4 (pg/mL)	2.26 (1.81–3.88)	2.10 (1.45–2.86)	2.42 (1.33–3.18)	0.826	2.22 (1.60–3.60)	2.24 (1.71–3.28)	2.18 (1.79–2.70)	0.874	2.17 (1.66–3.30)	2.34 (1.59–3.88)	2.19 (1.67–2.83)	0.461
IL-5 (pg/mL)	3.56 (2.45–6.77)	3.79 (2.68–7.48)	3.30 (2.07–7.48)	0.189	3.73 (2.39–6.23)	3.67 (2.38–7.53)	3.60 (2.92–5.91)	0.972	3.66 (2.39–6.54)	3.69 (2.66–7.48)	3.80 (2.34–7.70)	0.491

(Continues)

TABLE 4 | (Continued)

Features	rs1796520 [Parameter (M (P_{25}, P_{75}))]				rs3857550 [Parameter (M (P_{25}, P_{75}))]				rs3208733 [Parameter (M (P_{25}, P_{75}))]			
	CC	TC	TT	*p**	GG	AG	AA	*p**	CC	TC	TT	*p**
IL-6 (pg/mL)	11.52 (4.58–45.21)	11.55 (4.61–62.95)	9.71 (3.86–29.96)	0.174	11.46 (4.62–54.03)	12.30 (4.39–51.81)	6.58 (4.03–34.73)	0.375	9.92 (4.02–46.61)	12.07 (4.60–54.56)	19.97 (8.07–66.08)	0.097
IL-8 (pg/mL)	21.19 (7.43–79.51)	18.94 (4.54–52.20)	20.42 (3.79–40.98)	0.270	23.80 (7.45–81.95)	18.59 (4.97–49.41)	4.23 (2.14–46.53)	0.070	18.31 (5.92–53.25)	22.54 (6.60–62.06)	15.09 (3.31–69.43)	0.583
IL-10 (pg/mL)	2.82 (2.05–4.18)	2.66 (1.69–4.13)	2.53 (1.79–4.61)	0.666	2.82 (1.92–4.25)	2.74 (1.72–4.40)	2.95 (2.25–3.80)	0.848	2.59 (1.75–3.86)	3.04 (2.04–5.00)	2.52 (1.93–4.10)	0.058
IL-12p70 (pg/mL)	2.63 (1.45–4.23)	2.88 (1.45–4.36)	1.69 (1.13–3.23)	0.021	2.55 (1.39–4.36)	2.78 (1.42–4.09)	3.16 (2.05–4.49)	0.466	2.69 (1.62–4.09)	2.79 (1.28–4.36)	2.51 (1.43–4.56)	0.546
IL-17 (pg/mL)	12.97 (5.71–29.66)	9.62 (5.29–28.12)	13.20 (6.59–18.35)	0.663	13.06 (5.85–29.55)	10.12 (5.35–19.01)	17.28 (6.16–38.85)	0.316	11.29 (5.68–26.27)	11.29 (5.29–29.55)	17.87 (6.37–26.67)	0.676
TNF-α (pg/mL)	4.64 (2.41–15.07)	4.75 (2.05–11.59)	4.06 (1.62–18.73)	0.657	4.53 (2.29–16.58)	4.59 (2.09–14.00)	8.27 (3.65–10.57)	0.585	6.03 (2.42–16.43)	4.59 (2.29–17.82)	3.19 (0.98–4.42)	0.288
IFN-γ (pg/mL)	8.82 (3.61–15.54)	7.97 (4.28–21.07)	7.11 (5.33–16.41)	0.605	7.94 (4.06–19.11)	8.09 (3.79–17.63)	8.75 (6.22–16.18)	0.532	7.94 (4.50–17.71)	9.23 (3.37–20.58)	7.94 (4.21–22.20)	0.831
IFN-α (pg/mL)	4.24 (3.01–6.18)	4.35 (2.84–6.67)	3.34 (2.41–5.76)	0.037	4.10 (2.75–5.95)	4.23 (2.85–6.58)	3.96 (2.88–6.30)	0.887	3.97 (2.76–6.01)	4.23 (2.76–6.94)	4.24 (2.93–7.16)	0.393
HAQ score	10.00 (4.00–21.25)	12.00 (5.00–22.00)	15.00 (7.00–22.25)	0.186	10.00 (5.00–21.00)	13.00 (5.00–21.50)	13.50 (5.75–24.75)	0.422	11.00 (5.00–22.00)	11.00 (5.00–20.00)	15.00 (6.00–24.50)	0.566

*CC + TC versus TT genotype for rs1796520, rs3208733; GG + AG versus AA for rs3857550.

the A allele. For rs3208733, SLE patients with oral ulcers had a higher frequency of the C allele. For rs6912853, SLE patients with arthritis or hypocomplementemia had a lower frequency of the CC + TC genotype and a higher frequency of the C allele. Based on these differing findings regarding the association of BTN3A1 gene polymorphisms with clinical and laboratory features in SLE versus RA, we hypothesize that the same polymorphism may differentially correlate with clinical and laboratory manifestations depending on the disease. These inconsistencies may be attributed to several factors. First, different diseases present with distinct clinical and laboratory profiles, and these features are not identical between RA and SLE. Therefore, BTN3A1 gene polymorphisms may differentially regulate the development of these features, thereby variably contributing to the pathogenesis of RA and SLE. Second, the current findings are derived from statistical association analysis rather than causal relationship analysis. Thus, elucidating the precise mechanisms by which these clinical and laboratory features contribute to or inhibit RA development will require future functional studies [16–18].

To date, only a limited number of studies have investigated the association between BTN3A1 and inflammatory autoimmune diseases. In patients with ulcerative colitis, increased γδ T cell subsets exhibiting stem- like phenotypes and expressing TCF-1, BTN3A1, and PD- 1 have been observed in intestinal biopsies compared to controls [19]. In SLE patients, the expression of BTN3A1 in PBMCs, plasma, and $CD4^+$ T cells was elevated relative to controls. Moreover, knock- in of the BTN3A1 gene in lupus mice significantly induced lupus development, as evidenced by severe inflammation and increased proportions of Th1, Th2, and Th17 cells [12]. In psoriasis patients, the proportion of Vγ9Vδ2 T cells is decreased, yet these cells produce higher levels of IFN- γ and TNF-α compared to controls [20]. Monocytes from psoriasis patients expressed higher levels of BTN3A1 than those from healthy controls, and inhibition of BTN3A1 expression blocked Vγ9Vδ2 T cell activation. In type 1 diabetes (T1D) patients, the regulatory variant rs9379874 (T allele) promotes BTN3A1 expression by binding to FOXA1 [21], leading to excessive T cell activation and dysregulation of ATP and inflammatory cytokine metabolism. Collectively, these findings suggest that polymorphisms in the BTN3A1 gene may regulate BTN3A1 expression and thereby involve in the pathogenesis of autoimmune diseases. However, the specific mechanisms by which mutated loci within the BTN3A1 gene are involved in RA pathogenesis warrant further investigation.

This study has some limitations. First, the current study is derived from a single ethnic group (only Chinese Han) and lacks validation in diverse populations, limiting generalizability. Second, the current study is a statistical association analysis without mechanistic follow-up. Identifying a polymorphism does not explain how it affects gene expression, protein function, or immune pathways. Third, this study analyzed single polymorphisms independently, lacking genetic variants and environmental factors interaction analysis. This reductionist approach may miss synergistic effects critical to RA pathogenesis.

5 | Conclusion

This study showed that BTN3A1 gene polymorphisms (rs3208733, rs6912853, and rs10456045) are related to RA susceptibility and also correlate with RA clinical and laboratory features in the Chinese Han population.

Author Contributions

Study conception and design: Q.S., A.H., C.H. Acquisition of data, analysis and interpretation of data: Q.S., A.H., C.H. Drafting the article: Q.S., C.H. Final approval of the version of the article to be published: all authors, and that all authors agree to be accountable for all aspects of the work.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Datasets are available from the corresponding author on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Analysis of BTN3A1 gene polymorphisms (rs6912853 and rs10456045) in RA by clinical, laboratory features. **Table S2:** The disease activity parameters in relation to BTN3A1 gene genotypes (rs6912853, rs10456045) in RA patients.

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CLINICAL IMAGE

Paraneoplastic Adult-Onset Still's Disease Caused by Mediastinal Lymph Node Carcinoma of Unknown Primary Site, Most Likely Lung Adenocarcinoma

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A 73-year-old man presented with fever, polyarthritis and erythema on his back (Figure 1A) and extremities (Figure 1B). Laboratory findings showed leukocytosis and elevated CRP (24 mg/dL), white blood cell (11 800/μL; neutrophil (88%)), GOT (78 U/L), GPT (89 U/L), LDH (382 U/L), ferritin (1023 ng/mL) and IL-6 (143 pg/mL). Autoantibodies were all negative. Contrast enhanced CT revealed swollen mediastinal lymph nodes and pericardial effusion. Gastrointestinal endoscopic examinations

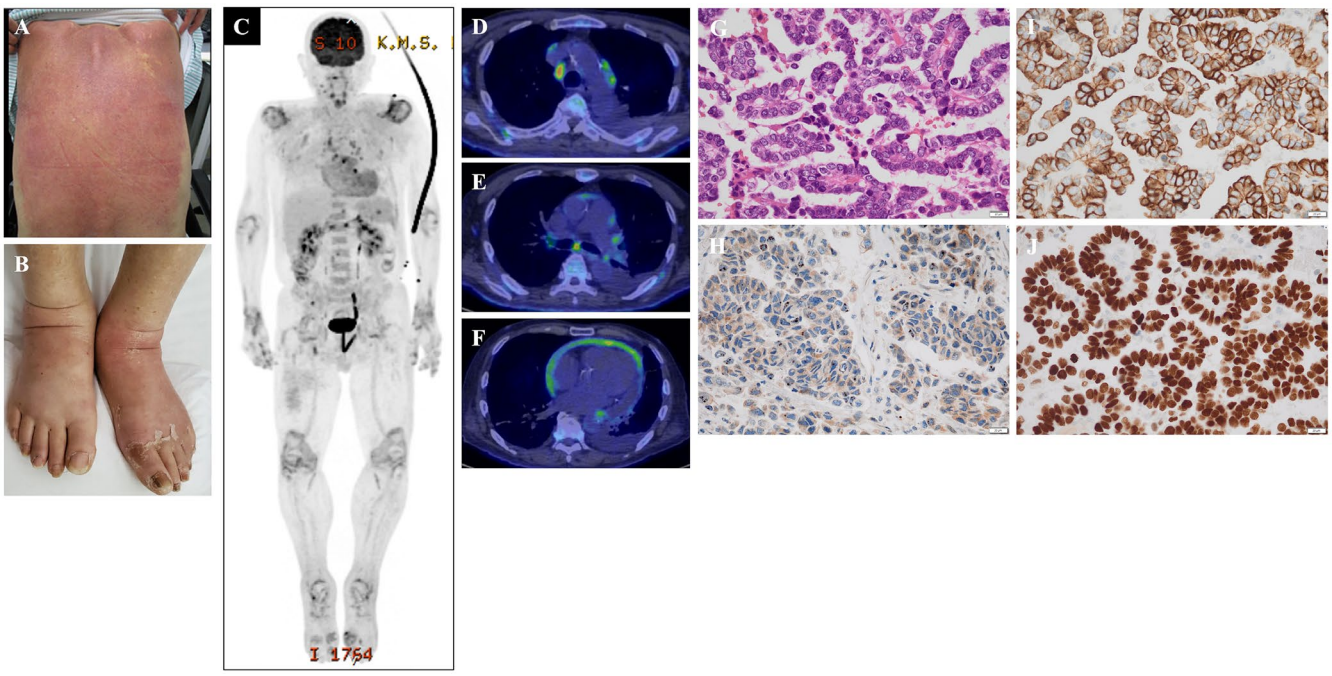

FIGURE 1 | Gross appearance of back and lower extremity showed erythema (A) and arthritis (B). FDG-PET/CT demonstrated high FDG uptake in mediastinal lymph nodes (C–E), multiple joints (C), and pericardium (F). Lymph node biopsy suggested adenocarcinoma (G) with positive immunostaining of IL-6 (H), Cytokeratin 7 (CK7) (I), and Thyroid transcription factor-1 (TTF-1) (J).

showed no specific findings. FDG-PET/CT demonstrated high FDG uptake in mediastinal lymph nodes (Figure 1C–E), multiple joints (Figure 1C), and pericardium (Figure 1F). These clinical, laboratory and imaging findings indicated adult- onset Still's disease (AOSD)- like manifestations and possible malignancy despite no organ lesions. In order to differentiate the possibility of paraneoplastic AOSD, thoracoscopic mediastinal lymph node biopsy was performed. Biopsy specimen suggested adenocarcinoma (Figure 1G) with EGFR gene mutation and positive immunostaining of IL- 6 (Figure1H), CK7 (Figure 1I), and TTF- 1 (Figure 1J). Thus, he was diagnosed with paraneoplastic AOSD caused by IL- 6-producing mediastinal lymph node carcinoma of unknown primary site, most likely lung adenocarcinoma. Treatment with osimertinib, EGFR inhibitor, was initiated, resulting in the remission of clinical manifestations and the improvement of laboratory and imaging findings.

Paraneoplastic syndrome mimicking AOSD is a rare condition with only a limited number in the literature [1, 2]. Previous reports have suggested that paraneoplastic cases may occur in relatively older patients. As the present case indicates, IL- 6 production by carcinoma might cause AOSD- like manifestations.

Lymphadenopathy is often revealed in AOSD patients, while adenocarcinoma of unknown primary in a mediastinal lymph node is rarely reported [3, 4].

Therefore, clinicians always need to note the possibility of adenocarcinoma of unknown primary in a mediastinal lymph node when the patients show AOSD- like manifestation, and FDG-PET/CT should be recommended to screen for occult malignancy in elderly patients presenting with AOSD- like manifestations.

Author Contributions

Y. Taniguchi and D. Okada equally contributed to this manuscript.

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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 analysed during the current study.

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LETTER TO THE EDITOR

Association Between Serum Matrix Metalloproteinase-3 Levels and Cognitive Function in Patients With Rheumatoid Arthritis

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Dear Editor,

We read with great interest the well-designed and thought-provoking article by Tada et al. [1], which demonstrates an independent association between serum matrix metalloproteinase-3 (MMP-3) levels and cognitive function in patients with rheumatoid arthritis (RA). The authors are to be commended for addressing a clinically important but understudied extra-articular complication of RA and for highlighting the potential role of MMP-3, a key mediator of synovial inflammation, in cognitive health. While this work provides valuable preliminary evidence, we wish to offer three constructive, mechanistically grounded considerations that may help refine the interpretation of the findings and inform future research.

First, the tissue source of elevated serum MMP-3 remains unclear. The authors interpret raised MMP-3 as a marker of RA-specific synovial inflammation. However, MMP-3 is not exclusively derived from synovial tissue. It is also abundantly produced by vascular endothelial cells, astrocytes, and neurons during systemic vascular inflammation and central neuroinflammation [2, 3]. Therefore, elevated circulating MMP-3 may equally reflect these alternative pathological processes rather than synovium-driven disease activity alone. This ambiguity limits the conclusion that RA-related inflammation is the primary driver of the observed cognitive association. Future studies would benefit from measuring MMP-3 in cerebrospinal fluid or including markers of neuroaxonal injury, such as neurofilament light chain, to distinguish peripheral versus central contributions.

Second, the study did not evaluate blood–brain barrier (BBB) integrity. A leading mechanistic hypothesis linking peripheral MMP-3 to cognitive impairment is its ability to degrade tight junction proteins (e.g., claudin-5, occludin), thereby compromising the BBB and promoting entry of inflammatory mediators into the central nervous system[3]. Without direct assessment of BBB integrity—using validated approaches such as the cerebrospinal fluid/serum albumin ratio, serum S100B, or dynamic contrast-enhanced MRI—this proposed pathway remains speculative [4, 5]. Incorporating at least one measure of BBB permeability would help clarify whether MMP-3-associated cognitive decline is mediated through barrier disruption.

Third, the potential impact of MMP-3 gene polymorphisms was not addressed. The functional promoter polymorphism rs3025058 (5A/6A) strongly influences MMP-3 transcription; the 5A allele confers higher promoter activity and greater MMP-3 expression [6, 7]. Genetic background may therefore substantially modify baseline and inducible MMP-3 levels. In the absence of genotyping, it is difficult to determine whether elevated serum MMP-3 in this cohort reflects disease-related inflammation, a genetic predisposition, or their interaction. Future work should include MMP-3 genotyping to assess whether genetic variation modifies the association between MMP-3 and cognitive outcomes.

In summary, Tada et al. present an important and hypothesis-generating link between serum MMP-3 and cognitive function in RA. However, interpreting MMP-3 as a specific marker of synovial pathology is complicated by its multiple cellular sources, the lack of BBB integrity assessment, and the absence of genetic stratification. Addressing these considerations in prospective mechanistic studies will help determine whether MMP-3 is merely a correlate of disease activity or a direct participant

in RA- associated cognitive impairment. Such research would strengthen the biological plausibility of this association and may support the development of MMP- 3 as a clinically relevant biomarker. Finally, we would be interested to see whether successful reduction of MMP- 3 through treat-to-target strategies or biologic/targeted synthetic DMARDs correlates with stabilization or improvement in cognitive function [8]. This would have direct clinical implications.

Author Contributions

Longchuan Xu conducted literature retrieval, drafted and revised the manuscript, and finalized the approved version independently.

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Conflicts of Interest

The author declares 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

SSGJ-613 Alleviates Pain and Reduces the Risk of Acute Flares in Patients With Gout: A Randomized, Double-Blind, Phase II Study

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Keywords: Acute gouty arthritis | Gout | Interleukin-1β (IL-1β) inhibition | SSGJ-613

ABSTRACT

Objectives: This phase II trial aimed to evaluate the efficacy and safety of SSGJ-613, a fully human anti-interleukin 1β monoclonal antibody, compared with compound betamethasone (CB) in Chinese patients with acute gouty arthritis.

Methods: In this multicenter, double-blind, double-dummy, active-controlled study, 90 adults with gout according to the 2015 American College of Rheumatology (ACR) criteria and who had experienced an acute gouty arthritis flare within 7 days were randomized (1:1:1) to receive a single subcutaneous injection of SSGJ-613 (200 mg or 300 mg) or intramuscular CB. The primary endpoint was the change from baseline in visual analog scale (VAS) pain scores at 72 h post-dosing. Safety was evaluated through monitoring of adverse events and laboratory parameters.

Results: The mean changes from baseline in VAS scores at 72 h were comparable between SSGJ-613200 mg (−45.8 mm), 300 mg (−40.4 mm), and CB (−48.2 mm) groups. However, SSGJ-613 significantly reduced the risk of new flares: 16.7% (200 mg) and 14.3% (300 mg) of patients experienced new flares versus 54.8% with CB ($p = 0.0037$ and $p = 0.0019$, respectively). The safety profile of SSGJ-613 was similar to CB, with no drug-related serious adverse events reported.

Conclusion: A single injection of SSGJ-613 demonstrated rapid pain relief comparable to corticosteroid therapy at 72 h and provided superior protection against gout flare recurrence over 12 weeks in Chinese patients, supporting its potential as an effective treatment option for acute gouty arthritis with prolonged therapeutic benefits.

Clinical Trials: NCT06169891.

Yu Xue and Xiaoxia Zhu contributed equally to this work and are co-first authors.

1 | Introduction

Gouty arthritis, a prevalent form of inflammatory arthritis, arises from the deposition of monosodium urate (MSU) crystals in the joints, typically in the setting of hyperuricemia. In China, gout affects an estimated 1%–3% of the population, with its prevalence continues to rise [1]. It presents as acute flares with erythema, swelling, severe pain and joint dysfunction. Recurrent flares and persistent low- grade inflammation may cause progressive joint damage and impaired quality of life. Poorly controlled gout and sustained hyperuricemia can also worsen comorbidities, emphasizing the need for effective flare prevention [2].

Conventional first- line treatments for acute gouty arthritis include colchicine, nonsteroidal anti- inflammatory drugs (NSAIDs) and corticosteroids. However, these drugs are not suitable for all patients due to contraindications, poor tolerability or insufficient efficacy. Patients with frequent flares urgently need better therapies to maintain remission [3]. This unmet need drives the development of new agents to suppress inflammation and reduce gout flare recurrence.

Interleukin- 1β (IL-1β) is a key driver of inflammation during gout flares, and IL- 1β blockade has proven effective for pain relief and relapse prevention [3–6]. Canakinumab, an anti- IL-1β monoclonal antibody, was approved by the EMA in 2013 and the FDA in 2023 for acute gouty arthritis. The 2020 ACR gout guidelines also recommend IL- 1 inhibitors for patients intolerant or unresponsive to conventional treatments and those with refractory disease [7]. Still, clinical evidence of IL- 1 inhibitors in Chinese patients remains limited, requiring further randomized controlled trials.

SSGJ- 613 is a fully human anti-IL-1β monoclonal antibody independently developed by Sunshine Guojian Pharmaceutical Co. This phase II, double- blind, double-dummy, active-controlled study was designed to evaluate the efficacy and safety of IL- 1β blockade in Chinese patients with gouty arthritis and to identify the optimal dosing strategy.

2 | Methods

2.1 | Study Design

The SSGJ- 613-AG-II study (NCT06169891) was a multicenter, randomized, double- blind, double-dummy, single-dose, active-controlled phase II clinical trial conducted in accordance with the Declaration of Helsinki and the relevant national and international ethical guidelines. The study protocol (Suppoting Information) was approved by the Ethics Committee of Huashan Hospital, Fudan University (Approval Number: 2023-LinShen-987).

All participants provided written informed consent after being fully informed of the study objectives, procedures, potential risks and benefits, and their rights. The trial adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

2.2 | Participants

The study enrolled adults aged 18–65 years with a confirmed diagnosis of gouty arthritis according to the 2015 ACR classification criteria. Eligible participants had to experience an acute gout flare within 7 days prior to dosing, report a baseline visual analog scale (VAS) pain score of 50 mm or higher, and have contraindications, intolerance, or inadequate response to NSAIDs and/or colchicine. Those on urate- lowering therapy were required to maintain a stable dose for at least 14 days prior to baseline and throughout the 12- week study period. Key exclusion criteria included prior exposure to IL- 1 antagonists, tumor necrosis factor (TNF) inhibitors, other biologics, or investigational drugs within 30 days prior to screening or within five half- lives of the drug.

2.3 | Randomization, Masking, and Interventions

The study consisted of a screening period (up to 48 h), a single treatment period (Day 1), and a 12- week follow-up period. A total of 90 patients with gouty arthritis were randomized in a 1:1:1 ratio to receive either SSGJ- 613200 mg, SSGJ- 613300 mg (both administered subcutaneously), or CB as the active control (intramuscular injection containing 5 mg betamethasone dipropionate and 2 mg betamethasone sodium phosphate). All injections were accompanied by a matching placebo to maintain blinding of patients and investigators. Rescue therapy, including methylprednisolone or prednisone, was permitted at the investigators' discretion for severe joint pain or new gout flares. Randomization was stratified according to the participants' ongoing use of urate- lowering medication.

2.4 | Outcomes

The primary endpoint was the change from baseline at 72 h post-dosing in pain intensity at the target joint using a 0–100 mm VAS. Secondary endpoints included the change from baseline in VAS pain scores at 6, 12, 24, 48, and 72 h post- dosing; median time to complete pain resolution at the target joint (VAS = 0 or "none" on); the need for rescue therapy; the time to the first new gout flare (defined as a flare in a previously unaffected joint or a recurrence in an affected joint where the previous flare had resolved completely); proportion of participants experiencing at least one new gout flare; mean number of new gout flares per patient; SF- 36 quality-of-life scores; and inflammatory markers (erythrocyte sedimentation rate [ESR], high- sensitivity C- reactive protein [hs-CRP], and serum amyloid A [SAA]) assessed at week 12.

Safety endpoints included the assessments of adverse events (AEs), serious AEs (SAEs), vital signs, physical examination findings, laboratory parameters, and other relevant safety measures. The severity of all AEs was graded according to the Common Adverse Event Evaluation Criteria (CTCAE version 5.0). Immunogenicity assessments focused on detecting anti-drug antibodies (ADAs) and neutralizing antibodies (NAbs) in the serum of participants across the treatment groups (Supporting Informations).

2.5 | Statistical Analysis

As an exploratory study, the sample size was not determined based on formal power calculations but was selected in accordance with Chinese regulatory guidelines and aligned with design of similar registered biologics. The study planned to enroll 30 patients per group (90 patients in total). Efficacy endpoints were performed on the Full Analysis Set (FAS). The primary endpoint was analyzed using on analysis of covariance (ANCOVA) model, with change in VAS scores from baseline to 72 h as the response variable, baseline VAS scores as a covariate, and urate- lowering therapy use (stratification factor) and treatment group as fixed effects. Least- squares mean differences and 95% confidence intervals were estimated for each SSGJ- 613 dose group versus the active control. Safety outcomes were summarized descriptively based on the safety set.

3 | Results

3.1 | Patient Disposition and Baseline Characteristics

A total of 90 patients from 23 sites were enrolled and randomized into three treatment groups: SSGJ- 613200 mg ($n = 30$), SSGJ-613300 mg ($n = 29$), and CB ($n = 31$). Overall, 84 patients (93.3%) completed the study, while six discontinued prematurely (one patient in 200 mg group, three in 300 mg group, and two in CB group). The leading reason for study discontinuation was adverse events ($n = 2$). Other reasons included withdrawal of informed consent, inadequate compliance, loss to follow-up, and participant (each $n = 1$) (Figure 1).

The demographic and baseline disease characteristics were generally well- balanced across groups (Table 1). The study population was predominantly of middle- aged males. The mean duration of gouty arthritis ranged from 4.7 to 7.6 years, with patients had approximately 5 to 8 acute flares in the preceding 12 months. The proportion of patients currently receiving urate-lowering therapy was 30.0% in the SSGJ- 613200 mg group, 32.1% in the 300 mg group, and 29.0% in the CB group, with febuxostat being the most commonly used agent across these patients. Contraindications, intolerance, or lack of response to NSAIDs were reported in 76.7%, 71.4%, and 67.7% of patients in the three groups, respectively; corresponding proportion for colchicine use were 80.0%, 75.0%, and 77.4%.

3.2 | Efficacy

3.2.1 | Pain Relief

Baseline mean VAS scores for target joint pain were 72.4 mm, 66.8 mm, and 67.4 mm in the SSGJ- 613200 mg, 300 mg, and CB groups, respectively. At 72 h post- dose, significant pain relief was observed across all treatment groups. The mean changes (±SD) from baseline in VAS scores (percentage changes) were −45.8 (±17.88) mm for SSGJ- 613200 mg, −40.4 (±17.35) mm for SSGJ-613300 mg, and −48.2 (±15.90) mm (−72.72 ± 23.949%) for CB. No statistical differences were detected between either SSGJ- 613 dose and CB (200 mg vs. CB: $p = 0.4532$; 300 mg vs. CB: $p = 0.0848$) using ANCOVA model.

Nevertheless, given the exploratory nature and limited sample size of this phase II trial, these findings warrant verification in larger population. Significant pain reduction relative to baseline

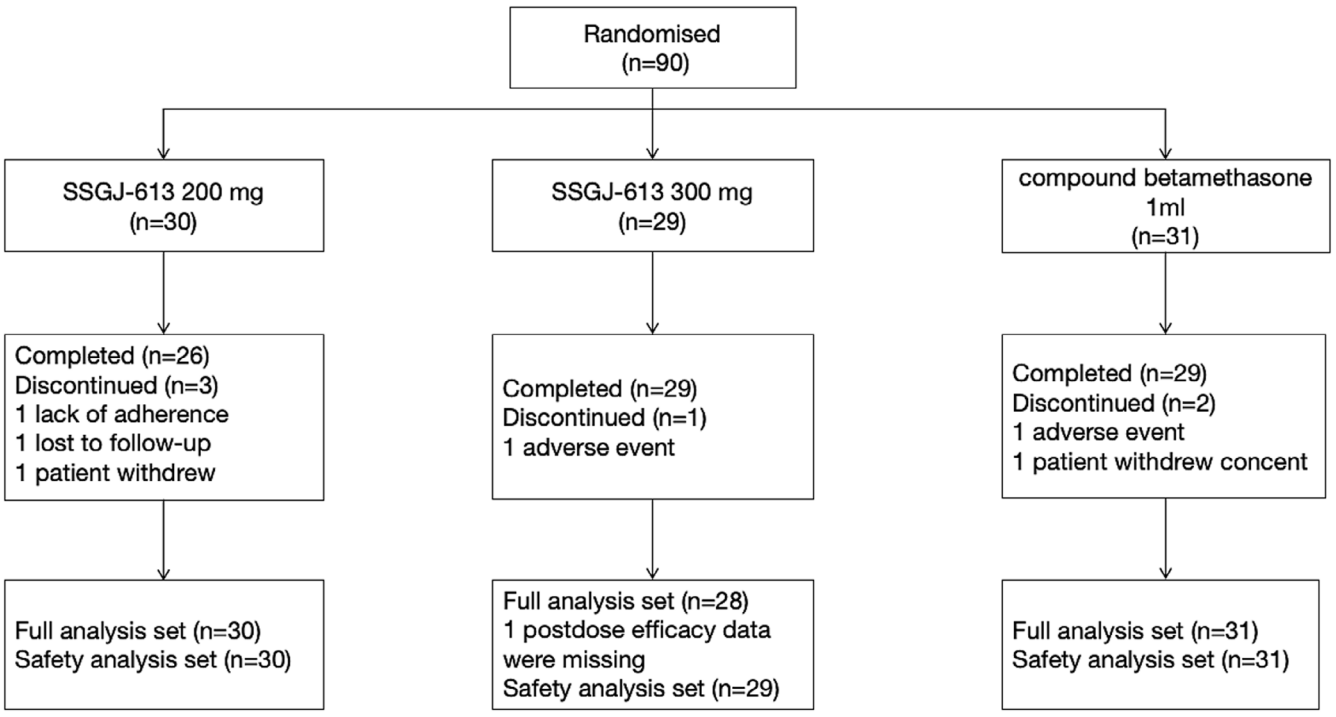

FIGURE 1 | Patient disposition.

TABLE 1 | Demographic and baseline characteristics.

	SSGJ-613200 mg (*N* = 30)	SSGJ-613300 mg (*N* = 28)	Compound betamethasone (*N* = 31)
Age (year) (mean, SD)	41.4 (10.94)	44.2 (11.93)	39.1 (9.57)
Men, *n* (%)	29 (96.7)	26 (92.9)	31 (100)
Weight (kg) (mean, SD)	88.86 (19.70)	82.13 (15.04)	87.34 (14.05)
BMI (kg/m^2) (mean, SD)	29.59 (5.59)	27.25 (3.66)	28.66 (4.13)
Duration of gouty arthritis (mean, SD)	7.621 (5.97)	4.736 (3.98)	6.136 (5.46)
Tophi present, *n* (%)	10 (33.3)	7 (25.0)	7 (22.6)
Number of acute gouty arthritis flares within the past 12 months (mean, SD)	8.0 (7.62)	5.0 (4.22)	5.7 (6.43)
Number of acute gouty arthritis flares within the past 12 months, *n* (%)			
< 3 times	6 (20.0)	11 (39.3)	9 (29.0)
≥ 3 times	24 (80.0)	17 (60.7)	22 (71.0)
Proportion of subjects currently receiving urate-lowering therapy, *n* (%)	9 (30.0)	9 (32.1)	9 (29.0)
Percentages of patients contraindicated for, intolerant of, or unresponsive to, *n* (%)			
NSAIDs	23 (76.7)	20 (71.4)	21 (67.7)
Colchicine	24 (80.0)	21 (75.0)	24 (77.4)
Both	17 (56.7)	13 (46.4)	14 (45.2)
Classification of gouty arthritis, *n* (%)			
Monoarticular	21 (70.0)	19 (67.9)	22 (71.0)
Polyarticular	9 (30.0)	9 (32.1)	9 (29.0)
Duration of first flare at time of treatment (day) (mean, SD)	4.1 (1.34)	4.3 (1.15)	3.9 (1.47)
Duration of first flare at time of treatment, *n* (%)			
< 4 Days	10 (33.3)	6 (21.4)	16 (51.6)
≥ 4 Days	20 (66.7)	22 (78.6)	15 (48.4)

Abbreviations: BMI, body mass index; NSAIDs, nonsteroidal anti- inflammatory drugs; SD, standard deviation.

was also observed at other assessed time points (6, 12, 24, 48 h) in each group (Figure 2).

The median time to (95% CI) complete pain remission of target joint was 8.0 (5.00, 21.00) days for the SSGJ- 613200mg group, 14.5 (6.00, 29.00) days for the 300 mg group, and 15.0 (6.00, 29.00) days for the CB group. The proportions of participants requiring rescue medication within the first 7 days post- dosing were 6.7% (2/30) with SSGJ- 613200mg, 14.3% (4/28) with 300 mg, and 9.7% (3/31) with CB.

3.2.2 | Reduction in Risk of New Flare

Within the 12- week study period, the median time to the first new gouty arthritis flare was not reached in either the SSGJ- 613 group, compared with 61 days in the CB group (Figure 3). The proportion of patients developing new flares within 12 weeks was 16.7% (5/30) with 200 mg (difference vs. CB: −35.2% [95% CI: −56.9 to −13.5]; $p = 0.0037$) and 14.3% (4/28) with 300 mg (difference vs. CB: −38.1% [95% CI: −59.3 to −16.9]; $p = 0.0019$), both of which were significantly lower than the 51.6% (16/31) observed in the CB group (Figure 4). The mean number of flares per patient over 12 weeks was markedly lower in the SSGJ- 613 groups, with (0.23 for 200 mg and 0.18 for 300 mg) than in the CB group (0.84). These findings demonstrate that treatment with SSGJ- 613 significantly delayed the time to the first new flare and reduced the incidence of new flares over 12 weeks compared with CB.

3.2.3 | Other Improvements

By Week 12, improvements in both physical and mental health components were observed across all groups as assessed by the SF- 36 questionnaire. Physical health scores increased by 13.98 (200 mg), 14.34 (300 mg), and 15.69 (CB) points, while mental health scores improved by 11.393, 7.434, and 8.174 points,

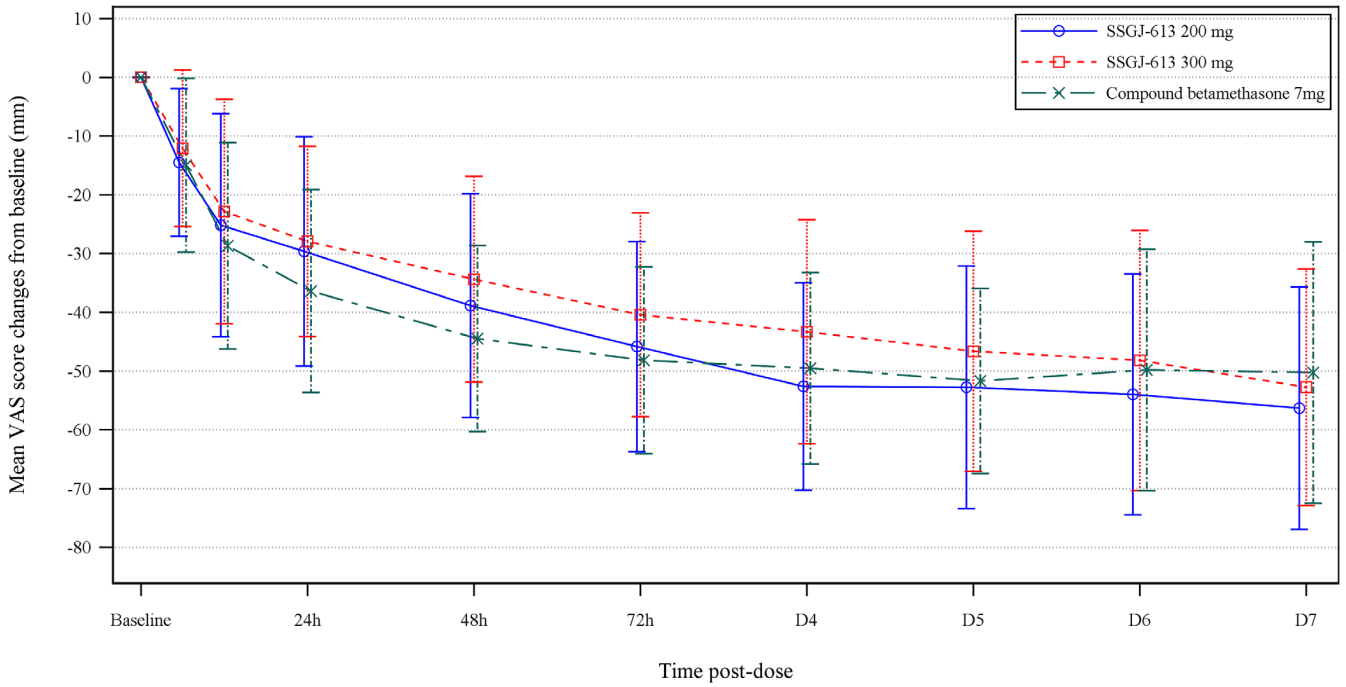

FIGURE 2 | Mean VAS score changes from baseline over 7 days post- dose for the first flare.

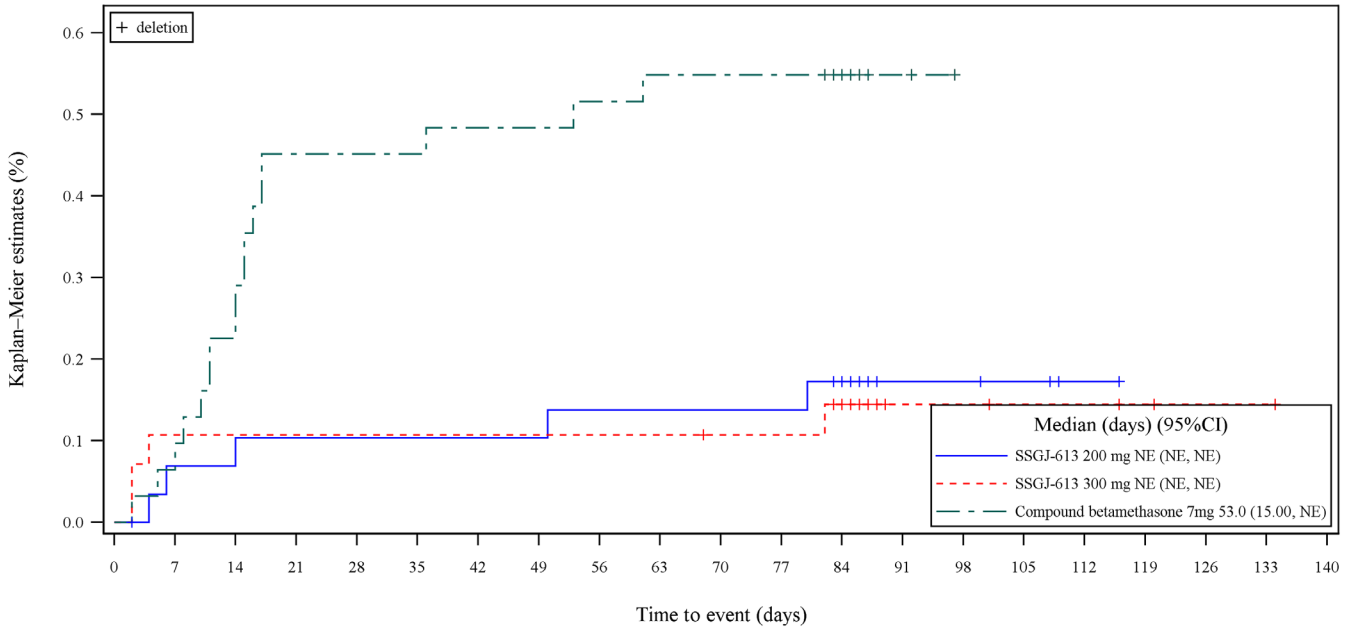

FIGURE 3 | Time to first new flare.

respectively. Inflammatory markers, including hs- CRP, SAA, and ESR decreased rapidly within 72 h post- dosing in all groups, with SSGJ- 613 treatment showing a more pronounced reduction in hs- CRP (200 mg: −11.0283 mg/L, 300 mg: −9.0928 mg/L) and SAA (200 mg: −64.9466 mg/L, 300 mg: −52.3828 mg/L) compared with CB (hs- CRP:−3.8774 mg/L; SAA: 13.9241 mg/L).

3.3 | Safety

The incidence of treatment- emergent adverse events (TEAEs) was 76.7% (SSGJ- 613200 mg), 65.5% (SSGJ- 613300 mg), and 77.4% (CB). Drug- related TEAEs were lower in the SSGJ-613 groups (36.7% for 200 mg and 31.0% for 300 mg) than in the CB group (45.2%). Most AEs were Grade 1 or 2 in severity according to CTCAE. No deaths, SAEs, or TEAEs leading to treatment discontinuation were considered related to SSGJ- 613. Infections were reported in 13.3% (200 mg), 20.7% (300 mg), and 19.4% (CB) of patients, with a low incidence of opportunistic infections in the SSGJ- 613 groups. The most common drug-related TEAEs (occurring in ≥ 5% of patients) were elevated alanine aminotransferase and hypertriglyceridemia, with similar frequencies observed between the SSGJ- 613200 mg and CB (Table 2).

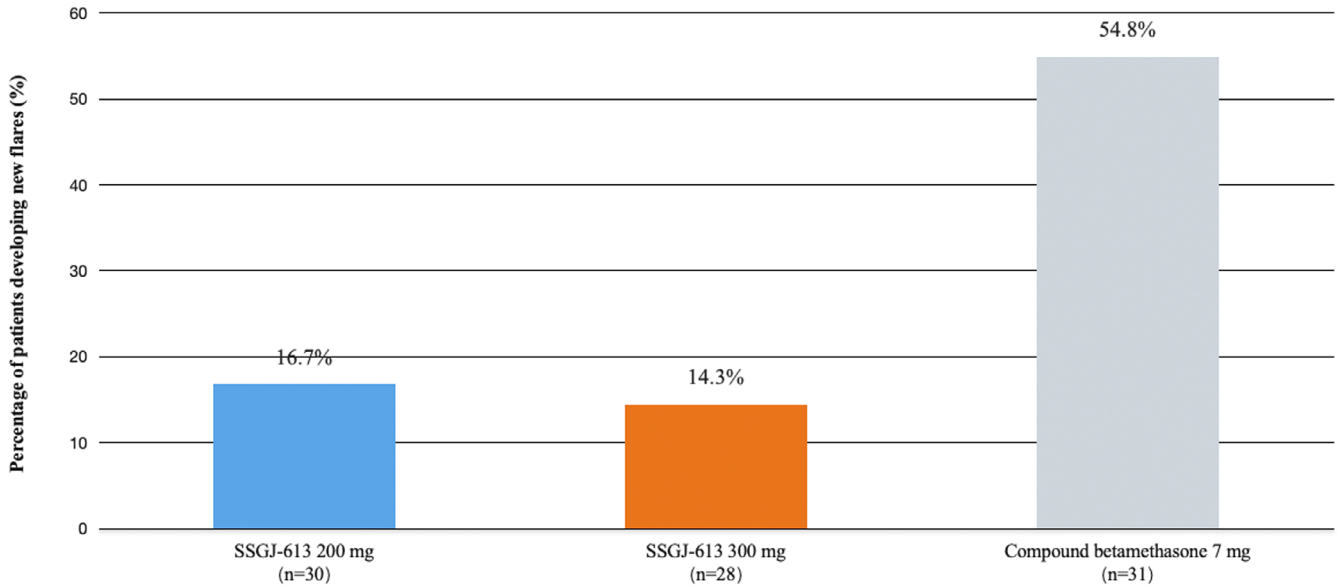

FIGURE 4 | Percentage of patients developing new flares within 12 weeks after single administration of SSGJ- 613.

TABLE 2 | Adverse events through week 12.

	SSGJ-613200 mg (*N* = 30)	SSGJ-613300 mg (*N* = 29)	SSGJ-613 pooled (*N* = 59)	Compound betamethasone (*N* = 31)
Any TEAEs	23 (76.7)	19 (65.5)	4 2 (71.2)	24 (77.4)
Infection AEs	4 (13.3)	6 (20.7)	10 (17.0)	6 (19.4)
AEs leading to withdrawal	1 (3.3)	0	1 (1.7)	1 (3.2)
SAE	1 (3.3)	1 (3.4)	2 (3.4)	0
Death	0	0	0	0
Any drug- related TEAEs	10 (33.3)	9 (31.0)	19 (32.2)	14 (45.2)
Infection AEs	0	3 (10.3)	3 (5.1)	2 (6.5)
Opportunistic infection	0	1 (3.4)	1 (1.7)	2 (6.5)
AEs leading to withdrawal	0	0	0	0
SAE	0	0	0	0
Drug- related TEAEs reported in≥ 5% of patients in any treatment group				
White blood cell count increased	1 (3.3)	0	1 (1.7)	5 (16.1)
Alanine aminotransferase increase	3 (10.0)	0	3 (5.1)	3 (9.7)
Neutrophil count increased	1 (3.3)	0	1 (1.7)	4 (12.9)
Monocyte count increased	1 (3.3)	0	1 (1.7)	2 (6.5)
Hypertriglyceridemia	2 (6.7)	1 (3.4)	3 (5.1)	2 (6.5)
Hyperlipidemia	2 (6.7)	1 (3.4)	3 (5.1)	2 (6.5)
Latent tuberculosis	0	0	0	2 (6.5)

Abbreviations: SAE, serious adverse events; TEAEs, treatment- emergent adverse events.

3.4 | PK, PD, and Immunogenicity

Following a single subcutaneous injection of SSGJ- 613 at doses of 200 mg and 300 mg, peak mean serum concentrations were observed within the scheduled sampling timeframe up to 168 h post- dosing. The 300 mg dose demonstrated higher average systemic exposure compared with the 200 mg dose. Even at 2016 h post- administration, mean serum concentrations in all groups remained elevated above baseline levels.

A substantial increase in total serum IL- 1β levels was observed across all dose groups following SSGJ- 613 administration, indicating effective binding to IL- 1β. This binding is hypothesized to form a complex that slows the clearance of IL- 1β, thereby sustaining elevated levels.

In the immunogenicity analysis conducted on 59 subjects, only one patient in the SSGJ- 613200 mg group tested positive for both anti- drug antibodies (ADA, with a titer of 120) and neutralizing antibodies (NAb) at 2016 h post- dosing (Day 84), reflecting a positivity rate of 3.3% (1/30). All other patients in the SSGJ- 613 groups tested negative for ADA.

4 | Discussion

This active- controlled phase 2 study highlights the advantages of SSGJ- 613, a recombinant humanized monoclonal antibody targeting IL- 1β, in managing symptoms and lowering risk of new flares in Chinese patients with acute gouty arthritis. Furthermore, it identifies the original dose for further investigation a phase III study.

Both CB and SSGJ- 613 demonstrated rapid efficacy in pain reduction, with observable and clinically meaningful effects (10 mm on the VAS) within 6 h post- administration. By 72 h, pain relief effects of SSGJ- 613 in the target joints were comparable to CB, showing no statistically significant differences, aligning with efficacy outcomes observed in pivotal studies of Canakinumab [8]. The number of patients with rescue medication use within the first 7 days post- dosing among treatment groups was similar across treatment groups, hence the impact on pain relief was negligible.

Gouty arthritis patients frequently experience recurrent flares despite traditional therapies, with some unable to tolerate or benefit from conventional treatments. As demonstrated in our study by the high rate of recurrent flares in the CB group, as over 50% of these patients developed at least one new flare within 12 weeks post- dosing. However, the higher incidence of flares in the CB group may not indicate a higher risk of rebound flares in this group, because half of the patients receiving CB did not experience flares until 61 days post- dosing. And because only 5 patients with 200 mg and 4 patients with 300 mg developed new flares within 12 weeks (less than half of the total per each group), the median time to the first new gouty arthritis flare was not reached in either SSGJ- 613 group. From a mechanistic perspective, CB offers rapid but short- lived anti-inflammatory effects due to its small- molecule properties, while SSGJ-613 provides sustained and precise IL- 1β blockade as a large-molecule biologic. These mechanistic differences may account for the higher flare rate in the CB group.

Both SSGJ- 613 dosages significantly lowered the proportion of patients developing new flares within 12 weeks compared to the CB group. SSGJ- 613 also exhibited consistent numerically superior trends across various efficacy endpoints, suggests that SSGJ- 613 may serve as a valuable additional therapy for long-term flare prevention.

Results for inflammatory markers (CRP, SAA, and ESR) indicate that SSGJ- 613 effectively and rapidly suppresses the inflammatory response. The 200 mg group demonstrated the most pronounced reduction in these markers, and the downward trend observed during the study was sustained, with effects comparable to or exceeding those of the 300 mg group. The 200 mg group had higher baseline CRP and SAA levels. Greater baseline inflammation led to larger marker reductions. Given that CRP and SAA are non- specific to AG and are affected by multiple clinical factors, this may explain why the 200 mg group showed greater biomarker reductions but similar pain relief compared to the 300 mg group. Furthermore, improvements in SF- 36 scores reflected a measurable therapeutic benefit.

SSGJ- 613 exhibited favorable safety and tolerability. No deaths, SAEs, or TEAEs leading to treatment discontinuation were reported as related to SSGJ- 613. The incidence of infection events was lower than that reported in the pivotal study of Canakinumab (17.0% with SSGJ- 613 versus 20.4% with Canakinumab) [8]. All infections were manageable, and no serious infections were reported.

The incidence of elevated alanine aminotransferase and hypertriglyceridemia exceeded 5% in the SSGJ- 613 group. However, these abnormalities did not exhibit a dose- dependent pattern, with a higher incidence observed in the CB group. Additionally, no lipid- related adverse reactions were reported in single- or multiple- dose studies of SSGJ-613 in healthy individuals. Considering that gout patients often present with metabolic abnormalities, these findings may be attributable to the underlying disease. Given the limited sample size, further evaluation in larger populations is warranted. Furthermore, only one patient developed anti- drug antibodies with a low ADA titer of 120, which had no impact on PK, PD, safety, or efficacy.

This study had a number of limitations. First, CB 1 mL was chosen as the active comparator based on the clinical experience of the investigators and because it is the standard dose used in China where the study was performed. Second, this study enrolled patients who have contraindications, intolerance, or inadequate response to NSAIDs and/or colchicine, based on their physician's assessment instead of prespecified rigorous criteria. This was an exploratory Phase II trial and the sample size (approximately 30 patients per group) was not determined based on formal statistical power calculations. Instead, it was based on regulatory expectations for early-phase clinical studies in China, feasibility considerations, and experience from previous similar biologic trials. Therefore, the study was not powered for confirmatory hypothesis

testing, and the observed *p* values, including the marginal difference between the SSGJ- 613300mg and compound betamethasone groups, should be interpreted as exploratory rather than definitive. The limited sample size also increases the risk of type II error and restricts the reliability of subgroup analyses, which were therefore not pre- specified or statistically powered. Consequently, these findings should be considered hypothesis- generating and primarily used to inform the de sign and sample size estimation of future adequately powered Phase III confirmatory trials. Because this was a phase II study, we chose to leave the definition of inadequate response or contraindication to the attending clinicians thus reflecting routine clinical practice, in order to gather data from this study to help define the patient population for inclusion in our phase III study.

In conclusion, a single dose of SSGJ- 613 effectively alleviated pain and inflammation associated with acute gouty arthritis flares, leading to rapid and clinically significant improvements in quality of life (as assessed using the SF- 36) for Chinese gout patients. Treatment with SSGJ- 613 was found to be safe and well- tolerated, with no new safety concerns identified compared to the established safety profile of Canakinumab. To validate the efficacy and safety of SSGJ- 613 in Chinese patients with gout, additional clinical trials are planned.

Author Contributions

This study was conceptualized and supervised by Yu Xue and Hejian Zou. Yu Xue and Xiaoxia Zhu contributed to data curation, patient recruitment, reviewing and editing the manuscript. Baoyu Zhang, Yanlin Li, Wei Gou, Lie Dai, Ning Zhang, Lin Tang, Zhenchun Zhang, Lingli Dong, Xiaobing Yang, Jian Xu, Zhijun Li, and Jing Yang participated in patient recruitment, clinical assessments, and data collection. All authors have read and approved the final version of the manuscript. Notably, Xiaoxia Zhu is marked with equal contribution alongside Yu Xue.

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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. **Data S1:** apl70798-sup-0001-supinfo.docx.

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ORIGINAL ARTICLE

Association Between Chikungunya Infection and Rheumatoid Arthritis in Taiwan—A Cross-Sectional Study

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ABSTRACT

Objectives: Chikungunya virus (CHIKV) infection might cause chronic chikungunya arthritis resembling rheumatoid arthritis (RA). The purpose of this study is to investigate the potential role of this virus in the pathogenesis of RA in Taiwan, a non-endemic area.

Methods: This cross-sectional study enrolled 98 RA patients and 200 healthy controls. Serum CHIKV IgM and IgG were tested using two commercial enzyme-linked immunosorbent assay (ELISA) kits (Abcam and Euroimmun) and an in-house neutralization assay. ELISA-IgM was performed only in patients with RA. ELISA or neutralization positive samples were further tested for CHIKV RNA by reverse-transcription-polymerase chain reaction. Associations between CHIKV seropositivity and various autoantibodies were analyzed. The agreements among these assays were also evaluated.

Results: Neutralizing antibodies were detected in 2 of 98 RA patients and in none of the controls ($p = 0.107$, Fisher's exact test; $p = 0.043$, chi-square). Both patients were absent from Abcam-IgM and -IgG, as well as Euroimmun-IgM and -IgG testing. There was no association between Abcam-IgM or Euroimmun-IgM and rheumatoid factor, anti-cyclic citrullinated peptide, antinuclear, or anti-extractable nuclear antigen antibodies. Poor agreements were noted between the neutralizing assay and Abcam-IgG or Euroimmun-IgG ($\kappa = 0$ and 0, respectively). A similar finding was also found between Abcam-IgM and Euroimmun-IgM ($\kappa = -0.38$).

Conclusion: Our study was not sufficiently powered to establish a causal association between CHIKV infection and RA development. Poor agreements were noted among different commercial ELISA kits and neutralization assays, underscoring the limitations of the serology assay.

Ping-Chang Lin and Ching-Yi Tsai made equal contributions.

Key Points

1. Our study was insufficiently powered to establish a definitive association between CHIKV infection and RA development due to low prevalence.
2. Testing results should be interpreted judiciously because of poor agreement among different assays.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory systemic autoimmune disease characterized by synovial inflammation, progressive joint damage, and long- term disability, with possible extra- articular involvement. The etiology is multifactorial, involving genetic predisposition, sex, and environmental factors, including smoking and viral infections such as parvovirus B19 (as suggested by our previous studies [1, 2]), retrovirus, and Epstein–Barr virus [3].

Chikungunya is a mosquito- borne disease caused by the chikungunya virus (CHIKV) infection. Acute CHIKV infection typically presents with fever, headache, rash, and debilitating arthralgia that resolves within weeks of infection in most cases, but a subset of patients develops chronic polyarthritis lasting months to years [4]. Imaging of the affected joints in these patients demonstrates synovial inflammation, pannus formation, and bone erosion resembling RA [5–7]. Epidemiological studies have shown that up to 10%–30% of CHIKV- infected individuals meet the classification criteria for RA [8, 9]. Moreover, being female and smoking are risk factors for developing chronic chikungunya arthritis, as in RA [10–12].

Similar to RA, chronic chikungunya arthritis can be effectively treated using conventional disease- modifying anti-rheumatic drugs (DMARDs) such as methotrexate, hydroxychloroquine, sulfasalazine, or biologics such as etanercept or adalimumab [13, 14].

Immunological studies further reveal a significant overlap between CHIKV- induced arthritis and RA, including shared proinflammatory cytokine patterns, matrix metalloproteinase, immune cell infiltration, and autoantibody profiles [15–19]. The transcriptomic analysis also shows a connection between CHIKV infection and RA [20].

Although convincing evidence links CHIKV infection to chronic arthritis and RA development, most of these studies were performed in endemic areas, and a similar study in non- endemic areas is still lacking. There are several methods to detect the CHIKV antibody, and the accuracy of commercial kits versus the neutralization test, a standard method, remains uncertain, particularly in autoimmune patients prone to cross- reactivity and false positivity [21].

The purpose of this study is to investigate whether CHIKV infection plays a role in the development of RA in Taiwan, a non- endemic area, and to compare the consistency of these measurement methods.

2 | Materials and Methods

2.1 | Study Design and Participants

We conducted a cross- sectional study at Kaohsiung Medical University Hospital (KMUH), Taiwan. A total of 98 patients with established RA and 200 healthy controls were enrolled from 2020 to 2022, with all RA patients meeting the 2010 EULAR- ACR classification criteria 22]. Written informed consent was obtained from all participants, while the study was approved by the KMUH Institutional Review Board (KMUHIRB- E(I)-20200061) and conducted in accordance with the Declaration of Helsinki.

2.2 | Rheumatologic Marker Testing

Rheumatoid factor (RF) was measured with the Siemens N Latex RF kit (Siemens Healthcare Diagnostics, Erlangen, Germany). Anti- cyclic citrullinated peptide (anti-CCP) and anti-extractable nuclear antigen (anti- ENA) antibodies were detected using the EliA CCP and EliA Symphony kits, respectively (Phadia AB, Uppsala, Sweden). Antinuclear antibodies (ANA) were assessed by the indirect immunofluorescence method (Euroimmun, HEp-20-10 EUROPattern).

2.3 | CHIKV Antibody Testing and Neutralizing Antibody Assay

CHIKV IgM and IgG were tested using Abcam Human ELISA Kits (Abcam, Cambridge, UK) and Euroimmun ELISA Kits (Euroimmun, Lübeck, Germany). IgM testing was performed only in RA patients, not in healthy controls, because healthy controls were typically free of any symptoms or signs of chikungunya infection, and Taiwan is a non- endemic area. In Euroimmun kits, the recombinant structural protein of CHIKV was used as the antigen with a ratio of the extinction value of the sample over the extinction value of the calibrator being calculated. The ratios $\geqq 1.1$, $\geqq 0.8$ to < 1.1, and < 0.8 were interpreted as positive, borderline, and negative, respectively. In Abcam kits, CHIK viral antigen was used to detect the anti- chikungunya antibody with the standard units of > 11, 9–11, and < 9 being interpreted as positive, gray zone, and negative, respectively. Neutralizing antibodies were detected using an in- house assay developed by Lin et al. [21].

2.4 | CHIKV RNA Detection

Reverse transcription- polymerase chain reaction (RT-PCR) was performed on samples positive for CHIKV IgM or neutralizing antibodies to evaluate whether the subjects had an ongoing infection. The SYBR Green assay was performed to detect CHIKV genomic RNA. This assay targeted the E1 gene using specific primers recommended by the Taiwan Centers for Disease Control: CHIK-forward (5′-AAGCT YCGCGTCCTTTACCAAG-3′) and CHIK- reverse (5′-CCAAATTGTCCYGGTCT TCCT-3′). A clinical sample was defined as CHIKV- positive if the cycle threshold (Ct) value was ≤ 35 and the melting temperature (Tm) was $\geq 79°C$. Samples with a $Ct > 35$ or $Tm < 79°C$ were considered negative.

2.5 | Statistical Analysis

For statistical testing, the SPSS software (IBM Corp., Armonk, NY) was used. The *t*- test was used to compare the age difference between RA patients and controls, while the chi- square or Fisher's exact test was used to evaluate the associations between categorical variables. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using the Haldane–Anscombe correction to account for zero cell counts, with Cohen's κ being used to assess the agreement between different assays. Statistical significance was defined as $p < 0.05$.

3 | Results

3.1 | Demographic Characteristics of RA Patients and Healthy Controls

The *t*- test and chi-square tests were used to evaluate the differences in age and sex distributions, respectively, in RA patients and controls (Table 1). There were no significant differences between these two groups.

3.2 | Chikungunya Antibody and RT-PCR Testing Results in RA Patients and Healthy Controls

There was no significant difference in Euroimmun- IgG seropositivity between RA patients and controls ($p = 0.476$; Table 2). Two of 98 RA patients had positive neutralizing- IgG antibody in comparison to none of 200 controls (Fisher's exact test, $p = 0.107$; Haldane–Anscombe correction, OR = 10.4, 95% CI = 0.5–217; chi- square test, $p = 0.043$). Although the OR was high, statistical significance could not be obtained due to the low number of cases with positive neutralizing- IgG antibodies. No IgM testing was performed in controls. RT-PCR was further performed on all samples that were positive for Abcam-IgM, Euroimmun-IgM, or neutralization-IgG, and all yielded negative results (data not shown).

3.3 | Association Between CHIKV IgM Positivity and Autoantibodies in RA

Abcam- IgM and Euroimmun-IgM showed no significant associations with RF, anti- CCP, ANA, or anti-ENA antibodies (Table3).

3.4 | Agreement Between Neutralization-IgG and ELISA IgG Results

Cohen's κ was 0 for both Abcam- IgG versus neutralizing-IgG and Euroimmun- IgG versus neutralizing-IgG, with an overall agreement of 98% for each comparison (Table 4). Although the overall agreements were high, the κ values were 0, indicating no agreement beyond chance. The positive agreements of 0% highlight a complete lack of concordance in identifying positive cases in these assays.

3.5 | Agreement Between Abcam-IgM and Euroimmun-IgM ELISA Kits

For IgM assays, the positive agreement between the two kits was 0%, the negative agreement was 91.5%, and the overall agreement was 89.3%. The κ value was −0.38, indicating poor concordance (Table 5).

TABLE 2 | Chikungunya antibody testing results in RA patients and controls.

	RA ($n = 98$)	Control ($n = 200$)	OR (95% CI)	*p*
Abcam (ELISA)-IgM				
Positive	7	NA		NA
Gray zone	14	NA		
Negative	77	NA		
Abcam (ELISA)-IgG				
Positive	0	0		NA
Gray zone	0	0		
Negative	98	200		
Euroimmun (ELISA)-IgM				
Positive	2	NA		NA
Borderline	0	NA		
Negative	96	NA		
Euroimmun (ELISA)-IgG				
Positive	0	1		0.476
Borderline	0	2		
Negative	98	197		
Neutralizing-IgG				
Positive	2	0	10.4 (0.5–217)	0.107[a]
Negative	96	200		

Note: IgM tests were not performed in controls.
Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio; RA, rheumatoid arthritis.
[a]Fisher's exact test was used for statistical analysis, and Haldane–Anscombe correction was used to measure the OR and 95% CI. The *p*- value would be 0.043 if the chi- square test was applied.

TABLE 1 | Demographic characteristics of RA patients and controls.

	Age (mean ± SD, years)	95% CI	*p*	Sex (Female: Male)	OR (95% CI)	*p*
RA ($n = 98$)	51.70 ± 13.96	−0.400~5.889	0.087	76: 22	0.974 (0.545~1.741)	0.930
Controls ($n = 200$)	48.96 ± 10.07			156: 44		

Note: The *t*- test was used for statistical analysis of the age difference between RA patients and controls. The chi-square test was used for statistical analysis of sex distribution between RA patients and controls.
Abbreviations: CI, confidence interval; OR, odds ratio; RA, rheumatoid arthritis.

TABLE 3 | Chikungunya ELISA IgM antibodies in rheumatoid arthritis patients by status of autoantibodies.

	Abcam-IgM				Euroimmun-IgM			
Autoantibodies	**Positive ($n=7$)**	**Gray zone ($n=14$)**	**Negative ($n=77$)**	***p***	**Positive ($n=2$)**	**Borderline ($n=0$)**	**Negative ($n=96$)**	***p***
RF (+)	5	12	58	0.663	2	0	73	0.429
RF (–)	2	2	19		0	0	23	
Anti-CCP (+)	6	12	63	0.916	2	0	79	0.513
Anti-CCP (–)	1	2	14		0	0	17	
ANA ≧1:40 (+)	3	4	35	0.502	1	0	41	0.837
ANA ≧1:40 (–)	4	10	42		1	0	55	
ANA ≧1:80 (+)	1	1	26	0.088	1	0	69	0.498
ANA ≧1:80 (–)	6	13	51		1	0	27	
Anti-ENA (+)	0	1	11	0.446	0	0	12	0.594
Anti-ENA (–)	7	13	66		2	0	84	

Abbreviations: ANA, antinuclear antibody; anti- CCP, anti-cyclic citrullinated peptide; anti-ENA, anti-extractable nuclear antigen; RF, rheumatoid factor.

TABLE 4 | Agreement between neutralizing- IgG and ELISA-IgG results in rheumatoid arthritis patients.

	Neutralizing-IgG		Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)	κ
	Positive ($n=2$)	**Negative ($n=96$)**				
Abcam-IgG						
Positive ($n=0$)	0	0	0.0 (0.0–65.8)	100 (96.2–100.0)	98.0 (92.9–99.4)	0
Negative ($n=98$)	2	96				
Euroimmun- IgG						
Positive ($n=0$)	0	0	0.0 (0.0–65.8)	100 (96.2–100.0)	98.0 (92.9–99.4)	0
Negative ($n=98$)	2	96				

Abbreviations: CI: confidence interval; κ: Cohen's kappa.

TABLE 5 | Agreement between Abcam- IgM and Euroimmun-IgM results in rheumatoid arthritis patients.

	Euroimmun-IgM		Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)	κ (95% CI)
	Positive	**Negative**				
Abcam-IgM						
Positive ($n=7$)	0	7	0.0 (0.0–65.8)	91.5 (83.4–95.8)	89.3 (80.9–94.3)	−0.38 (−0.081–0.004)
Negative ($n=77$)	2	75				

Abbreviations: CI, confidence interval; κ, Cohen's kappa.

3.6 | Clinical Profiles of Neutralizing Antibody- Positive RA Patients

Both RA patients with detectable neutralizing antibodies were RF- positive and anti-CCP-positive. ANA titers were elevated (1:160 and 1:40), and anti- ENA tests were negative.*HLA-DR* genotyping revealed *DR9/DR12* in one patient and *DR15/DR15* in the other. Radiography showed bone erosion in one patient and joint- space narrowing in both. Both patients were treated with hydroxychloroquine, sulfasalazine, and methotrexate; one also received abatacept (Table 6).

4 | Discussion

In this study, we did not have robust evidence to show an association between CHIKV infection and the development of RA.

TABLE 6 | Clinical characteristics of rheumatoid arthritis patients with positive Chikungunya neutralizing antibodies.

	Patient 1	Patient 2
Anti-chikungunya Ab		
Abcam-IgG	N	N
Abcam-IgM	N	N
Euroimmun-IgG	N	N
Euroimmun-IgM	N	N
Neutralizing Ab	P	P
RF	P	P
Anti-CCP	P	P
ANA titer	1:160	1:40
Anti-ENA	N	N
HLA-DR	*DR9/DR12*	*DR15/DR15*
X-ray findings		
Bone erosion	Present	Absent
Joint space narrowing	Present	Present
Treatment		
DMARDs	Hydroxychloroquine, sulfasalazine, methotrexate	Hydroxychloroquine, sulfasalazine, methotrexate
Biologics	Abatacept	None

Abbreviations: Ab, antibody; ANA, antinuclear antibody; anti- CCP, anti-cyclic citrullinated peptide; anti-ENA, anti-extractable nuclear antigen; DMARDs, disease-modifying antirheumatic drugs; N, negative; P, positive; RF, rheumatoid factor.

The presentation of chronic chikungunya arthritis in many patients could mimic RA clinically, and the pathogenesis of both diseases might be similar [23–25]. Besides sharing synovitis and bone erosion, elevated proinflammatory cytokines such as IL-1β, IL- 6, IL-17, and TNFα, etc. can be seen in the serum and synovium in both diseases [15, 26, 27]. Some differences also exist: RA patients have higher prevalences of RF and anti- CCP antibody than those in chronic chikungunya arthritis patients [15].

Up to half of CHIKV- infected patients might develop chronic chikungunya arthritis with some of these potentially developing RA later in life, fulfilling the 1987 ACR or 2010 ACR/EULAR classification criteria [22, 28–30]. In this study, two RA patients were found to be neutralizing antibody- positive, indicating a previous CHIKV infection. One of these two RA patients had lived in Zhuhai City, Guangdong Province, for more than 10 years, a province in southern China with a history of local outbreaks of chikungunya, and had come back to Taiwan due to the onset of polyarthritis. The other patient had a travel history to Thailand, an endemic area. Both cases had positive RF, anti-CCP, ANA, and typical radiographic findings. Similar findings can also be demonstrated in some patients with chronic chikungunya arthritis [30].

The linkages between chronic chikungunya arthritis and immune- related genes have been demonstrated [31]. Bouquillard et al. [30] demonstrated that two- thirds of RA patients following CHIKV infection have HLA- DRB1*04 or 01. Our previous study also suggested that the HLA- DR4 allele is related to the development of RA in Taiwan [32]. Thanapati et al. [33] revealed that the patients with DRB1*04/DQB1*03 haplotype are susceptible to CHIKV infection; however, neither of these two neutralizing antibody- positive RA patients had HLA-DR4. Owing to the small sample size of neutralizing antibody- positive RA patients, the association between HLA genotype and the development of RA after CHIKV infection could not be established.

In this study, we detected chikungunya antibody using various methodologies and compared them. We used the viral neutralizing antibody as the reference standard [21], which has a specificity and sensitivity of 100%. We also performed tests using the Euroimmun and Abcam kits for both IgM and IgG. It is known that the Euroimmun- IgM test for chikungunya tends to cross- react with dengue virus, Zika virus, and other non- chikungunya alphaviruses more readily than the IgG test [34, 35]; it is possible that similar results can be seen for the Abcam- IgM and -IgG kits. This hypothesis is supported by our RT-PCR results, which revealed that no CHIKV RNA could be detected in samples with positive IgM. Moreover, false- positive and false- negative results of the chikungunya ELISA IgM test might occur in patients with positive RF, as shown in the IBL CHIKV ELISA test manual. The sensitivity and specificity of the Euroimmun ELISA- IgG test are also lower than those of the neutralizing test, as indicated in the instruction manual, while the Abcam ELISA- IgG test may also yield similar results. The interpretation of the Chikungunya ELISA test should therefore be performed carefully. In this study, the positivity of Abcam-IgM and Euroimmun- IgM does not relate to the presence of RF.

This study showed an extremely poor agreement (Cohen's $\kappa = 0$) between neutralizing antibody and Abcam- IgG or Euroimmun-IgG, although they had high overall agreements, which were caused by high negative agreements, a common finding in a very low- prevalence area. A similar finding could also be found between Euroimmun- IgM and Abcam-IgM. The discordances between ELISA and neutralization assays appear related to cross- reactions among alphaviruses, with arboviruses, and non-specific reactions in autoimmune disease patients.

There are two major limitations in this study. First, the low prevalence of CHIKV- neutralizing antibody positivity limits the statistical power to establish an association between CHIKV infection and the development of RA. Second, the cross- sectional nature of this study masks the chronological information between exposure and outcome and prevents causal inference. Because Taiwan is a non- endemic area for chikungunya infection, it is difficult to solve these two problems.

In summary, this study does not have enough power to conclude that CHIKV infection might be an environmental trigger factor in RA development in Taiwan, although a potential association between CHIVK infection and RA cannot be excluded. The results in this study are preliminary and are merely at the hypothesis-generating stage in an attempt to investigate the possible relationship between CHIKV infection and RA. The discrepancies among serologic assays underscore the importance of confirmatory testing, such as a neutralizing antibody test, and careful interpretation is needed, especially in patients with autoimmune diseases.

Author Contributions

Chang- Yi Yen: writing – original draft, formal analysis, investigation. Chang- Chi Lin: validation, methodology, data curation. Ping-Chang Lin: methodology, formal analysis, data curation. Ching- Yi Tsai: methodology, data curation. Li- Teh Liu: methodology, formal analysis. Chen-Hsuan Lin: validation, formal analysis. Chun- Hong Chen: methodology, conceptualization. Chia- Chun Tseng, Hua-Chen Chan, Cheng-Chin Wu, and Tsan- Teng Ou: investigation. Jih-Jin Tsai: writing – review and editing, visualization, validation, resources, funding acquisition, conceptualization. Jeng- Hsien Yen: supervision, conceptualization.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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