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LETTER TO THE EDITOR

Unequal Risk Relationship Between Sjögren's Syndrome and Autoimmune Thyroid Disease: A Mendelian Randomization Study

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Dear Editor,

Autoimmune thyroid disease (AITD) is a chronic autoimmune disease characterized by thyroid dysfunction [1], primarily comprising two types: Hashimoto thyroiditis (HT) and Graves' disease (GD) [2]. HT, the most common form of autoimmune thyroiditis (AIT), is characterized by the presence of thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb) in serum [3, 4]. GD is the main cause of hyperthyroidism, characterized by positive serum thyroid stimulating hormone receptor antibodies (TRAb) [5, 6]. With the increasing research on AITD, some researchers have found a close association between Sjögren's syndrome (SS) and AITD [7]. Related studies have indicated that patients with primary SS (pSS) have a higher risk of AITD compared to the general population [8]. Additionally, the risk of SS is also higher in patients with GD [9]. However, some clinical reports have denied the causal relationship between SS and the risk of AITD [10, 11]. The inherent connection between SS and AITD remains a controversial topic. As a genetic analysis method that minimizes confounding factors and reverse causation, Mendelian randomization (MR) offers a new direction for addressing this issue [12]. This study employs bidirectional MR to elucidate the causal interplay between SS and AITD.

First, we obtained datasets for SS (code: finngen_R12_M13_SJOGREN; sample size: 487569 Europeans), AIT (code: finngen_R12_E4_THYROIDITAUTOIM; sample size: 425896 Europeans), and GD (code: finngen_R12_E4_GRAVES_STRICT; sample size: 500348 Europeans) from the FinnGen database (www.finngen.fi/fi). Subsequently, we identified single nucleotide polymorphisms (SNPs) closely associated with each dataset using a threshold of $p < 5 \times 10^{-8}$. However, this stringent criterion resulted in an insufficient number of SNPs for our analysis. Consequently, we relaxed the thresholds and identified SNPs associated with AIT, GD, and SS using thresholds of $p < 5 \times 10^{-5}$. While relaxing the significance threshold reduced the mean SNP strength, it significantly increased the total SNP strength. Next, we filtered the SNPs based on $R^2 < 0.01$, a distance of 1000 kb, and an F-statistic greater than 10 to exclude the influence of linkage disequilibrium and weakly associated variables. We then utilized LDlink to remove SNPs that contained confounding factors. Finally, we paired and excluded palindromic SNPs and applied the MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) method to identify and exclude outlier SNPs. Through these steps, we identified 78 SNPs associated with SS-AIT, 76 SNPs associated with SS-GD, 50 SNPs associated with AIT-SS, and 89 SNPs associated with GD-SS, as detailed in Tables S1–S4.

Subsequently, MR analysis was conducted using *R* 4.3.1 software, with inverse variance weighted (IVW) as the primary tool and MR-Egger and weighted median as secondary tools. The results showed that SS was associated with an increase in AIT (odds ratio [OR] 1.116, 95% confidence interval [CI] 1.002–1.245, $p = 0.047$)

and GD risk (OR 1.077, 95% CI 1.015–1.142, $p=0.014$); GD was associated with an increase in SS risk (OR 1.071, 95% CI 1.008–1.137, $p=0.027$), while AIT was not associated with SS risk (OR 1.004, 95% CI 0.97–1.04, $p=0.810$), as shown in Figure 1.

Then, horizontal pleiotropy was assessed using MR-Egger, where a $p \geq 0.05$ indicated compliance with the exclusivity assumption. The results revealed no evidence of horizontal pleiotropy for any of the analyses (SS-AIT: $p=0.376$; SS-GD: $p=0.292$; AIT-SS: $p=0.157$; GD-SS: $p=0.166$), as shown in Table S5. Furthermore, heterogeneity was examined using Cochran's Q tests, with a $p \geq 0.05$ indicating no significant heterogeneity. The results showed no heterogeneity in the analysis of SS-AIT ($p=0.404$) and AIT-SS ($p=0.116$); however, significant heterogeneity was detected in the analysis of SS-GD ($p<0.001$) and GD-SS ($p=0.006$), as shown in Table S6. Additionally, leave-one-out sensitivity analyses were performed to assess the robustness of the MR estimates. The stability of the results was confirmed for all causal relationships, as no single genetic variant disproportionately influenced the associations, as shown in Figure 2.

Published clinical studies have supported SS as a risk factor for AITD. A prospective cohort study in the United Kingdom showed that 15.8% of patients with pSS would develop AITD over a 25-year follow-up period [13]. A meta-analysis that included eight clinical studies showed that SS patients had a higher risk of AITD (OR 3.48, 95% CI 1.59–7.63), hyperthyroidism (OR 2.61, 95% CI 1.73–3.92), and hypothyroidism (OR 3.81, 95% CI 1.86–7.83) than the control groups [7]. These pieces of evidence point to SS as a risk factor for AITD, but they do not compare the role of SS on two different subtypes of AIT and GD. In an early clinical study, Hansen BU et al. [14] reported a ninefold higher prevalence of AIT in Swedish patients with pSS than in the general population. Pérez B et al. [15] also found that the prevalence of AIT in Mexican patients with pSS was approximately 24%, significantly higher than the North American prevalence of 7.8%. These pieces of evidence support that SS is a potential risk factor for AIT in Swedes and Mexicans, consistent with our findings. However, a case–control study in Turkey indicated that TPOAb and TGAb positivity rates in patients with pSS were comparable to those in the healthy population (11% vs. 8%) [10]. We hypothesize that the conflicting findings were due to regional differences, as the first two studies were conducted in Northern Europe and North America, whereas the latter study was located in Eastern Europe. This view was supported by Tunc R et al. [10], who found that the rate of antithyroid antibody positivity in Turkish patients with pSS was lower than in other countries. Therefore, although our MR analysis supports SS as a risk factor for AIT in Europeans, this causal relationship may not be applicable to Europeans in all regions.

Additionally, in published studies, Zeher M et al. [16] found no association between pSS and GD, whereas Ter Borg EJ et al. [17] reported a prevalence of GD of 2.7% in patients with pSS, similar to the prevalence of GD in the general female population (2.7% vs. 3.0%) [18]. These findings suggest that SS is not a risk factor for GD, which contrasts with our findings. It is worth noting that the studies on the impact of SS on the risk of GD published so far have all been based on small samples, and the negative results may be related to inadequate sample size. Although our MR analysis supports SS as a potential risk factor for GD, confirmation from clinical studies with larger samples is warranted.

Furthermore, in an Italian prospective study, Ferrari SM et al. [9] reported that patients with GD had a significantly higher risk of developing SS than the healthy population (0.8% vs. 0.1%). Coll J et al. [19] also showed that the prevalence of SS was much higher in Spanish patients with GD than in the general population (20% vs. 0.33%) [20]. These pieces of evidence suggest that GD is a potential risk factor for SS, which is consistent with our MR results.

Moreover, Scofield RH et al. [11] found no significant difference in the risk of SS among AIT patients compared to the same baseline level in the American population. In stark contrast, Fallahi P et al. [21] reported a significantly higher prevalence of SS in Italian patients with AIT than in the general population (0.9% vs. 0.2%), who suggested that AIT is a risk factor for SS. Concerningly, there is limited clinical research on this matter, and the current evidence is inadequate to ascertain the impact

Exposure	Outcome	SNPs	Method	P value	OR (95% CI)
SS	AIT	78	IVW	**0.047**	1.116 (1.002 to 1.245)
SS	AIT	78	MR Egger	0.844	1.023 (0.819 to 1.277)
SS	AIT	78	Weighted median	0.136	1.133 (0.961 to 1.336)
SS	GD	76	IVW	**0.014**	1.077 (1.015 to 1.142)
SS	GD	76	MR Egger	0.790	1.017 (0.901 to 1.148)
SS	GD	76	Weighted median	0.068	1.073 (0.995 to 1.157)
AIT	SS	50	IVW	0.810	1.004 (0.970 to 1.040)
AIT	SS	50	MR Egger	0.257	0.958 (0.891 to 1.030)
AIT	SS	50	Weighted median	0.862	1.004 (0.958 to 1.053)
GD	SS	89	IVW	**0.027**	1.071 (1.008 to 1.137)
GD	SS	89	MR Egger	**0.026**	1.162 (1.021 to 1.323)
GD	SS	89	Weighted median	0.228	1.051 (0.969 to 1.139)

FIGURE 1 | Forest plot of MR analysis on the causal relationship between SS and AITD. SS, Sjögren's syndrome; AITD, autoimmune thyroid disease; AIT, autoimmune thyroiditis; GD, Graves' disease. IVW, inverse variance weighted.

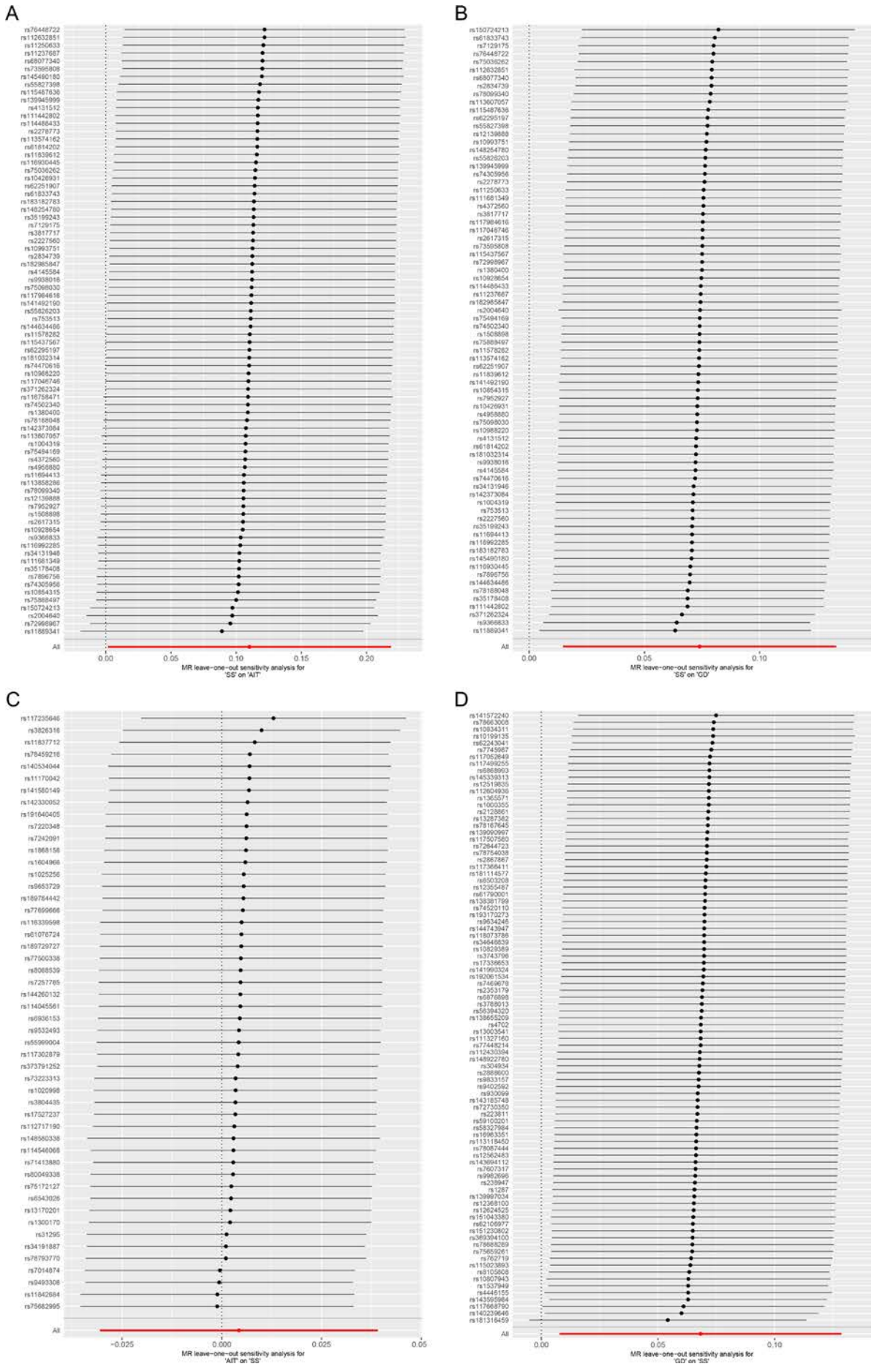

FIGURE 2 | Legend on next page.

FIGURE 2 | Leave-one-out sensitivity analyses on causal relationships between SS and AITD. (A) SS on AIT; (B) SS on GD; (C) AIT on SS; (D) GD on SS. SS, Sjögren's syndrome; AITD, autoimmune thyroid disease; AIT, autoimmune thyroiditis; GD, Graves' disease.

of AIT on SS. At least in this MR analysis, we did not find a role for AIT on SS risk.

There are some limitations to this MR analysis. First, the generalizability of our findings across ethnicities is limited. This study focused primarily on individuals of European ancestry, establishing causal relationships between SS and AITD within this population. However, these causal effects may differ substantially across ethnic groups, such as Asian and African populations. Despite an extensive search across multiple databases, we were unable to identify datasets for SS that included individuals of Asian or African descent, which prevented us from conducting MR analyses specific to these populations. It is important to note that factors such as regional, dietary, and cultural heterogeneity can lead to markedly different causal estimates in MR analyses based on different data sources. Even within European populations, genetic variation may differ significantly—for example, between Northern Europeans (primarily of Germanic descent) and Eastern Europeans (primarily of Slavic descent). Therefore, our findings should be interpreted with caution and require validation through independent cohorts and cross-ancestry replication. Second, while our MR analysis revealed no significant genetic association between AIT and SS risk, this null finding currently lacks corroborating evidence from prospective clinical cohorts and functional studies interrogating shared immunological pathways. Therefore, further research is needed to explore the potential underlying mechanisms and validate these findings in larger and more diverse cohorts. Finally, there may be unrecognized pathways or confounding factors between exposure factors and outcome indicators, which may increase the bias risk of MR results. Given these limitations, we expect to advance MR analysis of different races in the future and explore the causal relationships between SS and AITD through large-scale clinical studies.

In conclusion, this MR analysis demonstrates that SS is a potential risk factor for AIT and GD, whereas GD rather than AIT is a potential risk factor for SS. However, due to limited clinical evidence, these results need to be further confirmed in subsequent clinical studies with large samples.

Author Contributions

Jingyi Wu: writing – original draft, methodology, data curation. **Chenxi Yang:** writing – original draft. **Yunfeng Yu:** writing – original draft. **Gang Hu:** writing – original draft. **Rong Yu:** writing – review and editing. **Chuanlong Zhou:** writing – review and editing, conceptualization, supervision.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

Jingyi Wu
Chenxi Yang
Yunfeng Yu
Gang Hu
Rong Yu
Chuanlong Zhou

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1.** SNPs from GWAS on the exposure (SS) and outcome (AIT). **Table S2.** SNPs from GWAS on the exposure (SS) and outcome (GD). **Table S3.** SNPs from GWAS on the exposure (AIT) and outcome (SS). **Table S4.** SNPs from GWAS on the exposure (GD) and outcome (SS). **Table S5.** The MR-Egger intercept analysis of horizontal pleiotropy. **Table S6.** The Cochrane Q test of heterogeneity.

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ORIGINAL ARTICLE

Exploring the Targets of Gulao Yukang Pill in Rheumatoid Arthritis via the Hippo Signaling Pathway: An Integrated Network Pharmacology and Experimental Validation Study

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Keywords: Gulao Yukang pill | Hippo signaling pathway | network pharmacology | rheumatoid arthritis | treatment

ABSTRACT

Objective: Investigating the Mechanism of Gulao Yukang Pill (GLYK) in Treating Rheumatoid Arthritis (RA) via the Hippo signaling pathway and Its Regulatory Effects on *Th17*/*Treg* Cell Balance.

Methods: Network pharmacology was applied to construct a network mapping the interactions between drug active ingredients and target genes, pinpointing the crucial active components and genetic targets through which GLYK exerts its effects on RA. Using Gene Set Variation Analysis (GSVA) in R4.2.2, we predicted the pathway activity scores of 7 Hippo pathways between the disease and control groups and selected those with significant differences. Key genes were correlated with the selected pathways, and target genes related to the Hippo pathway were screened. The binding capacity of crucial active components and their corresponding target genes was predicted using the Deep Purpose algorithm framework. Targets acting on RA were screened, and a CIA (Collagen-Induced Arthritis) animal model was developed for the purpose of further demonstrating the therapeutic impact of GLYK on RA and to verify the results of network pharmacology through histopathological observation, ELISA method detection, flow cytometry detection, Western blot detection, and qRT-PCR detection.

Results: Network pharmacology has identified nine key targets in the Hippo pathway associated with rheumatoid arthritis (RA) treatment, including Mst1 and TAZ, which are critical for GLYK's therapeutic effects as they are linked to its core effective ingredients that mediate the treatment of RA. Animal experiments validated the network pharmacology predictions, confirming that Mst1 and TAZ in the Hippo pathway are pivotal therapeutic targets for RA. GLYK suppresses inflammation and bone destruction by inhibiting the Hippo pathway components Mst1/TAZ, thereby reducing the *Th17*/*Treg* ratio.

Abbreviations: AI, Arthritis Index; CFA, complete Freund's adjuvant; CIA, collagen-induced arthritis; CTD, Comparative Toxicogenomics Database; DisGeNET, Disease Gene Network; DL, deep learning; DTI, drug–target interaction; ELISA, Enzyme-Linked Immunosorbent Assay; FLS, fibroblast-like synoviocytes; GLYK, Gulao Yukang Pill; GSVA, Gene Set Variation Analysis; IL-17, interleukin-17; IL-21, interleukin-21; IL-22, interleukin-22; JAK, Janus kinase; Mst1, mammalian sterile twenty1; NF-κB, nuclear factor kappa B; OPG, osteoprotegerin; PCNA, proliferating cell nuclear antigen; PVDF, polyvinylidene fluoride; qRT-PCR, quantitative real-time polymerase chain reaction; RA, rheumatoid arthritis; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; STAT, signal transducer and activator of transcription; SymMap, integrative database of traditional chinese medicine enhanced by symptom mapping; TAZ, transcriptional coactivator with PDZ-binding motif; TCM, traditional Chinese medicine; TCM-ID, Traditional Chinese Medicine Information Database; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; *Th17*, T helper cell 17; TNF-α, tumor necrosis factor-α; TRAP, tartrate-resistant acid phosphatase; *Treg*, regulatory T cell.

Conclusion: GLYK can influence the expression of upstream core molecules Mst1 and downstream effector molecules TAZ in the Hippo pathway, which can reduce the swelling and arthritis index of CIA rats, lower the proportion of *Th17*/*Treg* cells, inhibit inflammation, and bone destruction. The Hippo pathway is a goal of GLYK in the intervention of RA.

1 | Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammatory responses and progressive bone destruction [1]. Epidemiological studies [2, 3] indicate a global incidence rate of 0.5%–1%, with China's prevalence approximating 0.42%. Evidence-based medical research confirms that 50% of untreated RA patients develop disabilities within 2 years of disease onset, escalating to 70% by the third year [4]. RA-associated bone destruction progresses rapidly, ultimately leading to joint stiffness, deformity, and loss of function, severely compromising patients' quality of life. The primary therapeutic objective for RA involves delaying existing bone destruction and preventing its further progression. Therefore, it is crucial to explore effective measures for preventing bone destruction initiation and minimizing joint damage. Traditional Chinese Medicine (TCM) demonstrates potential in modulating immune responses, alleviating clinical symptoms, and enhancing physical constitution, offering novel approaches for the comprehensive management of RA comorbid conditions. Research findings indicate that TCM not only alleviates RA-related joint pain and swelling while reducing disease activity, but also holistically regulates systemic manifestations such as fatigue and cold intolerance. Furthermore, TCM demonstrates potential in delaying cardiopulmonary complications. Critically, it enables gradual dose reduction or discontinuation of conventional medications in certain RA patients while maintaining long-term therapeutic efficacy [5]. This therapeutic approach synergizes well with modern medical paradigms including the "treat-to-target (T2T)" strategy and "deep remission" objectives in RA management [6]. TCM posits that blood stasis obstruction constitutes a pivotal pathogenic factor in rheumatoid arthritis (RA) development. Blood stasis serves dual roles as both an etiological contributor and a persistent pathological product throughout the disease continuum. Particularly, the syndrome pattern of kidney deficiency with blood stasis has been identified as a critical determinant in RA pathogenesis and progression [7]. A retrospective cohort study showed that long-term TCM treatment not only had a positive effect on the occurrence time of extra-articular diseases in RA patients, but also helped to reduce the risk of extra-articular diseases. TCM can be flexibly applied to the whole treatment process of RA patients [8]. A matched cohort study based on 1383 individuals also confirmed that the use of TCM may be associated with a reduced risk of recurrent exacerbations in RA patients. These findings provide evidence to recommend TCM treatment for RA patients [9]. Clinical studies have demonstrated that GLYK, a representative agent of the kidney-tonifying and collateral-dredging therapeutic approach, exhibits favorable therapeutic efficacy in managing low-disease-activity rheumatoid arthritis (RA). This treatment modality demonstrates multidimensional benefits including symptom amelioration, quality of life enhancement, and a favorable safety profile with minimal adverse effects. Mechanistic investigations suggest its therapeutic actions may correlate with downregulation of serum interleukin-17 (IL-17) levels in RA patients, potentially modulating key inflammatory pathways implicated in disease progression [10]. GLYK, a hospital-manufactured preparation from the Affiliated Hospital of Gansu University of Chinese Medicine, has been extensively used in clinical practice for years. This formulation demonstrates significant therapeutic effects in improving clinical symptoms during active RA phases, reducing inflammatory markers, and ameliorating synovitis and bone destruction [11]. Studies have found that there are many main components of TCM in the treatment of RA, including alkaloids, flavonoids, terpenes, phenylalanine, etc. [12]. However, the potential molecular mechanism of GLYK in the treatment of RA is still unclear. Network pharmacology of TCM investigates the interactions among structure-efficacy-bioactive molecules as its entry point, employing multidisciplinary approaches including genomics, bioinformatics, and molecular biology to establish a component-target-disease regulatory network through disease-associated genes. This methodology enables deeper and more comprehensive understanding of TCM's mechanisms of action. Compared with conventional research paradigms, this biological network-oriented pharmacological intervention not only elucidates the working mechanisms of herbal formulas but also reveals drug effects on biological networks from a macro/systemic regulatory perspective. Consequently, this innovative conceptual framework and technical approach will provide novel research strategies and methodologies for investigating the mechanisms of multi-component herbal formulas [13]. GLYK, an effective traditional Chinese medicinal pill for clinical treatment of rheumatoid arthritis (RA), was investigated in this study through network pharmacology analysis to explore its active ingredients, therapeutic targets, related signaling pathways, and interactions with RA-associated genes. By establishing a collagen-induced arthritis (CIA) model, we validated the therapeutic effects and underlying mechanisms of GLYK in RA treatment, providing evidence-based support for its clinical application. This research not only comprehensively interprets the modern biological characteristics and inherent principles of the "Bushen Tongluo" (kidney-tonifying and collateral-dredging) therapeutic strategy, but also offers novel insights into traditional Chinese medicine approaches for RA management. The findings significantly contribute to elucidating the contemporary biological foundations of TCM theory while advancing innovative perspectives for integrative RA treatment methodologies.

2 | Methods

2.1 | Building the Drug-Active Ingredient-Target Gene Interaction Network

Based on the keywords of RA "RA," genes associated with the disease were predicted in the CTD [14] (https://ctdbase.org), DisGeNET [15] (https://www.disgenet.org/), and GeneCards [16] (http://www.genecards.org/) databases, and the intersection

of the three databases' prediction results was taken to select genes associated with the disease. In the HIT2 [17] (http://hit2.badd-cao.net), TCM-ID [18] (http://tcm.czgenomics.com:8000/tcmid), TCMSP [19] (http://tcmspw.com/tcmsp.php), and SymMap [20] (http://www.symmap.org) databases, the effective components of GLYK (Antler, Scorpion, Davallia trichomanoides Blume, Cinnamon, Centipede, Turtle Shell, Astragalus, JixueTeng, Panax notoginseng, Asini Corii Colla, Dried ginger, Qingfeng Teng, Glycyrrhiza uralensis Fisch) were retrieved, and effective active ingredients were finally selected based on the drug-likeness indicator QED (Quantitative Estimate of Drug-likeness) > 0.2 [21, 22]. According to the PubchemID of the selected GLYK active ingredients in HIT2, the target genes of the compounds were retrieved, and compounds without target genes identified in the database were removed. Using the Venn Diagram in R4.2.2, the overlap of RA-associated genes and GLYK target genes was taken, and the drug-active ingredient-target gene relationship network was set up utilizing Cytoscape software. Key active ingredients and RA-targeting genes were identified based on network topology metrics including Betweenness Centrality, Closeness Centrality, Degree, and Radiality.

Target Gene Screening: Select genes that simultaneously meet all three criteria of exceeding threshold values for (1) Betweenness Centrality, (2) Closeness Centrality, and (3) Degree. In cases of conflicting metrics, prioritize genes with higher Betweenness Centrality rankings (as it reflects global regulatory functions).

Active Compound Screening: Compounds fulfilling either condition: (1) Directly connected to ≥ 5 key target genes; (2) Ranked in the top 10% by comprehensive score (0.4\times Degree + 0.6\times Radiality).

Threshold determination criteria are as follows: The betweenness centrality threshold was established by plotting cumulative distribution curves and identifying points of abrupt slope change (inflection point analysis). The connectivity threshold was derived from Barabási's network science theory, where critical hubs are typically defined as nodes with ≥ 3 times the average degree. Parameter weight assignments followed the guidelines outlined in the Cytoscape official tutorial "Network Centrality Measures."

2.2 | GSVA Enrichment Analysis Based on the Hippo Pathway

Through keyword searches in the WikiPathways sub-database of the authoritative MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp), we identified entries containing "HIPPO" using the Boolean search query: '(gene_set_name LIKE "%HIPPO%") AND (subcollection = "WP")'.

This approach yielded 7 Hippo signaling pathway-related gene sets, as detailed below:

- GOBP_HIPPO_SIGNALING.
- GOBP_REGULATION_OF_HIPPO_SIGNALING.
- GOBP_NEGATIVE_REGULATION_OF_HIPPO_SIGNALING.
- GOBP_POSITIVE_REGULATION_OF_HIPPO_SIGNALING.
- REACTOME_SIGNALING_BY_HIPPO.
- WP_HIPPO_SIGNALING_REGULATION_PATHWAYS.
- WP_LEUKOCYTEINTRINSIC_HIPPO_PATHWAY_FUNCTIONS.

The Gene Set Variation Analysis (GSVA) algorithm (R version 4.2.2) was employed to estimate pathway activity scores of these seven pathways in samples from the GSE89408 dataset (https://www.ncbi.nlm.nih.gov/geo/). Kernel intensity estimation was performed using the default Gaussian kernel. The gene expression matrix was normalized using $\log_2$(FPKM+1) transformation, with the minimum gene set size set to 10 genes. A two-sample independent *t*-test was conducted on GSVA enrichment scores to identify pathways exhibiting significant differences between disease and control groups. Subsequently, Pearson correlation analysis was performed between the 18 key genes identified in section 2.1 and the filtered Hippo pathway, utilizing both GSVA enrichment scores and gene expression levels (TPM values).

2.3 | Prediction of the Binding Capacity of Key Active Ingredients and Genes Through Machine Learning Algorithm

Deep learning (DL) models have shown good performance in drug–target interaction (DTI) prediction. Deep Purpose [23] (https://github.com/kexinhuang12345/DeepPurpose) is a versatile and user-friendly Python library for deep learning, primarily utilized for DTI prediction. Within the scope of this research, we deployed the Deep Purpose algorithmic framework to forecast the binding strength between core active ingredients and their corresponding target genes.

2.4 | Experimental Animals and Drugs

The experimental animals were SPF-grade SD rats, purchased from the Experimental Animal Center of Gansu University of Chinese Medicine, and the animal experiment plan was approved by the Experimental Animal Ethics Committee of Gansu University of Chinese Medicine (Approval No.: 2023-421). Methotrexate tablets (Batch No.: 197240205) were purchased from Shanghai Shangyao Xinyi Pharmaceutical Co. Ltd, and GLYK (Approval No.: Z20190001000) was purchased from the affiliated hospital of Gansu University of Chinese Medicine. Animal experiments were conducted in accordance with ARRIVE guidelines 2.0. Rats were randomly divided into 6 groups using a random number table, and researchers were blinded to group assignments during data collection.

2.5 | Establishment of Animal Model [24]

The CIA (Collagen-Induced Arthritis) model is a widely utilized in vivo for studying rheumatoid arthritis (RA),

characterized by two critical features: first, the breakdown of immune tolerance, the severity of which correlates with disease activity and progression; second, the production of autoantibodies. Consequently, the CIA model has become the gold standard for RA research in vivo [24]. This model closely mimics the clinical manifestations observed in RA patients, exhibiting symptoms such as reduced muscle mass, exacerbated fatigue, and generalized weakness in locomotor tests. Additionally, it effectively replicates the rheumatoid arthritis-associated cachexia syndrome (RA-CDP), offering clinicians and researchers a more clinically relevant framework to investigate therapeutic strategies and prognostic predictions for RA-CDP [25, 26]. As a polyarthritis model, the CIA model induces synovial inflammation and bone destruction, making it particularly valuable for elucidating the mechanisms underlying RA-related bone damage.

Dissolve 10 mg of bovine type II collagen in 5 mL of acetic acid. Add 5 mL of Complete Freund's Adjuvant (CFA) and place the mixture in an ice bath (4°C). Slowly drip an additional 5 mL of CFA into the solution while emulsifying at 200 *g* until a water-in-oil emulsion forms. Combine the emulsified mixture with the bovine type II collagen solution to prepare a 1 g/L collagen emulsion. After 1 week of adaptive feeding, inject 0.2 mL of the collagen emulsion intradermally at the base of the rat tail. Administer a booster immunization 7 days after the primary injection. Rats in the normal control group receive an equal volume of physiological saline at the same injection site.

Arthritis Index (AI) Assessment:

Evaluate rats on day 7 post-booster immunization using the following criteria:

- 0 = No swelling;
- 1 = Mild redness/swelling in toe joints;
- 2 = Moderate swelling of toes and ankle;
- 3 = Severe swelling below ankle;
- 4 = Pan-articular swelling including ankle.

The AI score for each rat is calculated as the mean score of all four paws. A successful arthritis model is defined by an AI score ≥ 2 [27, 28].

2.6 | Experimental Grouping and Drug Administration Method

After successful model establishment, the rats were randomly divided into 6 groups using a random number table method. The sample size was calculated using G*Power 3.1 software. Based on one-way ANOVA (analysis of variance) with 6 comparison groups, an effect size (Cohen's *f*) of 0.25 (medium effect), $\alpha = 0.05$, and power = 0.80, the calculation indicated that 6 rats were required per group, resulting in a total sample size of 36 rats. The experimental groups are as follows: Normal Control (NC) group, Model Control (model) group, GLYK Low Dose (GL) group, GLYK Medium Dose (GM) group, GLYK High Dose group (GH group), Methotrexate (MTX) group. According to the methods in "Experimental Animal Science," the equivalent dose of GLYK was calculated to be 1.54 g/kg, and the high, medium, and low doses were 1.08, 0.54, and 0.27 g/kg, respectively, made into a suspension for intragastric administration [29, 30]. The control group and the model group underwent intragastric administrations of physiological saline, while methotrexate was given at an amount of 0.1 mg/kg once weekly. Treatment was given after successful modeling, and all treatments were administered orally for 42 days. SD rats ($n = 6$ per group) were used. All procedures were repeated in two independent cohorts (biological replicates).

2.7 | Histopathological Observation

The rats were anesthetized via intraperitoneal injection of 3% sodium pentobarbital (1 mL/100 g body weight). After anesthesia, the ankle joints were harvested and fixed in 4% paraformaldehyde solution. The decalcified tissues were rinsed with PBS, then gradually dehydrated through a graded alcohol series (80%, 90%, 95%-I, 95%-II, 100%-I, 100%-II), with each gradient maintained for 60 min. The specimens were embedded in paraffin at 55°C using a tissue embedding machine after wax impregnation. Serial sections of 4 μm thickness were prepared and stained with hematoxylin and eosin (HE) followed by dehydration and mounting. Pathological changes in ankle joints were examined under a light microscope. Three independent observers assessed the severity of inflammation using the following criteria: Synovial inflammation (0: normal; 1: mild diffuse inflammatory infiltration; 2: moderate infiltration; 3: severe inflammation with abscess formation) [31]. For osteoclast observation, sections were stained with TRAP staining solution for 1 h, counterstained with hematoxylin for 2 min, then dehydrated, cleared, and mounted with resinous mounting medium before microscopic examination. The number of TRAP-positive multinucleated cells was quantified per unit area (mm^2 of trabecular bone surface). Only cells exhibiting strong TRAP staining with ≥ 3 nuclei were counted, and overlapping fields were systematically excluded to prevent double-counting within the same microscopic region.

2.8 | Experimental Reagents

Primary reagents	Manufacturer (catalog number)
Hematoxylin and Eosin (HE) Staining Kit	Beyotime (C0105S)
TRAP Staining Kit	Servicebio (G1050-50T)
Rat IL-17A ELISA Kit	Biosharp (BSER-021-96T)
Rat IL-21 ELISA Kit	Cusabio (CSB-E11843r)
Rat IL-22 ELISA Kit	R&D Systems (M2200)
MST1 Antibody (Rabbit Polyclonal)	Abcam (ab231138)

Primary reagents	Manufacturer (catalog number)
TAZ Antibody (Rabbit Polyclonal)	Abcam (ab110239)
RORγt Antibody (Rabbit Polyclonal)	Thermo Fisher Scientific (PA5-86733)
Foxp3 Antibody (Rabbit Monoclonal)	Abcam (ab215206)
CD4 antibody (APC)	Sino Biological (orb248714)
Goat Anti-Rabbit IgG H&L (HRP)	Abcam (ab6721)

2.9 | ELISA Method Detection

Blood was collected from rat hearts via cardiac puncture and centrifuged using a high-speed centrifuge to obtain serum supernatant. Detection of IL-17, IL-21, and IL-22 levels was performed strictly according to the manufacturer's protocols provided with the respective ELISA kits. Following kit instructions, recombinant protein standards were prepared through two-fold serial dilutions, with both standard concentrations and test samples analyzed in duplicate wells to reduce experimental variability. Standard curves were generated using a four-parameter logistic (4PL) regression model, demonstrating excellent correlation coefficients ($R^2 > 0.99$ for all assays; a representative standard curve is provided in the Supporting Information). Technical replicates showed coefficients of variation (CV) below 10%, ensuring methodological reliability.

2.10 | Flow Cytometry Detection

The spleen was ground to fully crush and mix it with 4 mL precooled PBS solution to obtain a single-cell suspension of the spleen; the mouse spleen single-cell suspension was carefully added to the liquid surface of 4 mL separation liquid and centrifuged at 2500 rpm for 30 min, collecting the cells at the interface; the spleen cells were carefully washed with PBS solution, then centrifuged at 1000 rpm for 10 min, discarding the supernatant and leaving the cell precipitate, repeating the operation twice; RPMI1640 culture liquid was used to resuspend and count the spleen cells, and the cell thickness was regulated to 5×10^6/mL for standby. In vitro, the cells were activated using a stimulant composed of ionomycin, PMA, and BFA, and then cultured in a 37°C incubator for a duration of 4 h. Following a single wash with PBS, 0.5 μL of anti-rat CD4-APC antibody was introduced, and the cells were allowed to incubate at ambient temperature in darkness for 30 min. Post-centrifugation, the supernatant was decanted, and the cells underwent membrane permeabilization in accordance with the cell membrane permeabilization kit's guidelines. Subsequently, the addition of 1 μL anti-rat IL-17/FoxP3 flow antibody was followed by an incubation period of 20 min in the dark at room temperature. An additional 1 mL of permeabilization buffer was introduced, followed by centrifugation and removal of the supernatant. Subsequently, the cells were reconstituted in PBS for subsequent analysis using the detection equipment.

2.11 | Western Blot Detection

The synovial tissue was collected, and protein samples were extracted, concentrated gel and separating gel were prepared, protein bands were separated by electrophoresis, semi-dry transfer to PVDF membrane, blocked with blocking solution for 2 h. The PVDF membranes were incubated with rabbit-derived Mst1 antibody (Abcam, catalog number ab231138, 1:1000 dilution) and rabbit-derived TAZ antibody (Abcam, catalog number ab110239, 1:1000 dilution) overnight at 4°C; TBST washed the membrane, added HRP-labeled goat anti-rabbit IgG H&L (HRP) and visualized using chemiluminescence detection. Band intensities were quantified (1:2000), incubated on a shaker for 1 h; after washing, the membranes were incubated with chemiluminescent substrate, exposed for detection, and scanned to visualize protein bands. The ECL chemiluminescence signals were developed, and band intensities were quantified using ImageJ software. GAPDH was selected as the internal reference protein due to its relatively stable expression in ankle joint tissues and insensitivity to experimental treatments. Pre-experimental validation confirmed distinct molecular weight separations between GAPDH and target proteins (Mst1/TAZ: GAPDH: 37 kDa; Mst1: 60 kDa; TAZ: 55 kDa), effectively preventing band overlap. Each membrane was simultaneously probed for GAPDH to verify consistent sample loading and uniform transfer efficiency across all specimens. Using ImageJ 1.53c software in "Gel Analysis" mode, target bands (Mst1/TAZ and GAPDH) were outlined with local background subtraction (Rolling Ball Radius = 50), and integrated intensity values were recorded. The relative expression ratio was calculated as: (Target protein intensity—Background) / (GAPDH intensity—Background). Control group values were normalized to 1, with experimental group results expressed as fold changes relative to controls.

2.12 | qRT-PCR Detection

Detection of Mst1 and TAZ gene expression in the ankle joint: The ankle joint was frozen with liquid nitrogen and placed in a frozen grinder to grind evenly, and total RNA of the bone tissue was extracted through an RNA extraction kit, and RNA concentration was determined by spectrophotometric quantification. cDNA was synthesized using a reverse transcription kit and amplified with a real-time fluorescent quantitative PCR instrument. GAPDH served as the internal reference gene. Primers were synthesized by Abcam Company.

Primer Sequence.

Gene Name	Primer sequence (5′-3′)	Amplicon size (bp)
Mst1	Forward: TGCTTCTGGCACAGTGTTCA	588
	Reverse: CTACCTCGCCACGGTAATCC	
TAZ	Forward: TCCTGCGACCCCTCTTATCA	342
	Reverse: TCAGCAATCAGCCGTCCAAT	

Gene Name	Primer sequence (5′-3′)	Amplicon size (bp)
GAPDH	Forward: GCGAGATCCCGCTAACATCA Reverse: CTCGTGGTTCACACCCATCA	178

2.13 | Data Processing

Statistical analyses were conducted using GraphPad8.0 software. Initially, quantitative data underwent the Shapiro–Wilk test to assess normal distribution. If all data are normally distributed, they are presented as mean ± SD ($n = 6$ rats; technical triplicates per sample). Conversely, they were depicted using the interquartile range. One-way ANOVA served as the primary method for statistical analysis. For data that were normally distributed, the F test was initially applied to check for variance homogeneity, followed by the SNK test for between-group comparisons when variances were homogeneous. In cases of heterogeneous variances, the Welch test was initially employed, followed by Dunnett's T3 test for between-group comparisons. The Kruskal–Wallis one-way ANOVA was utilized for comparing quantitative data that did not conform to a normal distribution. A $p < 0.05$ was taken to denote statistical significance.

TABLE 1 | HPLC method for determining active components of standards.

Standard	Time of peak (min)	Content (mg/mL)
Naringenin	22.628	0.244
Notoginsenoside R1	23.097	0.213
Cinnamyl alcohol	26.277	0.1004
Ononin	28.854	0.452

TABLE 2 | HPLC method for determining active components in samples.

Samples	Time of peak (min)	Content (mg/mL)
Naringenin	22.738	0.245214
Notoginsenoside R1	23.202	0.197219
Cinnamyl alcohol	26.383	0.0469673
Ononin	28.952	0.0909454

3 | Results

3.1 | Quantitative Analysis of GLYK Components

The HPLC method was used to determine the active components in the extract of Gulao Yukang Pill, which includes naringenin, notoginsenoside R1, cinnamyl alcohol, and ononin. The extract primarily contains naringenin and notoginsenoside R1, while the concentrations of cinnamyl alcohol and ononin are relatively low. The retention times and concentrations are summarized in Tables 1 and 2 and Figure 1A,B. GLYK is composed of multiple traditional Chinese medicines. This study only identified several bioactive compounds contained in GLYK (e.g., Naringenin, Notoginsenoside R1, Cinnamyl alcohol, Miscanthus glycoside, etc.), while some components remain uncharacterized. The pharmacological mechanisms will be further explored in future studies when conducting detailed investigations into its pharmacological effects.

3.2 | Prediction of Targets for RA Treatment With GLYK

A cumulative total of 1746 RA-associated genes were predicted in the CTD, DisGeNET, and GeneCards databases (Figure 2A), and 911 effective active ingredients of GLYK were retrieved from

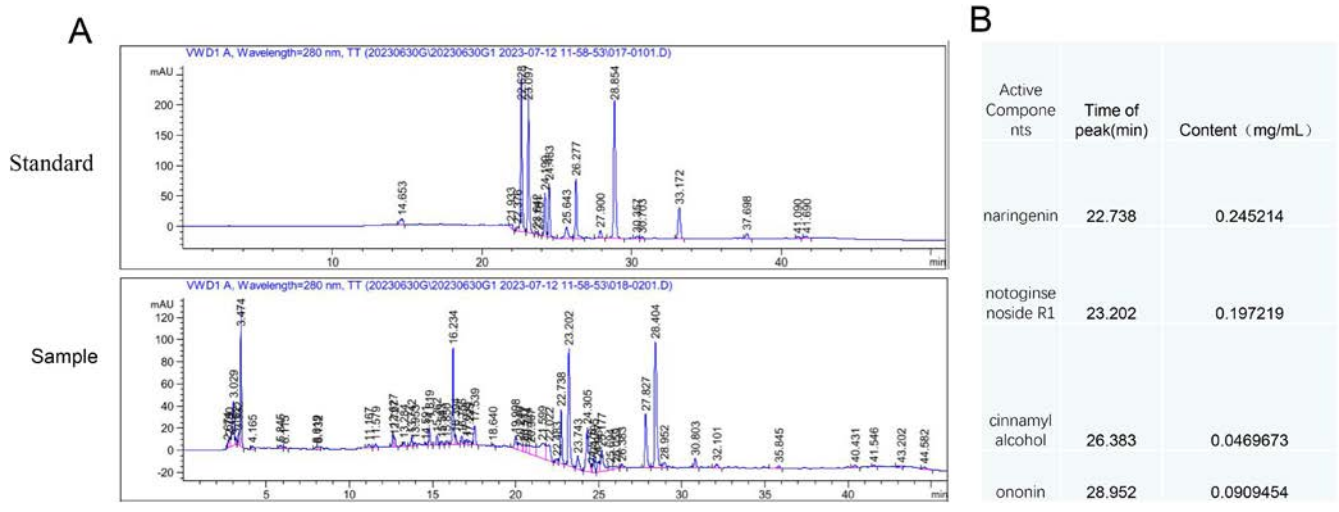

FIGURE 1 | Quantitative analysis of GLYK components a represents the HPLC determination of active components in the GLYK extract, including naringenin, cinnamyl alcohol, ononin, and notoginsenoside R1. B shows the retention times and concentrations of the GLYK components.

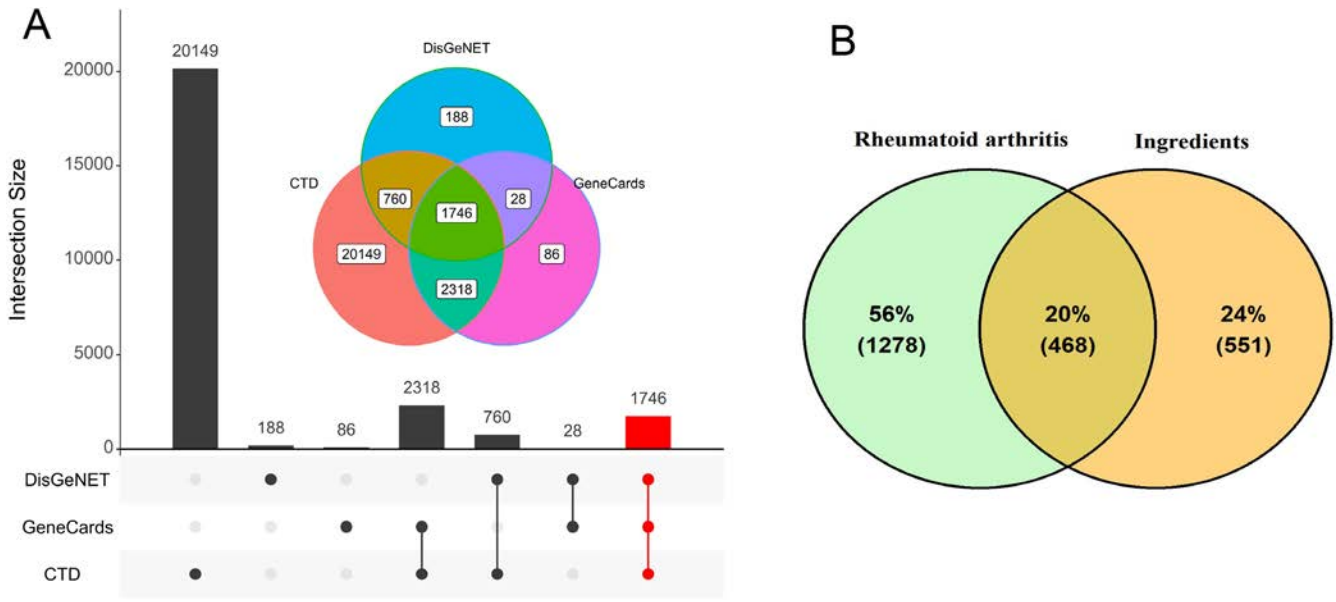

FIGURE 2 | Prediction of RA treatment targets for GLYK A represents the Venn diagram of the intersection of RA genes from three databases, with 1746 common genes associated with RA predicted in CTD, DisGeNET, and GeneCards; B represents the Venn diagram of the intersection between rheumatoid arthritis genes and drug target genes, with a total of 468 genes potentially acting on RA being screened.

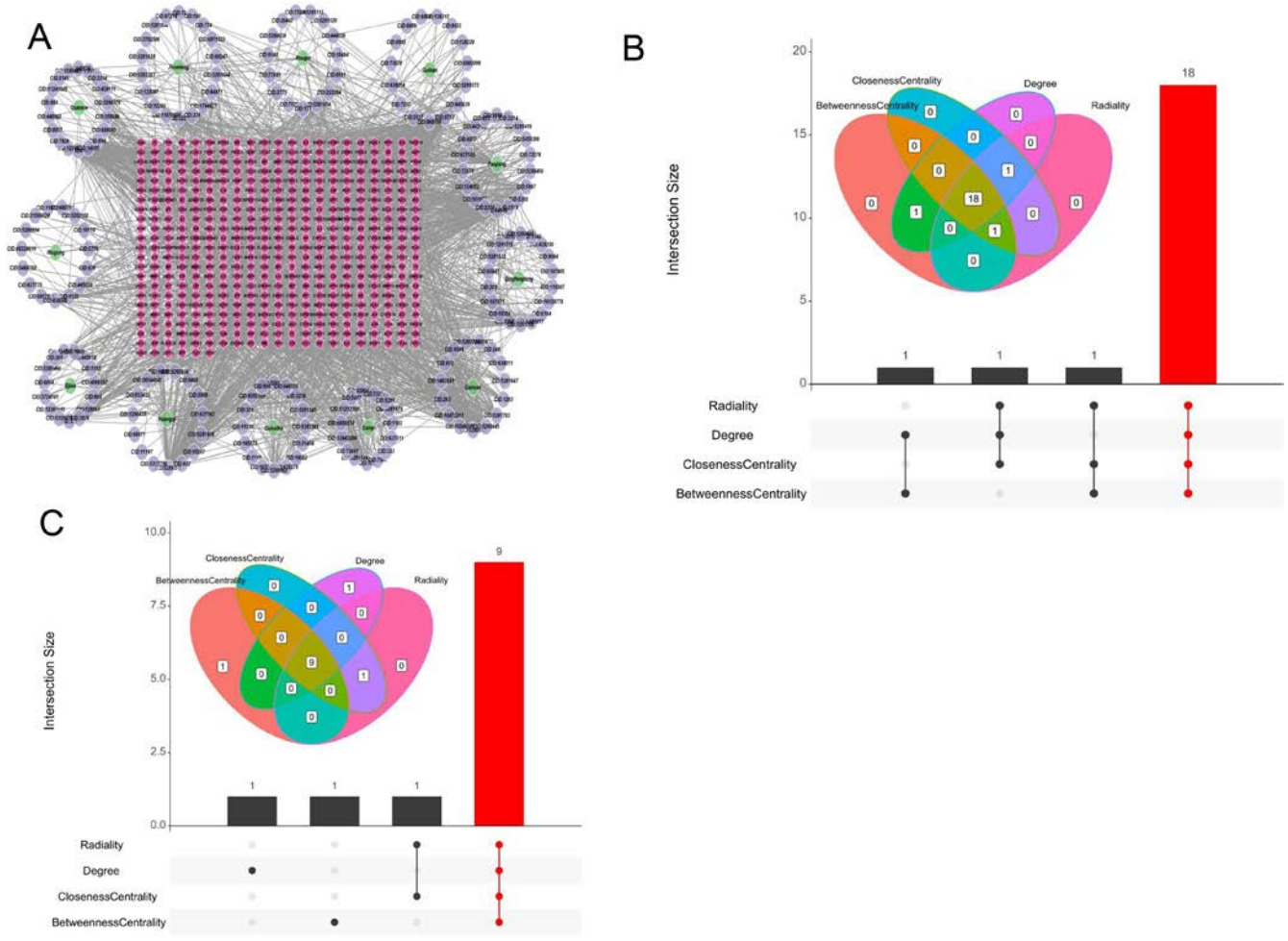

FIGURE 3 | Construction of the drug-active ingredient-target gene network. A is the drug- active ingredient-target gene network diagram, where green represents drugs, purple represents active ingredients, and red represents target genes. B represents the Venn diagram of key genes selected based on network topology parameters Betweenness Centrality, Closeness Centrality, Degree, and Radiality, with a total of 18 genes. C represents the Venn diagram of the key active ingredients identified, totaling 9.

TABLE 3 | Topological parameters of 18 key genes.

Name	Betweenness centrality	Closeness centrality	Degree	Radiality
CASP3	0.050842356	0.414153846	46	0.989967648
Mst1	0.044724068	0.411117899	42	0.98984119
NOS2	0.022702785	0.396582204	26	0.989208898
MAPK1	0.021843844	0.393797542	27	0.98908244
AKT1	0.015999753	0.385673352	25	0.988703066
BCL2	0.025045199	0.383913292	31	0.98861876
PPARG	0.018956727	0.382603752	24	0.988555531
JUN	0.011573791	0.380011293	22	0.988429073
BAX	0.018258392	0.379582628	27	0.988407996
PTGS2	0.024247646	0.37915493	29	0.98838692
RELA	0.012814871	0.37915493	21	0.98838692
NFKB1	0.023084386	0.376187814	28	0.988239385
TAZ	0.009624383	0.374930362	19	0.988176156
MMP9	0.013095739	0.372853186	23	0.988070774
MAPK3	0.011770027	0.372440509	18	0.988049698
IL1B	0.011657375	0.372028745	19	0.988028622
VEGFA	0.008356924	0.370798898	16	0.987965393
IL6	0.011896314	0.367960634	20	0.987817858

the HIT2, TCM-ID, TCMSP, and SymMap databases, with 911 effective active ingredients finally selected based on the drug-likeness indicator QED > 0.2. According to the PubchemID of the selected GLYK active ingredients in HIT2, the target genes of the compounds were retrieved, and compounds without target genes identified in the database were removed, resulting in a total of 911 effective active ingredients corresponding to 1019 target genes. A Venn analysis of genes associated with RA and drug effective active ingredient target genes resulted in 468 genes that may act on RA (Figure 2B).

3.3 | Building the Drug-Active Ingredient-Target Gene Interaction Network

A network depicting the interactions between drug active ingredients and target genes was established utilizing Cytoscape software (Figure 3A), with green representing drugs, purple standing for active ingredients, and red symbolizing target genes. According to the network topology parameters Betweenness Centrality, Closeness Centrality, Degree, and Radiality, 18 key genes were screened: CASP3, Mst1, NOS2, MAPK1, AKT1, BCL2, PPARG, JUN, BAX, PTGS2, RELA, NFKB1, TAZ, MMP9, MAPK3, IL-1β, VEGFA, and IL-6 (Figure 3B, Table 3). Nine key active ingredients were Quercetin, Curcumin, Oestradiol, Genistein, Apigenin, Ursolic Acid, Vitamin E, Luteolin, and Daidzein (Figure 3C, Table 4).

3.4 | GSVA Enrichment Analysis

Seven Hippo-related signaling pathways were selected from MSigDB, and gene set variation analysis (GSVA) was used to predict the pathway activity scores of these 7 pathways in the GSE89408 dataset samples. The findings revealed that two specific pathways exhibited markedly distinct activity scores when comparing the disease group to the control group ($p < 0.05$), which were WP_HIPPO_SIGNALING_REGULATION_PATHWAYS and WP_LEUKOCYTEINTRINSIC_HIPPO_PATHWAY_FUNCTIONS (Figure 4A). Pearson correlation analysis revealed that among the 18 key genes, 9 genes, including NFKB1, CASP3, RELA, AKT1, PPARG, TAZ, Mst1, and MAPK3, exhibited a substantial association with the Hippo pathway ($p < 0.05$) (Figure 4B).

3.5 | Prediction of the Binding Capacity of Key Active Ingredients and Genes Through Machine Learning Algorithm

The Deep Purpose algorithm predicted the binding capacity of 9 active ingredients of GLYK (Quercetin, Curcumin, Oestradiol, Genistein, Apigenin, Ursolic Acid, Vitamin E, Luteolin, Daidzein) and 9 genes related to the Hippo pathway (MAPK1, NFKB1, CASP3, RELA, AKT1, PPARG, TAZ, Mst1, MAPK3). The greater the scores, the more potent the combination with capacity between the active ingredients and genes, indicating

TABLE 4 | Topological parameters of 9 key active ingredients.

CPD	Pubchem_id	Betweenness centrality	Closeness centrality	Degree	Radiality
Quercetin	CID:5280343	0.171097163	0.43224149	141	0.990684244
Curcumin	CID:969516	0.125300077	0.407138536	114	0.989672579
Oestradiol	CID:5757	0.102656331	0.384351799	94	0.988639836
Genistein	CID:5280961	0.075063925	0.397989355	80	0.989272128
Apigenin	CID:5280443	0.072637046	0.398460628	83	0.989293204
UrsolicAcid	CID:64945	0.043655594	0.376187814	63	0.988239385
VitaminE	CID:14985	0.038514834	0.374513077	50	0.98815508
Luteolin	CID:5280445	0.034551152	0.368363437	56	0.987838934
Daidzein	CID:5281708	0.03225235	0.380871534	45	0.988471225

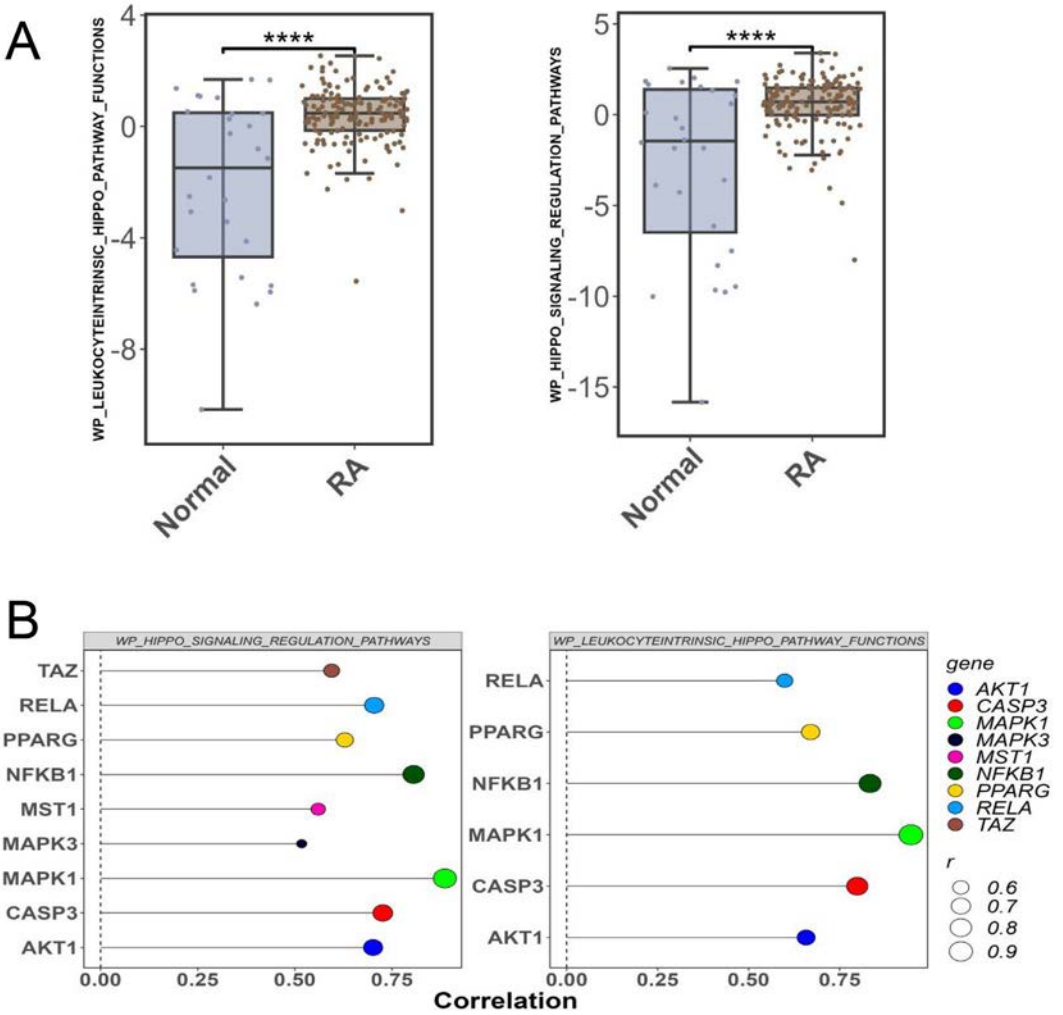

FIGURE 4 | Gene set variation analysis: seven Hippo-related signaling pathways were selected from MSigDB. Using R4.2.2, GSVA was employed to predict the activity scores of these 7 pathways in the GSE89408 dataset samples. The expression levels of 18 key genes were correlated with the activity scores of these two pathways using Pearson correlation analysis, revealing that 9 genes had significantly correlated scores with the Hippo pathway. A represents the boxplot of the activity scores for two selected Hippo pathways, WP_HIPPO_SIGNALING_REGULATION_PATHWAYS and WP_LEUKOCYTE_INTRINSIC_HIPPO_PATHWAY_FUNCTIONS, showing significant differences in pathway activity scores between rheumatoid arthritis and normal groups, with *** indicating $p < 0.05$. B is a scatter plot of the correlation between Hippo pathway activity scores and gene expression, where different colored points correspond to different genes, and the size of the points corresponds to the absolute value of the correlation coefficient R.

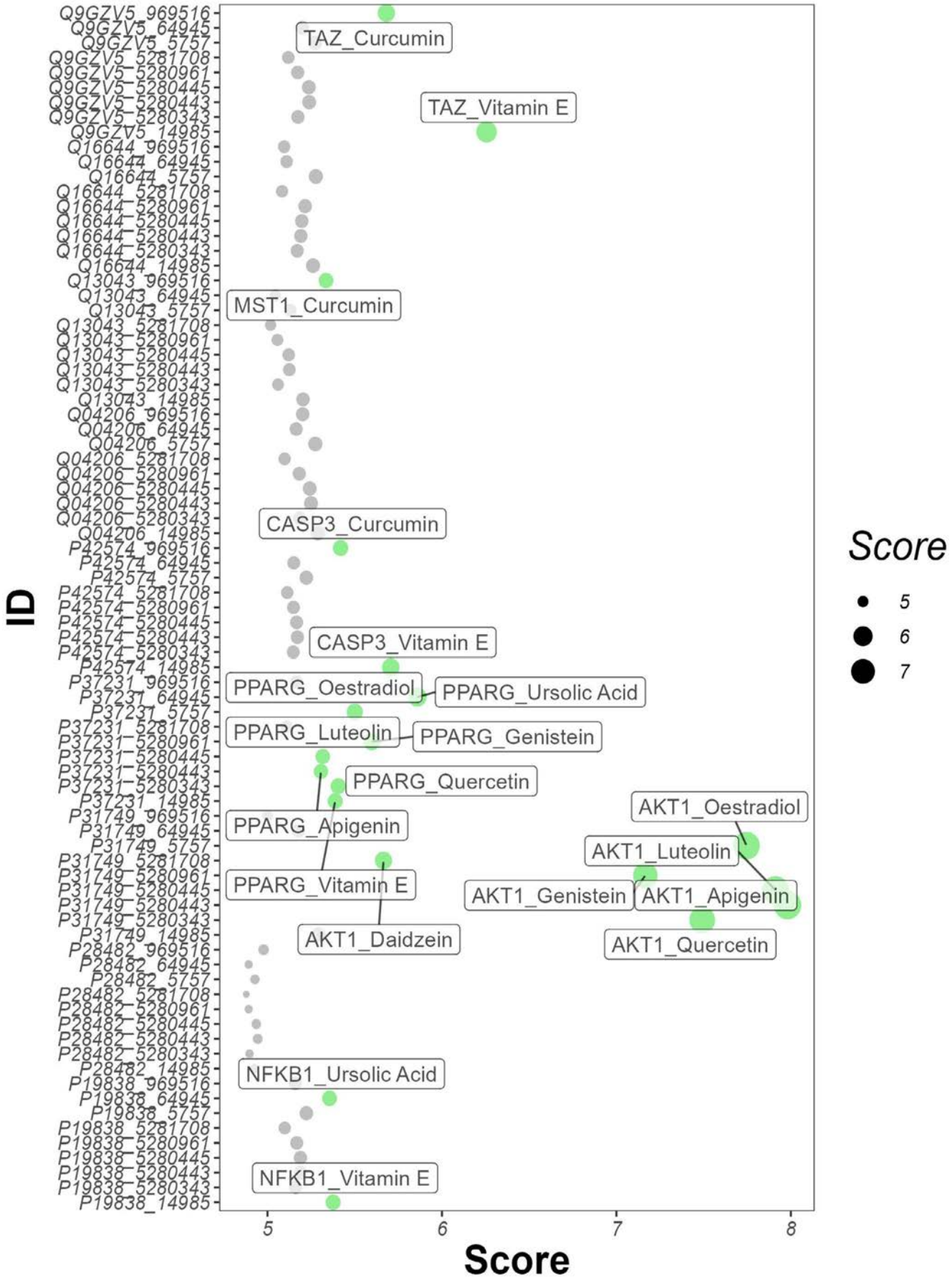

FIGURE 5 | Legend on next page.

FIGURE 5 | Scatter plot of drug-target binding affinity predictions based on the Deep Purpose algorithm framework to predict the binding affinity scores of the selected 9 key active ingredients and 9 target genes. The stronger the binding affinity, the stronger the interaction between the active ingredients and the genes, suggesting that the active ingredients may influence the pathway through these genes. In the plot, the green points represent the top 20 results sorted by predicted scores.

TABLE 5 | Top 20 prediction results from deep purpose.

Pubchem_ ID	Compound	Uniprot	Gene	Score
5280443	Apigenin	P31749	AKT1	7.981
5280445	Luteolin	P31749	AKT1	7.9121
5757	Oestradiol	P31749	AKT1	7.7458
5280343	Quercetin	P31749	AKT1	7.4919
5280961	Genistein	P31749	AKT1	7.1659
14985	VitaminE	Q9GZV5	TAZ	6.2556
64945	UrsolicAcid	P37231	PPARG	5.8588
14985	VitaminE	P42574	CASP3	5.7066
969516	Curcumin	Q9GZV5	TAZ	5.6812
5281708	Daidzein	P31749	AKT1	5.665
5280961	Genistein	P37231	PPARG	5.5976
5757	Oestradiol	P37231	PPARG	5.5004
969516	Curcumin	P42574	CASP3	5.419
5280343	Quercetin	P37231	PPARG	5.4056
14985	VitaminE	P37231	PPARG	5.388
14985	VitaminE	P19838	NFKB1	5.3761
64945	UrsolicAcid	P19838	NFKB1	5.3557
969516	Curcumin	Q13043	Mst1	5.3354
5280445	Luteolin	P37231	PPARG	5.3167
5280443	Apigenin	P37231	PPARG	5.3064

that the active ingredients may affect the pathway through these genes. According to the scores, AKT1, TAZ, PPARG, CASP3, NFKB1, and Mst1 were selected (Figure 5, Table 5), with Mst1 being the upstream core molecule of the Hippo pathway and TAZ being its downstream effector molecule.

3.6 | Effects on CIA Rat Arthritis-Related Symptoms and Cytokines

The assessment of AI scores in the CIA models demonstrated successful model establishment. Compared with the NC group, the model group exhibited a statistically significant increase in AI scores ($p < 0.05$). In contrast, the GL, GM, GH, and MTX groups showed statistically significant reductions in AI scores compared to the model group ($p < 0.05$) (Figure 6A).

Following GLYK intervention, the paw volume values of CIA rats in each group decreased progressively with increasing dosage and prolonged treatment duration. Compared with the NC group, the model group exhibited significantly elevated paw volume ($p < 0.05$). In contrast, the GL, GM, GH, and MTX groups demonstrated statistically significant reductions in paw volume compared to the model group ($p < 0.05$) (Figure 6B).

After GLYK intervention, Tarlov scores in CIA rats demonstrated a dose- and time-dependent decrease across treatment groups, accompanied by progressive behavioral improvement. Compared with the NC group, the model group exhibited significantly elevated Tarlov scores ($p < 0.05$). Relative to the model group, statistically significant reductions in Tarlov scores were observed in the GL, GM, GH, and MTX groups ($p < 0.05$) (Figure 6C).

The ELISA assay for cytokine detection revealed that compared to the NC group, the model group exhibited significantly elevated expression levels of IL-17, IL-21, and IL-22 with statistical significance ($p < 0.05$). When compared to the model group, the GL, GM, GH, and MTX groups demonstrated statistically significant reductions in the expression levels of IL-17, IL-21, and IL-22 ($p < 0.05$) (Figure 6D).

3.7 | Effects on Histopathology of CIA Rats

HE staining revealed that the ankle joints of rats in the NC group maintained normal morphology and anatomical structure with smooth surfaces. The subchondral trabecular bones exhibited regular size and orderly arrangement. In contrast, the model group showed significantly aggravated ankle joint lesions compared to the NC group, characterized by substantial inflammatory cell infiltration in synovial tissues leading to pannus formation. The superficial cartilage layer demonstrated extensive cellular degeneration and necrosis, accompanied by disorganized trabecular architecture. Compared with the model group, the GL, GM, GH, and MTX groups exhibited dose-dependent reductions in ankle joint lesions, diminished inflammatory cell infiltration in synovial tissues, decreased cellular degeneration/necrosis in superficial cartilage layers, and normalized trabecular bone organization (Figure 7A). Synovial inflammation scores were significantly elevated in the model group compared to the NC group ($p < 0.05$). Conversely, all treatment groups (GL, GM, GH, MTX) showed statistically significant reductions in synovial inflammation scores compared to the model group ($p < 0.05$) (Figure 7B).

TRAP staining results demonstrated minimal staining areas in the normal control (NC) group, with sparsely distributed osteoclasts displaying regular morphology and smooth trabecular surfaces free of erosive lacunae. In contrast, the model group exhibited a significant increase in osteoclast quantity, with multiple TRAP-positive multinucleated giant cells observed on trabecular surfaces. These cells featured dark red granular cytoplasm and blue-stained nuclei, predominantly

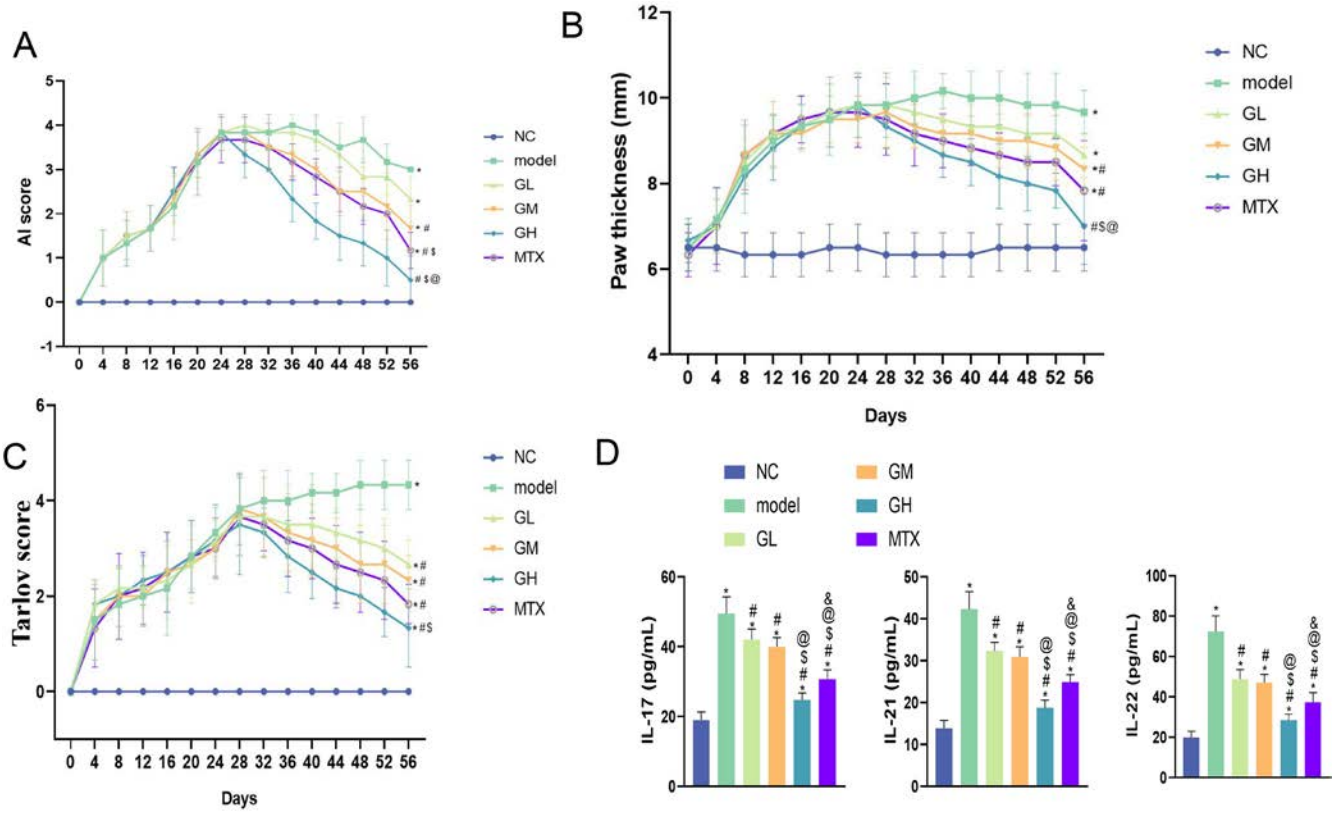

FIGURE 6 | The impact of GLYK on arthritis-related symptoms in CIA rats. A represents the AI scores of the arthritis index for each group of rats, B represents the foot volume measurements for each group of rats, C represents the Tarlov scores for each group of rats, and D shows the impact on IL-17, IL-21, and IL-22. * indicates $p < 0.05$ versus NC; # indicates $p < 0.05$ versus model; $ indicates $p < 0.05$ versus GL; @ indicates $p < 0.05$ versus GM; & indicates $p < 0.05$ versus GH ($n = 3$ biological replicates).

localized within bone resorption lacunae. Both the number of TRAP-positive cells and staining intensity were markedly enhanced. GLYK intervention effectively reduced the quantity and staining intensity of TRAP-positive multinucleated giant cells (Figure 7C). Comparative analysis revealed a statistically significant elevation of osteoclast numbers in rat ankle joints in the model group compared to the NC group ($p < 0.05$). Relative to the model group, gradual dose-dependent reductions in osteoclast numbers were observed in GL, GM, GH, and MTX treatment groups, with statistically significant differences at lower doses ($p < 0.05$) (Figure 7D).

3.8 | Effects on *Th17*/*Treg* Ratio

Flow cytometry was performed to assess the expression levels of *Th17* and *Treg* cells in the spleen. Compared with the NC group, the *Th17*/*Treg* ratio was significantly increased in the model group ($p < 0.05$). Notably, the GL, GM, GH, and MTX groups all exhibited statistically significant reductions in the *Th17*/*Treg* ratio compared to the model group ($p < 0.05$) (Figure 8).

3.9 | Effects on Mst1 and TAZ in the Hippo Signaling Pathway

Western blot analysis was performed to detect the expression of Mst1 and TAZ proteins in synovial tissues. The results demonstrated that compared with the NC group, the model group exhibited a significant decrease in Mst1 expression and a marked increase in TAZ expression, with statistically significant differences ($p < 0.05$). In comparison to the model group, the GL, GM, GH, and MTX groups showed elevated Mst1 expression and reduced TAZ expression, all displaying statistical significance ($p < 0.05$) (Figure 9A,B). Quantitative real-time PCR (qRT-PCR) assessment of Mst1 and TAZ mRNA levels in the ankle joint exhibited a significant reduction in Mst1 mRNA and a corresponding increase in TAZ mRNA in the model group in contrast to the NC group, with these diversity being statistically considerable ($p < 0.05$). In contrast, the GH group displayed an upregulation of Mst1 mRNA and a downregulation of TAZ mRNA relative to the model group, and these changes were also statistically considerable ($p < 0.05$) (Figure 9C).

4 | Discussion

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovitis and pannus formation, with inflammatory bone destruction being the primary factor contributing to disability in RA patients [32]. Current treatment challenges lie in the insensitivity or suboptimal response of some patients to existing medications, leading to difficulties in disease control. For instance, methotrexate achieves an ACR70 response rate of approximately 20%–40% in RA treatment, while combination therapy with biologics (TNF inhibitors

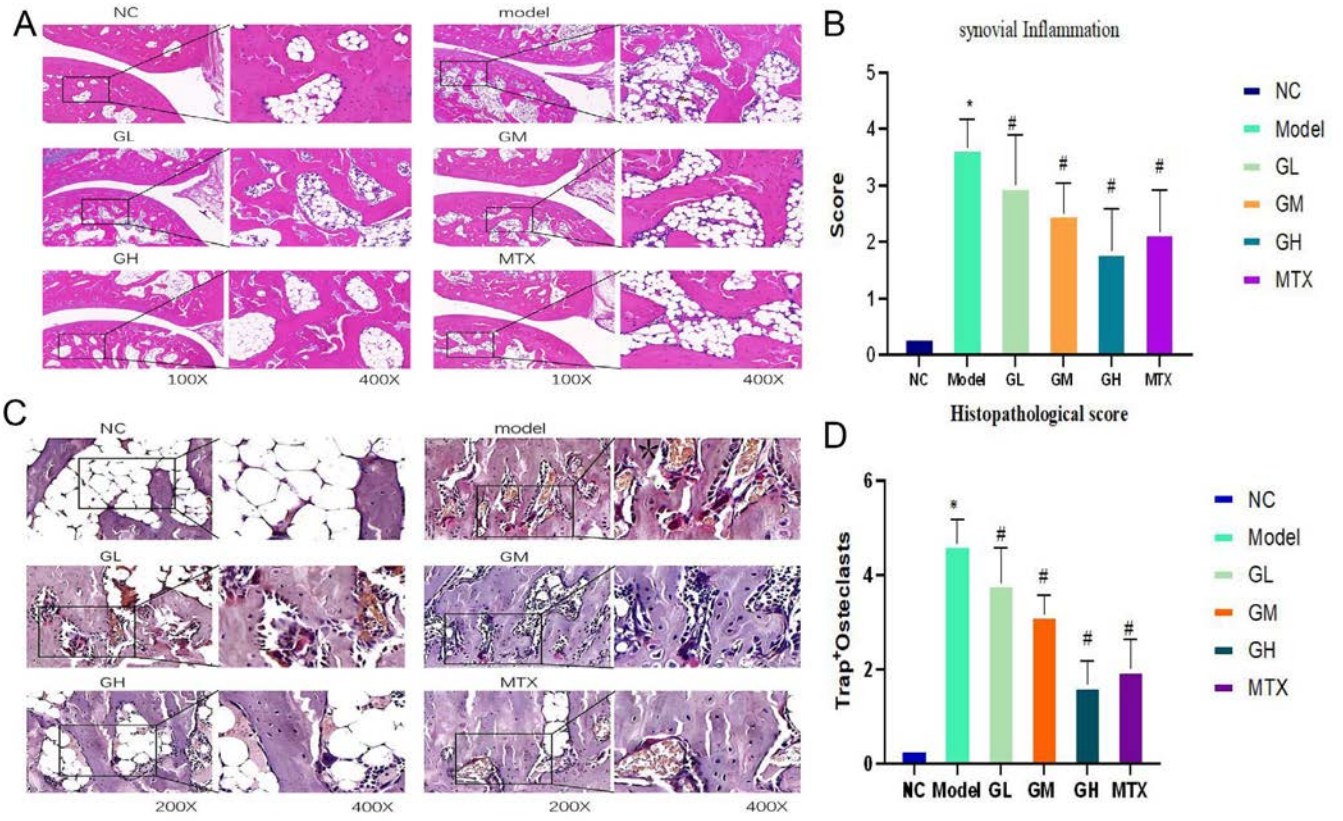

FIGURE 7 | Results of histopathological examination: a shows H&E staining of ankle joints from rats in each group, B represents the score for synovial inflammation, C shows TRAP staining of ankle joints from rats in each group, and D is a comparison chart of osteoclast counts. indicates $p < 0.05$ versus NC; # indicates $p < 0.05$ versus model ($n = 3$ biological replicates).

such as infliximab, IL-6 receptor inhibitors like tocilizumab, and T-cell co-stimulation blockers including abatacept) increases the ACR70 response rate to approximately 40%–50% [33]. Conventional immunosuppressants demonstrate limited efficacy in rapidly progressive and refractory RA cases, along with frequent adverse effects. Moreover, prolonged use of biologics has revealed emerging issues of drug resistance [34, 35]. Another therapeutic dilemma manifests in the incomplete prevention of joint destruction by current treatment approaches [36]. The management of RA remains particularly challenging in clinical practice, especially regarding the lack of effective interventions for persistent synovitis and bone destruction. This study, through integrated network pharmacology and experimental validation, reveals that the traditional Chinese herbal compound GLYK may inhibit inflammation and bone destruction by regulating key Hippo pathway molecules Mst1 and TAZ, thereby restoring *Th17*/*Treg* balance.

4.1 | Regulatory Effects of GLYK on Key Molecules of the Hippo Pathway

In the CIA model, GLYK intervention significantly upregulated the expression of Mst1, an upstream kinase in the Hippo pathway, while suppressing the activity of its downstream effector TAZ. As a central regulator of immune homeostasis, elevated Mst1 expression may enhance immunosuppressive function by promoting *Treg* cell differentiation [37, 38]. Conversely, TAZ downregulation likely reduces *Th17* cell differentiation through inhibition of RORγt activity [39, 40]. Our data support prior reports that Mst1 deficiency impairs *Treg* function [41], while TAZ overexpression exacerbates inflammation [39]. Notably, this study establishes a direct link between GLYK and the Hippo pathway. Our findings demonstrate a consistent dose-dependent effect of GLYK on both Mst1 expression and *Treg* cell numbers: western blot analysis demonstrated dose-dependent upregulation of Mst1 protein and downregulation of TAZ protein in synovial tissues. Similarly, GLYK exerted a parallel dose-dependent effect on TAZ expression and *Th17* cell numbers: TAZ expression decreased with higher GLYK doses, while *Th17* cell numbers declined accordingly. These results indicate that GLYK likely corrects the *Th17*/*Treg* imbalance by restoring the dynamic balance between Mst1 and TAZ. This multi-target mechanism contrasts with single-agent biologics.

4.2 | Downstream Effects of *Th17*/*Treg* Imbalance and Therapeutic Implications

This study revealed that GLYK intervention significantly reduced *Th17* cell proportion while simultaneously increasing *Treg* cell proportion, thereby inhibiting joint swelling and bone destruction. The *Th17*/*Treg* ratio demonstrated significant positive correlations with both joint swelling severity and bone destruction. These findings confirm the critical role of *Th17*/*Treg*

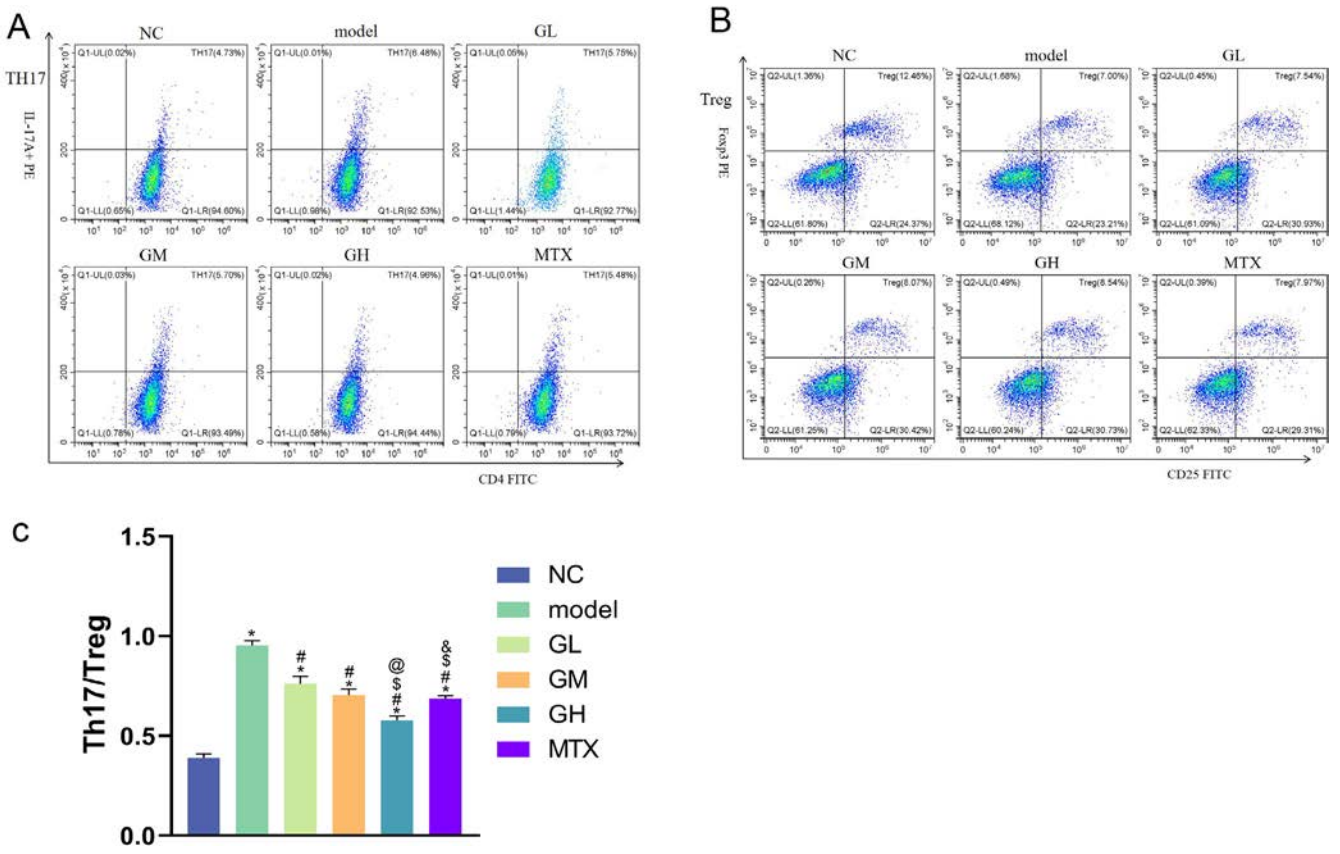

FIGURE 8 | The impact of GLYK on Th17/Treg ratio, a for the quantity of Th17 detected by flow cytometry in the spleen, B for the quantity of Treg cells detected by flow cytometry in the spleen, C for the ratio of Th17/Treg, * indicates $p < 0.05$ versus NC; # indicates $p < 0.05$ versus model; $ indicates $p < 0.05$ versus GL; @ indicates $p < 0.05$ versus GM; & indicates $p < 0.05$ versus GH ($n = 3$ biological replicates).

imbalance in RA progression [42]. Notably, IL-17 secreted by *Th17* cells was shown to directly activate synovial fibroblasts, stimulating the release of matrix metalloproteinases (MMPs) that accelerate cartilage degradation [43]. Conversely, the elevated *Treg* cell population likely alleviates bone destruction through IL-10-mediated suppression of osteoclast differentiation [44]. *Th17*/*Treg* imbalance manifests as synovial inflammation, joint destruction, and bone erosion, thereby exacerbating RA pathogenesis [45]. Compared with existing therapies, GLYK demonstrates distinctive advantages through its multi-targeted modulation of *Th17*/*Treg* balance to simultaneously suppress inflammation and bone destruction. This mechanism may address the osteoprotective deficiencies commonly observed in traditional immunosuppressants.

4.3 | Comparative Advantages Over Existing Therapies

Compared to conventional therapies like methotrexate or TNF-α inhibitors, GLYK demonstrates unique value through two key mechanisms: First, its multi-component synergistic action targeting both Mst1 and TAZ may reduce the likelihood of drug resistance commonly seen with single-target agents. Second, while GLYK's modulation of the *Th17*/*Treg* balance partially overlaps with IL-6 receptor antagonists (e.g., tocilizumab), conventional IL-6 inhibitors show limited efficacy in mitigating bone erosion. In contrast, this study revealed that GLYK significantly reduced TRAP-positive osteoclast counts, suggesting superior bone-protective benefits. This dual-action profile positions GLYK as a potential foundation for novel combination therapeutic strategies.

4.4 | Limitations and Future Directions of Mechanistic Validation

This study has the following limitations: First, although Hippo pathway-related targets were identified through network pharmacology, classical pathway molecules such as *YAP* and *LATS1*/2 were not experimentally validated. Second, the absence of Mst1/TAZ gene knockout models or specific inhibitor experiments prevented full establishment of a direct causal relationship between GLYK and its targets. Furthermore, the binding patterns between GLYK's active components and Mst1/TAZ require further verification through molecular docking or surface plasmon resonance (SPR) analysis. Future research could employ single-cell sequencing to elucidate GLYK's precise regulatory effects on T-cell subsets and combine organoid models to evaluate its therapeutic potential in restoring the synovium–bone interface microenvironment.

In summary, through network pharmacology and experimental validation, this study revealed that GLYK could modulate the expression of Mst1 (a core upstream molecule in the Hippo

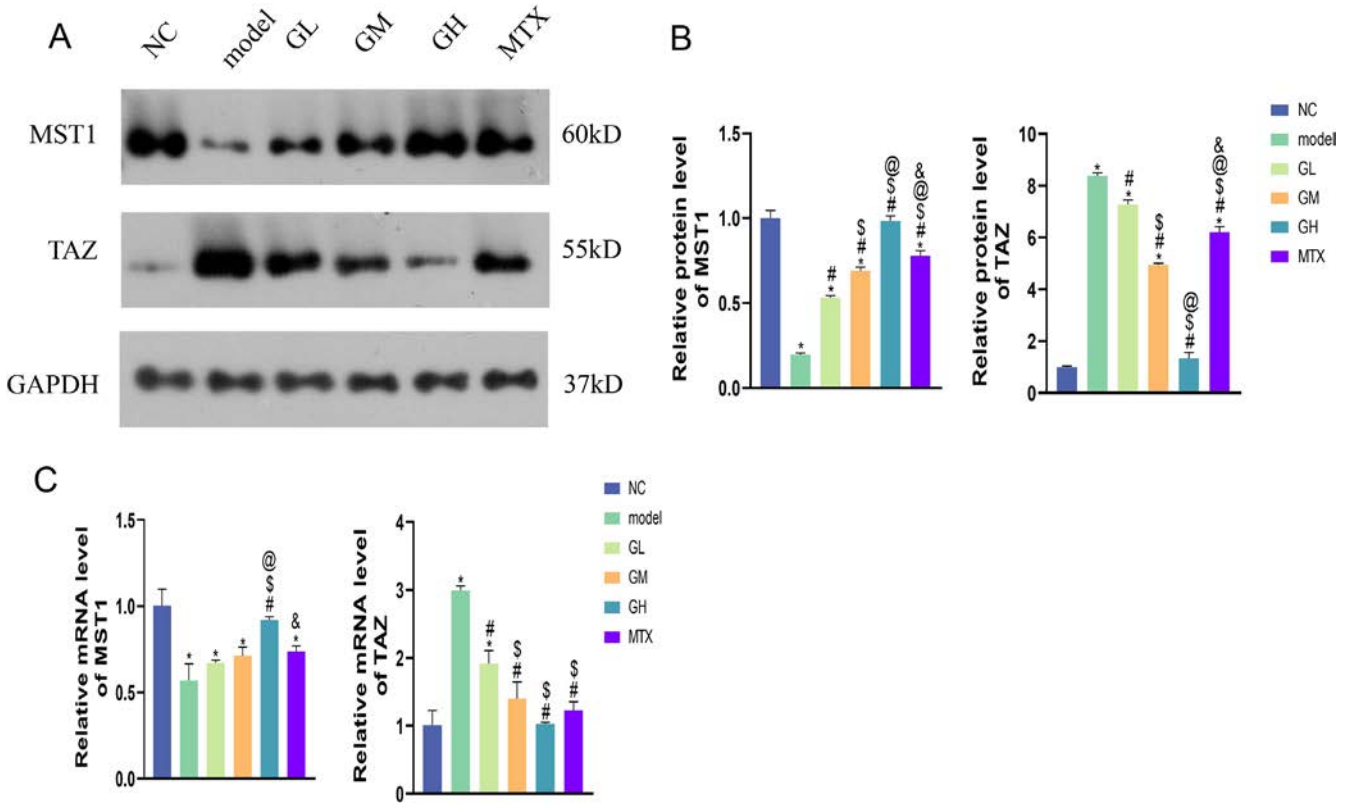

FIGURE 9 | The impact of GLYKW on Mst1 and TAZ in the Hippo signaling pathway, A and B for Western blotting analysis of protein expression in synovial tissue, C for qRT-PCR detection of Mst1 and TAZ gene expression in the ankle joint, * indicates $p < 0.05$ versus NC; # indicates $p < 0.05$ versus model; $ indicates $p < 0.05$ versus GL; @ indicates $p < 0.05$ versus GM; & indicates $p < 0.05$ versus GH ($n = 3$ biological replicates).

signaling pathway) and its downstream effector TAZ, alleviate swelling and arthritis indices in CIA rats, reduce the *Th17*/*Treg* cell ratio, inhibit inflammatory responses, and suppress bone destruction. The Hippo signaling pathway serves as a therapeutic target for GLYK in the treatment of rheumatoid arthritis.

Author Contributions

Tao Wang analyzed the experimental data and wrote the manuscript. Gang Wang and Jia Wang designed the study and the article structure. Hailong Liu and Ganggang Jiang provided experimental data. Gang Wang and Jia Wang critically reviewed and edited the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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Supporting Information

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

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ORIGINAL ARTICLE

The APLAR Recommendations for the Management of Psoriatic Arthritis

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Keywords: psoriatic arthritis | quality of evidence | treatment recommendation

ABSTRACT

Objectives: Under the auspices of the Asia-Pacific League of Associations for Rheumatology (APLAR), we aimed to develop broad, evidence- and consensus-based guidelines to aid health professionals managing patients with psoriatic arthritis (PsA) in the region.

Methods: A working group of 35 members comprising rheumatologists, dermatologists, and patient research partners from 18 APLAR countries was convened. The working group conducted systematic literature reviews to derive the quality of evidence via GRADE methods in supporting the efficacy and safety of classes of therapeutic agents for the management of active PsA, its comorbidities, and screening for specific infection concerns in the region. Recommendation statements on the principles of management and the best use of therapeutic drugs were developed. Consensus within the working group was achieved. An external voting panel, five from each of the 18 APLAR countries, was convened to confirm further agreement on the recommendation statements.

Results: The main literature review included 178 articles from clinical trials for PsA. Additional articles on the evidence for managing comorbidities, uveitis, inflammatory bowel disease, and screening for chronic hepatitis B and latent tuberculosis were reviewed. The working group discussed and reached consensus on eight management principles and 16 recommendation statements for managing PsA. Endorsement from an external voting panel ($n = 90$, response rate 80%) was achieved.

Conclusion: These first recommendations for the management of PsA patients in the APLAR regions were developed based on the best available evidence and region-specific considerations through discussion among rheumatologists, dermatologists, and patients, with strong agreement from an external expert panel.

For affiliations refer to page 8.

1 | Introduction

Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory disease with diverse manifestations, including articular (peripheral arthritis and axial disease), extra-articular (enthesitis, dactylitis), and extra-musculoskeletal features (psoriasis, nail disease, inflammatory bowel disease [IBD], uveitis) along with myriad comorbidities [1]. In addition to joint damage and chronic disability, it has detrimental effects on quality of life [1] and accelerated atherosclerosis [2]. In the past two decades, the prognosis and outcomes for PsA have been revolutionized by early diagnosis and treatment with disease-modifying antirheumatic drugs (DMARDs) [3].

The Asia and Pacific region is home to 60% of the world's population and is where 4.7 billion people reside [4]. This region is diverse in patients' demographics, sociocultural practices, and healthcare systems. In particular, some areas lack medical insurance and subsidies for newer and more effective medications for PsA. The region is also characterized by different patterns of endemic infectious diseases that pose challenges for the use of new biological/targeted synthetic (b/ts-) DMARDs. Moreover, geographic or ethnic differences in the epidemiology of PsA in the region have been described [5, 6].

To date, no recommendations have been developed for the management of PsA for the Asia-Pacific region. Given the change in treatment paradigm and the availability of new DMARDs, there is an unmet need for expert practitioners from the region to develop recommendations to provide updated guidance on the management of PsA patients, based on the best available evidence and with consideration of the specific needs of the region. Therefore, under the auspices of the Asia-Pacific League of Associations for Rheumatology (APLAR), we aimed to develop recommendations on the care of PsA for the region. These recommendations are intended for use by rheumatologists, as well as other clinicians and prescribers taking care of patients with PsA.

2 | Methods

2.1 | Working Group

A steering committee, followed by a working group of 35 members from 18 countries, was convened by the Spondyloarthritis Special Interest Group (SpA-SIG) under APLAR. A maximum of two rheumatologist members from each country was included. For countries without a regular member in the SpA-SIG, nominations were solicited from the presidents of the National Member Organizations. In addition, two dermatologists (YWY and AM) and two patients (JC and AR) were invited to the working group. Two engagement webinars were conducted by the project leader (YYL) with the patients, familiarizing them with the work processes.

2.2 | Principles of Management

We formulated the principles of management based on best clinical practice and a review of existing recommendations [7–10] and their previous versions. Thorough discussions among working group members, soliciting missing concepts, and refinement of statements were conducted, and consensus was achieved through Delphi exercises according to the OMERACT methods [11]. Agreement was considered achieved when 70% or more of participants rated ≥ 7 on a scale of 1 to 9 ('not important' to 'critically important').

2.3 | Evidence Development and Recommendation Statements

We developed this guideline using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system [12]. The main research question was formulated based on the population, intervention, comparison, and outcomes (PICO). We focused on patients with active PsA who: were (1) naïve to therapies, (2) had an inadequate response to conventional synthetic (cs-) DMARDs, and (3) had an inadequate response to b/ts-DMARDs. We evaluated the efficacy and safety of classes of drugs. We did not distinguish between branded versus biosimilar or generic formulations as differences in clinical efficacies are not expected. We searched the literature using PubMed from inception to 16 February 2023, focusing primarily on randomized controlled trials (RCT) and observational trials if necessary. The details of the search strategies and search terms for the main research question are listed in Table S1. Two working group members conducted the data extraction and quality assessment of each included article according to the standard protocol. Separate subgroups were convened to focus on the axial domain, cardiovascular and other comorbidities, and infection concerns in APLAR regions, with separate literature search strategies applied. All protocols were pre-defined and outlined in PROSPERO (CRD4202339498, CRD42023416470, and CRD42023451338).

The quality of evidence to support the use of each class of drugs in the defined PsA population was derived by members of the subgroups. The working group met via a webinar to review evidence on efficacy and adverse effects of therapies, and balance against values, preference, and resource use. Follow-up discussions were conducted via email. The proposed recommendations were made on a four-point scale: strong for/weak for/weak against/strong against, according to the GRADE system [13]. A strong recommendation implies that most individuals under these circumstances should benefit from and receive the recommended course of action and vice versa.

Consensus on the proposed recommendation statements was achieved by up to three rounds of Delphi exercises among the working group, until consensus is achieved according to OMERACT standards [11]. The quality of evidence by GRADE and the rationale for the proposed statements were presented, and the working group members were asked to rate their level of agreement for each statement from 1 to 10 ('not agree' to 'fully agree'). Achievement of agreement was considered if 70% or more of the working group members gave a level of agreement of ≥ 7 out of 10. All voting and endorsement criteria were prespecified.

2.4 | External Voting Panel

An external voting panel was engaged to provide their opinions on the proposed recommendation statements. The same voting rules were applied to Delphi exercises for the voting panel. The voting panel ($n = 90$) consisted of up to five practicing rheumatologists who care for patients with PsA from each participating country.

3 | Results

3.1 | Principles of Management

Eight statements of overarching principles for managing PsA were drafted for eight concept areas: goals of treatment; shared decision-making; regular assessment of PsA domains; treat-to-target; considering comorbidities; considering extra-musculoskeletal manifestations; multidisciplinary care; and facilitating early diagnosis (Table 1). The statements were rephrased and refined, and no missing concepts were identified. Consensus was achieved through two rounds of anonymized Delphi exercises [11]. Detail of phrasing of statements of the first and second Delphi exercises is shown in Table S2.

3.2 | Literature Search and Evidence Development

The literature search for the main research question on classes of drugs for PsA treatment yielded 3437 articles, from which 178 articles from 113 studies were included (Figure S1). From the literature search for comorbidities in PsA, 21 of 1449 identified articles were included (Figure S2), and the search for therapies for uveitis and IBD yielded 113 from 746 identified articles (Figure S3).

A total of 16 statements for the management of PsA were developed, including seven on the treatment of arthritis, enthesitis, or dactylitis, two on the treatment of axial involvement, one for uveitis, two for IBD, two on cardiovascular and other comorbidities, and two on specific infectious diseases. All statements were discussed in a 4-h webinar conducted on 23 March 2024.

TABLE 1 | Principles of the management of psoriatic arthritis in the APLAR regions.

Concepts	Statements	Agreement (%)[a]	
		First Delphi	Second Delphi
1. Goals of treatment	The goals of treatment for PsA are to: 1. Control disease activity; 2. Restore physical and social roles; 3. Prevent joint damage and disability; 4. Improve quality of life	97.0	90.6
2. Shared decision making	The therapeutic decision should be based on the best available evidence, be individualized, and be made with a shared decision with the patient, and with consideration of the best available resources	100	87.5
3. Regular assessment of PsA domains	All PsA domains should be assessed at regular intervals for ongoing disease activity	90.9	84.4
4. Treating to target	A treat-to-target strategy should be adopted aiming for remission or a low disease activity state, if remission is not achievable	97.0	90.6
5. Considering comorbidities	Rheumatologists should assess and coordinate with appropriate specialties in the management of comorbidities, including obesity, Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), cardiovascular disease and its risk factors, fibromyalgia, and depression	97.0	96.9
6. Considering extra-musculoskeletal manifestations	Extra-musculoskeletal manifestations, including psoriasis, nail disease, uveitis, and inflammatory bowel disease (IBD), should be considered in the care of patients	90.9	87.5
7. Multidisciplinary care	Multidisciplinary care with other specialties is encouraged wherever feasible for proper care of extra-musculoskeletal manifestations	90.9	78.1
8. Facilitating early diagnosis	Rheumatologists should facilitate early diagnosis and treatment of psoriatic arthritis utilizing the best resources available within their medical system	90.9	100

[a]Agreement was considered when 70% or more of participants rated the principle ≥ 7 on a scale from 1 to 9 ('not important' to 'critically important'). Response rates for first and second Delphi exercises were 94% and 91%, respectively.

TABLE 2 | Recommendation statements for management of psoriatic arthritis in APLAR regions.

Recommendation statements	Quality of evidence by GRADE	Working Group		
		First Delphi	Second Delphi	External panel
		% Agreement[a] [mean score[b]]		
For peripheral arthritis, enthesitis, and dactylitis				
1. We strongly recommend MTX, conditionally recommend SSZ and leflunomide for cs-DMARD–naïve patients with active peripheral arthritis	⊕⊕⊖⊖ Low	100 [8.55]	75.0 [7.56]	95.8 [8.56]
2. We strongly recommend apremilast in cs-DMARD–naïve or –experienced PsA patients with active peripheral arthritis, enthesitis, dactylitis, and/or psoriasis	⊕⊕⊕⊕ High	96.6 [8.45]	—	84.7 [7.93]
3. In cs-DMARD–naïve PsA patients, we conditionally recommend either TNFi, IL-17i, IL-23i, or IL-12/23i for PsA with active peripheral arthritis (high-quality evidence), enthesitis (moderate-quality evidence), and dactylitis (moderate-quality evidence)	⊕⊕⊕⊕ Moderate to High	96.6 [8.83]	81.3 [7.56]	93.1 [8.39]
4. There are currently no data to support the use of JAKi in cs-DMARD–naïve PsA patients. However, given strong evidence for use of JAKi in cs-DMARD–experienced patients, their potential low production cost, and high accessibility in APLAR regions, we conditionally recommend the use of JAKi[c] for cs-DMARD–naïve patients for all domains	NA	75.8 [7.30]	—	84.7 [7.63]
5. In PsA patients who have prior experience with cs-DMARDs, we strongly recommend TNFi, IL-17i, iL-23i, IL-12/23i, or JAKi[c] over no change of treatment for active peripheral arthritis (high-quality evidence), enthesitis (moderate-quality evidence), and dactylitis (moderate-quality evidence)	⊕⊕⊕⊕ Moderate to High	96.6 [9.38]	—	100 [9.53]
6. In patients who had inadequate response to cs-DMARDs, evidence is supportive of similar efficacies for IL-17i compared to TNFi (high-quality evidence); IL-12/23i compared to TNFi (low-quality evidence); and JAKi compared to TNFi (moderate-quality evidence) for active PsA. We strongly recommend TNFi, IL-17i, iL-23i, or JAKi[c] for peripheral arthritis, enthesitis, and dactylitis equally; and conditionally recommend IL-12/23i for enthesitis. The decision should be made to balance the risks and benefits of therapies with consideration of patient characteristics	⊕⊕⊕⊕ Moderate to High	93.1 [8.72]	—	97.2 [8.63]
7. In PsA patients who have prior experience with b-DMARDs, we strongly recommend switching to a drug from a different class. We strongly recommend TNFi, IL-17i, IL-23i, or JAKi[c] (moderate−/high-quality evidence for peripheral arthritis; moderate-quality evidence for enthesitis/dactylitis) and conditionally recommend switching to IL-12/23i (low−/moderate-quality evidence for peripheral arthritis; low-quality evidence for enthesitis/dactylitis) over no change of treatment for PsA with active peripheral arthritis, enthesitis, and dactylitis.	⊕⊕⊕⊕ Moderate to High	93.1 [8.90]	—	98.6 [8.90]
For axial involvement				
8. Based on strong evidence in axial spondyloarthritis, we strongly recommend NSAIDs as the first option for management of inflammatory axial symptoms in PsA.	⊕⊕⊕⊖ Moderate	96.6 [8.66]	—	97.2 [9.17]

(Continues)

TABLE 2 | (Continued)

Recommendation statements	Quality of evidence by GRADE	Working Group: First Delphi	Working Group: Second Delphi	External panel
		% Agreement[a] [mean score[b]]		
9. For PsA patients with significant axial disease who do not respond well to or are intolerant of NSAIDs, we strongly recommend TNFi, IL-17i, or JAKi[c] over no change in treatment. For those who have not responded adequately to and/or are intolerant of TNFi, IL-17i, and JAKi, we conditionally recommend IL-12/23i and IL-23i over no change of treatment	⊕⊕⊕⊖ Moderate	96.6 [8.66]	—	95.8 [9.03]
For management of cardiovascular comorbidities				
10. We strongly recommend screening for and managing cardiovascular risk factors to improve cardiovascular outcomes for all PsA patients.	⊕⊕⊖⊖ Low	93.1 [9.17]	—	98.6 [9.39]
11. Comorbidities, including obesity, metabolic syndrome, metabolic dysfunction-associated steatotic liver disease (MASLD), and fibromyalgia, may affect both the effectiveness of a given therapy and the potential for adverse events. We conditionally recommend assessing the presence of comorbidities prior to therapy selection.	⊕⊕⊖⊖ Low	100 [9.34]	—	98.6 [9.46]
For management of extra-musculoskeletal manifestations				
12. As MTX is commonly prescribed for uveitis, we conditionally recommend it for PsA patients with uveitis. We conditionally recommend the use of TNFi (monoclonal antibodies) for treatment of uveitis over other b/ts-DMARDs	⊕⊕⊖⊖ Low	100 [8.69]	—	98.5 [8.99]
13. Based on moderate- to high-quality evidence from the studies in IBD, we strongly recommend TNFi (monoclonal antibodies); IL-12/23i for Crohn's disease (CD) and ulcerative colitis (UC). In addition, we strongly recommend IL-23i for CD. Lastly, in the case of UC, we strongly recommend Upa and Tofa, and for CD, Upa[c]	⊕⊕⊕⊖ Moderate	93.1 [8.41]	96.9 [8.41]	100 [8.90]
14. There is high-quality evidence suggestive of IL-17i exacerbates Crohn's disease. We strongly recommend against IL-17i for IBD	⊕⊕⊕⊕ High	100 [8.86]	—	95.8 [8.94]
Consideration of specific infections in APLAR regions				
15. In view of the high prevalence of latent tuberculosis (LTB) across the APLAR regions, we strongly recommend screening for LTB prior to any b-/tsDMARD therapy, following local guidelines. In the absence of local guidelines on screening, we conditionally recommend either Interferon-Gamma Release Assays (IGRA) or Tuberculin Skin Test (TST), whichever is locally accessible, together with chest radiography	⊕⊖⊖⊖ Very low	93.1 [8.90]	100 [9.41]	97.2 [9.64]
16. We recommend prescribers follow their national guidelines on screening for hepatitis B and C. In the absence of local guidelines, we strongly recommend serological screening for HBV infection with at least HBsAg prior to initiation of all b-/ts-DMARD therapies for patients with PsA	⊕⊖⊖⊖ Very low	96.6 [9.00]	—	95.8 [9.42]

Note: The response rates for the first and second Delphi exercises in the working group were 85.7% and 91.4%, respectively. The response rate for the external voting panel was 80.0%.

Abbreviations: b, biological; CD, Crohn's disease; cs, conventional synthetic; DMARDs, disease-modifying antirheumatic drugs; HBsAg, hepatitis B surface antigen; IBD, inflammatory bowel disease; IGRA, Interferon-Gamma Release Assays; IL-12/23i, interleukin-12/23 inhibitors; IL-17i, interleukin-17 inhibitors; IL-23i, interleukin-23 inhibitors; JAKi, Janus kinase inhibitors; LTB, latent tuberculosis; MASLD, metabolic dysfunction-associated steatotic liver disease; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; PsA, psoriatic arthritis; SSZ, sulfasalazine; TNFi, tumor necrosis factor inhibitors; Tofa, tofacitinib; ts, targeted synthetic; TST, tuberculin skin test; UC, ulcerative colitis; Upa, Upadacitinib.

[a]The % agreement was defined as the proportion of participants who rated the agreement as ≥ 7/10. Agreement is achieved if ≥ 70% of participants voted ≥ 7/10 for agreement.

[b]The mean scores were based on agreement ratings from 1 to 10 ('not agree' to 'fully agree').

[c]JAK inhibitors should be used with caution in patients aged 65 years or older, those at increased risk of major cardiovascular problems (such as myocardial infarction or stroke), those who smoke or have smoked, those at increased risk of cancer, and those with blood clots in the lungs and deep veins (venous thromboembolism, VTE). The doses should be reduced in these patient groups where possible.

Consensus on these 16 statements from the working group was achieved through two rounds of Delphi exercises (response rate 85.7% and 91.4%) (Table 2). After the first Delphi exercise, further comments were solicited from all working group members and discussed through email correspondence. Frequent dialogue among stakeholders for four statements was made with relevant refinements and taken to the second Delphi exercise. The statements for the first Delphi and the rephrased statements for the second Delphi are shown in Table S3. In addition, endorsement of these 16 statements was achieved through a Delphi exercise with the external voting panel ($n = 90$, response rate 80.0%). A schematic diagram for the management of patients with PsA in the APLAR regions was constructed (Figure 1).

3.3 | Management of PsA Patients Who Are naïve to Treatment

For treatment-naïve PsA patients with active inflammation in peripheral arthritis, we strongly recommend the use of methotrexate (MTX) and conditionally recommend the use of sulfasalazine (SSZ) and leflunomide (Recommendation Statement 1). Although the quality of evidence supporting the efficacy of these cs-DMARDs was low in RCTs, this decision is balanced against the long experience of using cs-DMARDs in PsA. These cs-DMARDs are more accessible in most APLAR countries at affordable prices. This is further supported by observational data from four studies of MTX monotherapy [14–17], with reasonable numerical short-term ACR responses at 12–24 weeks ranging from 40.8% to 63.2%. Therefore, the working group strongly recommends treatment initiation with MTX, conditionally with SSZ and leflunomide (Statement 1, Table 2), while assessing response and upgrading to other effective therapies according to the treat-to-target strategy as outlined in the principles of management (Table 1). This consensus was achieved after balancing the low quality of evidence for efficacy, with the low cost and high accessibility of cs-DMARDs in the APLAR regions. The working group also acknowledges the importance of close monitoring of adverse events related to cs-DMARDs, particularly those for the liver with leflunomide, with or without the combination with MTX [18]. In addition, a five-fold higher risk of interstitial lung disease associated with leflunomide in some Asian ethnicities has been reported compared to data from the Caucasian populations [19].

There is moderate- to high-quality evidence to support the beneficial effects of tumor necrosis factor inhibitors (TNFi) for active peripheral arthritis, enthesitis, and dactylitis in PsA patients who were naïve to cs-DMARDs, particularly in those with early disease [15, 16], which may indirectly apply to interleukin (IL)-17i and IL-12/23i. There was a lengthy discussion as TNFi, IL-17i, and IL-12/23i are less accessible than cs-DMARDs because of their cost. In many APLAR regions, these b-DMARDs are classified in the non-standard drug list, patients pay outof-pocket with minimal subsidies, and most do not have insurance coverage. After the second Delphi, the working group finally reached a consensus to conditionally recommend either TNFi, IL-17i, or IL-12/23i for treatment-naïve patients with active PsA (Statement 2, Table 2). This is highly relevant for Asians who have a poorer tolerance of MTX with a higher risk of lymphoproliferative disorders [20], as well as a higher prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) or fatty liver despite their relatively lean body build [21]. In addition, patient partners in the working group have expressed loud and clear that patients deserve the right to know about and be offered a choice of the best available treatments.

Despite the lack of direct evidence for the use of janus kinase inhibitors (JAKi) in cs-DMARD–naïve PsA patients, the working group conditionally recommends them based on the good efficacy and safety indirectly from cs-DMARD–experienced patients, as well as the potential high accessibility from generic products due to low production cost in the APLAR regions (Statement 4, Table 2). The working group highlighted the caution in the use of JAKi at the lowest dose possible for patients above 65 years of age, those at risk of major cardiovascular problems such as myocardial infarction or stroke, those who smoked, and those at increased risk of cancer and thromboembolic events. This caution applies to JAKi use in all patient groups, and the balance of risks and benefits should be considered.

3.4 | Management of PsA Patients Who Had Inadequate Response to Cs-DMARDs or b-DMARDs

For patients with inadequate response to cs-DMARDs, the working group strongly recommends TNFi, IL-17i, IL-23i, or JAKi equally for peripheral arthritis, enthesitis, and dactylitis, given similar high-quality evidence (Statement 6, Table 2). For patients with prior experience with b-DMARDs, we strongly recommend switching between TNFi, IL-17i, IL-23i, or JAKi, preferably to a drug from a different class. A switch to IL-12/23i was only conditionally recommended given the low quality of evidence (Statement 7, Table 2).

3.5 | Management of Axial Domain

Based on indirect evidence derived from axial spondyloarthritis (axSpA) [22], we strongly recommend nonsteroidal anti-inflammatory drugs (NSAIDs) as the first pharmaceutical option for active axial PsA. For those with persistent axial symptoms despite NSAIDs, we strongly recommend TNFi, IL-17i, or JAKi over no change in treatment (Statements 8 and 9, Table 2).

In view of limited evidence for the use of IL-23i in ankylosing spondylitis [23], but potentially favorable results for axial PsA with imaging-confirmed sacroiliitis [24], we conditionally recommend IL-12/23i and IL-23i over no change of treatment only for those who have not responded adequately to and/or are intolerant of TNFi, IL-17i, and JAKi, and when the choice of treatment is limited (Statement 9, Table 2).

3.6 | Management of Comorbidities

The comorbidities subgroup concluded from the review of 21 observational studies, including a meta-analysis of five PsA studies involving 35 907 PsA patients, that PsA patients have a 21% increased risk of cardiovascular mortality compared with the general population [25]. Although the quality of evidence was considered low based on observational studies, the evidence is

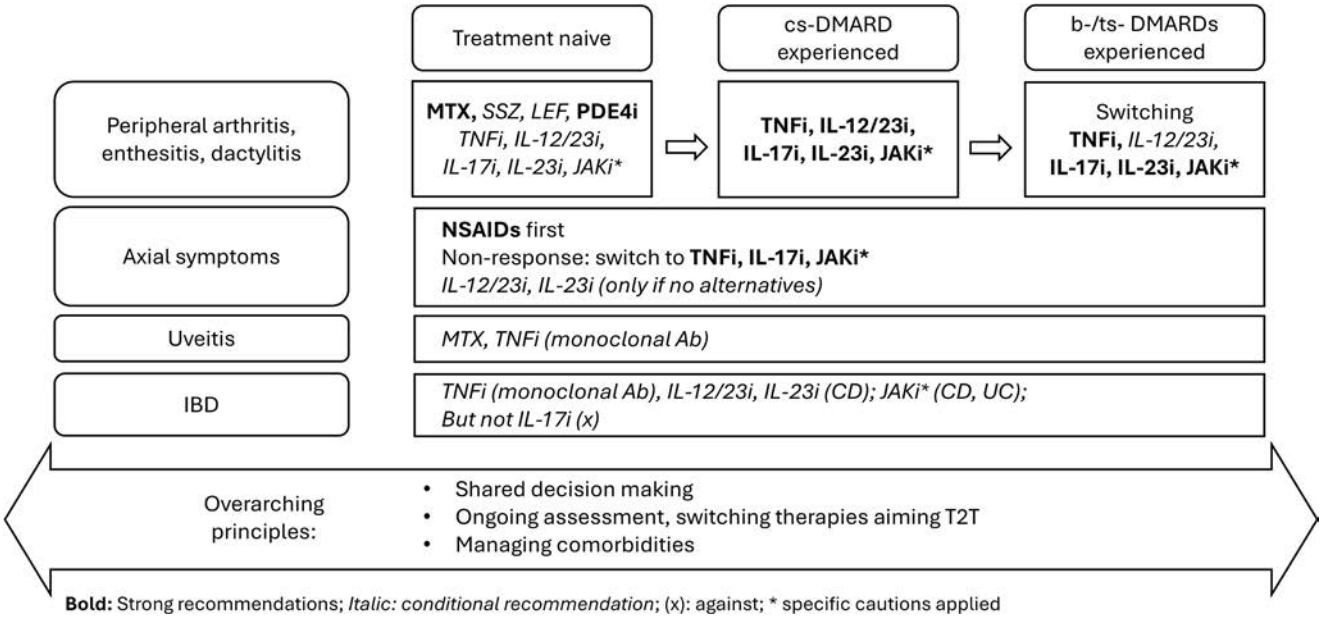

FIGURE 1 | The APLAR Recommendation for PsA. *JAK inhibitors should be used with caution in patients aged 65 years or older, those at increased risk of major cardiovascular problems (such as myocardial infarction or stroke), those who smoke or have smoked, those at increased risk of cancer, and those with blood clots in the lungs and deep veins (venous thromboembolism, VTE). The doses should be reduced in these patient groups where possible. b, Biological; CD, Crohn's disease; cs, Conventional synthetic; DMARDs, Disease-modifying antirheumatic drugs; IL-12/23i, Interleukin-12/23 inhibitors; IL-17i, Interleukin-17 inhibitors; IL-23i, Interleukin-23 inhibitors; IBD, Inflammatory bowel disease; JAKi, Janus kinase inhibitors; MTX, Methotrexate; NSAIDs, Non-steroidal anti-inflammatory drugs; PsA, Psoriatic arthritis; SSZ, Sulfasalazine; TNFi, Tumor necrosis factor inhibitors; Tofa, Tofacitinib; ts, Targeted synthetic; UC, Ulcerative colitis; Upa, Upadacitinib.

convincing and has a high impact. Therefore, we recommend screening and managing cardiovascular risk factors to improve outcomes for all PsA patients (Statement 10, Table 2). In addition, comorbidities, including obesity, metabolic syndrome, MASLD, and fibromyalgia, may affect both the effectiveness of a given therapy and the potential for adverse events. We conditionally recommend assessing the presence of comorbidities before therapy selection (Statement 11, Table 2).

Evidence and recommendations regarding treatment of uveitis and IBD were extrapolated from the primary conditions rather than PsA studies. We conditionally recommend MTX and TNFi (monoclonal antibodies) for uveitis (Statement 12, Table 2). For IBD, we recommend TNFi (monoclonal antibodies), IL-12/23i, and JAKi according to the evidence. Further details are available in Statement 12, Table 2.

3.7 | Specific Concerns for the APLAR Regions

Due to the high prevalence of tuberculosis (TB) and latent tuberculosis (LTB) across the APLAR regions [26], we strongly recommend screening for LTB before the use of any biological therapy, following the respective country guidelines. The infection subgroup asked whether Interferon-Gamma Release Assays (IGRA), such as T-SPOT or QuantiFERON, should be used over the tuberculin skin test (TST) to diagnose LTB prior to biologic therapy. The former is more costly and less accessible in some APLAR regions. Overall, based on low-quality evidence, the working group concluded that there was fair agreement between IGRA and TST. The quality of evidence was limited by heterogeneity in the types of IGRAs used, varying endemicity of TB in different studies, indirectness of data derived from other inflammatory arthritis conditions, and different methods for endorsing the agreement. We, therefore, conditionally recommend the use of either IGRA or TST for screening of LTB prior to initiating biological therapy (Statement 15, Table 2).

There is a high prevalence of chronic hepatitis B across the APLAR regions [27] and a low to moderate risk of hepatitis B reactivation is recognized with all DMARDs [28]. After review, the working group concluded that the data are inadequate to support the superiority of the combination of hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBcAb) over HBsAg alone in predicting the risk of reactivation. We suggest stakeholders follow their national guidelines; where absent, we strongly recommend screening for chronic hepatitis B with at least HBsAg prior to initiation of all DMARD therapies for patients with PsA therapy (Statement 16, Table 2).

4 | Discussion

These are the first treatment recommendations developed for the management of PsA for the APLAR regions and provide up-to-date guidance for practitioners on the care for PsA. These recommendations involved comprehensive evaluation of evidence on the efficacy and safety of classes of drugs in PsA. We conducted comprehensive systematic literature reviews and derived the quality of evidence according to the well-defined GRADE methods. To achieve consensus, we have balanced values, preferences, and resource use through thorough discussions among the experts providing care for PsA patients across a broad area across the APLAR regions. This supplemented the existing

recommendations developed by consensus for smaller regional areas across Asia [29–33]. The APLAR regions comprises populations with diverse cultures, demographics, socioeconomic development, and healthcare systems. Particularly, for many places within APLAR, medical insurance is less developed, and patients have limited subsidies for the use of biologics. On the other hand, generic ts-DMARDs have become more accessible in some regions. The APLAR regions are also characterized by the high prevalence of infectious diseases like chronic hepatitis B [27] and TB [26], as well as the higher prevalence of metabolic syndromes [21]. These factors challenged the consensus process for the current guideline. The composition of the working group was the key to obtaining balanced perspectives on the specific needs across countries. Our current working group comprises rheumatologists from 18 countries across the APLAR regions. The invitation to participate was sent via the APLAR SpA-SIG network, with the additional effort of recruiting members from countries without representation in the SpA-SIG. To ensure equity of representation, we limited participation to two members from each country. Dermatologists and patients were also engaged in the working group to give a more holistic perspective on PsA care. The endorsement from the external panel demonstrates the recommendations' practicality and generalizability to the region.

Other recommendation guidelines have been developed and updated from international societies for the care of PsA [7, 9, 34]. Similar to these recommendations, we advocate a treat-to-target strategy, with regular assessments and adjustments towards achieving remission or low disease activity. We emphasize a shared decision-making approach for therapeutic decisions with patients and consider the best available options, supported by evidence within the available resources. Compared with the GRAPPA recommendations that emphasized numerous domains [7], we took a simple approach towards combined peripheral domains (arthritis, enthesitis, and dactylitis) and axial involvement. We have included comprehensive reviews into extra-articular manifestations including uveitis and IBD, although the evidence derived was indirectly extrapolated from the primary conditions. We did not specifically evaluate evidence on the skin and nail domains, yet dermatologists' perspectives were included in our discussions. Our principles of management highlight the need to consider these extra-articular manifestations and emphasize the importance of multidisciplinary care.

In these APLAR recommendations, we strongly recommend MTX and conditionally recommend other cs-DMARDs as the first line, while being cognizant of monitoring for adverse events. In contrast to the EULAR guideline that recommended b−/ts-DMARDs after failing cs-DMARDs [34], we conditionally recommend b-DMARDs as the first line, if accessible, given the quality of evidence. We also conditionally recommend JAKi as the first line (with a caution on adverse events) given the potential benefits and relative accessibility. However, considering resource use, our level of recommendation for b-/ts-DMARDs as the first line is conditional, in contrast to the strong recommendation given by GRAPPA [7]. The current APLAR recommendations built on current evidence and consensus will serve as the basis for further updates in the near future. It would be of great interest when more evidence on different types of JAKi and new oral tyrosine kinase 2 inhibitors are available for updated review.

5 | Conclusion

We have developed the first recommendations on the care of PsA for the APLAR regions based on the best available evidence, adjusted for practicality and the situations in APLAR regions. The agreement has been achieved by experts and patients across the countries, as well as from a broader voting panel. These recommendations for PsA should provide guidance for practitioners caring for PsA patients in the APLAR regions.

Author Contributions

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Conflicts of Interest

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Data Availability Statement

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

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1.** Flow diagram of the main literature search on efficacy and safety of therapies for psoriatic arthritis. **Figure S2.** Flow diagram of the literature search on impact of comorbidity on treatment outcomes of patients with PsA. **Figure S3.** Flow diagram of the literature search on the efficacy and safety of advanced therapies in IBD and uveitis which have been used in PsA. **Table S1.** Main research question for the efficacy and safety of therapies in PsA. **Table S2.** Principles of the management of psoriat c arthrit s in the APLAR. **Table S3.** Recommendation statements for management of psoriatic arthritis in APLAR regions, phrasing of first and second Delphi.

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REVIEW

Vitamin D Is Associated With Susceptibility and Disease Severity in Systemic Lupus Erythematosus: A Systematic Review and Meta-Analysis

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ABSTRACT

Objectives: Vitamin D, recognized for its immunomodulatory properties, is potentially associated with autoimmune diseases like systemic lupus erythematosus (SLE). This systematic review and meta-analysis assessed the relationship between Vitamin D levels and SLE, highlighting its role in modulating disease activity and immunological markers like anti-dsDNA, C3, and C4.

Methods: Case–control studies reporting Vitamin D, disease activity indices, C3, C4, and anti-dsDNA antibody levels in SLE patients and healthy controls were evaluated. Systematic searches in PubMed, Scopus, ScienceDirect, Web of Science, and Embase were last updated on April 6, 2024. Inclusion criteria targeted studies on SLE patients and healthy controls reporting these specific markers. Study quality was assessed with the Newcastle-Ottawa Scale (NOS), and publication bias was checked using Egger's test and Begg's funnel plot. The meta-analyses were conducted with CMAv4.

Results: Analysis of 43 studies with 2940 SLE patients and 2458 healthy controls showed significantly lower Vitamin D levels in SLE patients (mean difference: −10.070 ng/mL; 95% CI: −12.85 to −7.28; $p < 0.001$). Vitamin D levels negatively correlated with SLEDAI scores (correlation: −0.427; 95% CI: −0.541 to −0.298; $p < 0.001$) and anti-dsDNA antibodies (correlation: −0.397; 95% CI: −0.611 to −0.130; $p = 0.004$), and positively correlated with complement components C3 (correlation: 0.268; 95% CI: 0.077–0.440; $p = 0.006$) and C4 (correlation: 0.299; 95% CI: 0.192–0.400; $p < 0.001$). Sensitivity analyses confirmed these findings.

Conclusions: The findings revealed a strong association between low Vitamin D levels and increased SLE severity. However, limitations like some inconsistency, small-study biases, and possible language restrictions in study inclusion necessitate cautious interpretation.

1 | Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with multisystem involvement, characterized by the dysregulated production of autoantibodies that target cells and tissues, resulting in significant morbidity and mortality [1, 2]. Despite advancements in therapeutic approaches, the etiology and pathogenesis of SLE remain incompletely understood. They are attributed to a multifactorial interplay of genetic, environmental, and immune system factors [3, 4].

Vitamin D, a fat-soluble steroid hormone, is an environmental factor primarily known for its role in calcium and phosphate homeostasis, essential for bone health [5]. Beyond its skeletal functions, Vitamin D exerts multiple immunomodulatory effects. The biologically active form of Vitamin D, calcitriol (1,25-dihydroxy Vitamin D3), supports immune regulation by enhancing T regulatory (Treg) cell function [6, 7], inducing apoptosis in activated B cells and dendritic cells, and inhibiting plasma cell differentiation and autoantibody production [8–10]. Furthermore, the effects of Vitamin D are mediated through the Vitamin D receptor (VDR), with genetic polymorphisms in VDR linked to SLE susceptibility and disease severity [11, 12].

Epidemiological studies have produced conflicting results regarding the relationship between Vitamin D levels and SLE. Some research indicates that Vitamin D deficiency may elevate the risk of developing SLE and is inversely associated with important markers of disease severity, like anti-dsDNA, and positively correlates with complement levels (C3, C4). Some studies do not support these observations [9, 13–51].

Earlier meta-analyses had explored the relationship between Vitamin D and SLE, but they were constrained by either a limited number of studies or the omission of critical correlation analyses: Guan et al. considered 19 studies [52], Islam et al. examined 34 studies [53]. None analyzed the correlations with disease severity parameters like SLEDAI scores and immunological markers. The rising concerns regarding redundant and contradictory systematic reviews and meta-analyses felt the necessity for a comprehensive and up-to-date analysis [54].

To address these gaps, we conducted an updated meta-analysis to comprehensively assess the relationship between Vitamin D and SLE. This analysis will include correlations with disease activity scores, anti-dsDNA, and complement levels.

2 | Materials and Methods

2.1 | Literature Search for Meta-Analysis

A comprehensive literature search was performed using PubMed, Scopus, Web of Science, Embase, and ScienceDirect databases to identify relevant studies. The search spanned all articles published until April 6, 2024, without setting a lower time limit to ensure an exhaustive literature review. Keywords used included 'Vitamin D,' '25-hydroxyvitamin D,' 'C3', 'C4', 'dsDNA', 'systemic lupus erythematosus,' and 'SLE,' combined using Boolean operators (AND/OR). Manual searches of references from previous systematic reviews and meta-analyses on this topic were also conducted to identify additional eligible studies. The detailed search strategy is presented in Table S1.

2.2 | Inclusion and Exclusion Criteria

Eligible studies were required to meet the following inclusion criteria: (1) Study Design: Only case–control studies were considered; (2) Participants: Studies included patients diagnosed with systemic lupus erythematosus (SLE) according to the criteria established by the American College of Rheumatology (ACR) or European Alliance of Associations for Rheumatology (EULAR); (3) Outcomes: Reports needed to provide data on Vitamin D levels, presented either as mean and standard deviation or as median and interquartile range, in both SLE patients and healthy controls; and (4) Language and Publication Type: Only studies published in English as full-length journal articles were included. Other publication types, such as conference abstracts, master's theses, or dissertations, were excluded.

The exclusion criteria included the following: (1) review articles and case studies, (2) studies lacking data on either SLE patients or healthy controls, (3) reports with incomplete information, and (4) animal studies or investigations involving SLE patients who were pregnant, had infections, had juvenile SLE, or overlaps.

2.3 | Study Screening

Two independent reviewers (S.R. and A.K.P.) screened the study. Titles and abstracts were initially reviewed to exclude irrelevant studies. Full-text articles of potentially eligible studies were retrieved for further assessment. Discrepancies between reviewers were resolved through discussion or adjudicated by a third reviewer. No attempt was made to contact the original authors for missing data.

2.4 | Data Extraction

Two authors (A.K.P. and S.R.) independently extracted data from the included studies using a standardized form. The information extracted included: (1) Study details: First author, year of publication, and study location; (2) Participant characteristics: Sample size, age, and BMI of SLE patients and healthy controls; (3) Vitamin D levels: Mean and standard deviation (or converted from median and interquartile range); (4) Statistical data: Correlations (r-values, p-values) between Vitamin D levels and SLEDAI, C3, C4, and anti-dsDNA; and (5) Assay methods used to measure Vitamin D levels. In cases of disagreement, a consensus was reached through discussion.

2.5 | Quality Assessment

Study quality was assessed independently by two reviewers using the Newcastle-Ottawa Scale (NOS) for case–control studies. The NOS evaluates three criteria: selection of study groups (0–4 stars), comparability of groups (0–2 stars), and exposure ascertainment (0–3 stars). Studies receiving five or more stars were deemed to be of moderate or good quality. Discrepancies in quality assessments were resolved through discussion. The NOS was selected due to its widespread use and ability to evaluate methodological quality in observational studies [55]. The graphical presentation of the NOS was performed by the RobVis tool [56].

2.6 | Statistical Analysis

Statistical analysis was performed using Comprehensive Meta-Analysis (CMA) v4 software. Vitamin D values reported as

medians with interquartile ranges were converted to means and standard deviations using established formulas to ensure uniformity. Effect sizes (mean differences or correlations) were calculated with 95% confidence intervals. A two-tailed p-value < 0.05 was considered statistically significant. Publication bias was evaluated using Begg's funnel plot and Egger's regression analysis [57]. Heterogeneity across studies was assessed using the Cochrane Q test, with significant heterogeneity indicated by p-heterogeneity < 0.05 and I^2 values $> 50\%$ [58]. A random-effects model was applied in cases of significant heterogeneity, whereas a fixed-effects model was used otherwise. Sensitivity analysis was conducted by excluding one study at a time to test the robustness of the results [59].

3 | Results

3.1 | Literature Search and Eligible Studies for Meta-Analysis

We identified a total of 5126 records through database searches, with 43 studies ultimately included in the final analysis. In compliance with PRISMA 2020 guidelines [60], a detailed overview of the search process is presented in Figure 1 as a PRISMA flowchart. Furthermore, the item- and study-level results from the NOS assessment were also done (Figure S1). The general characteristics and data for all studies included in this meta-analysis are shown in Table S2.

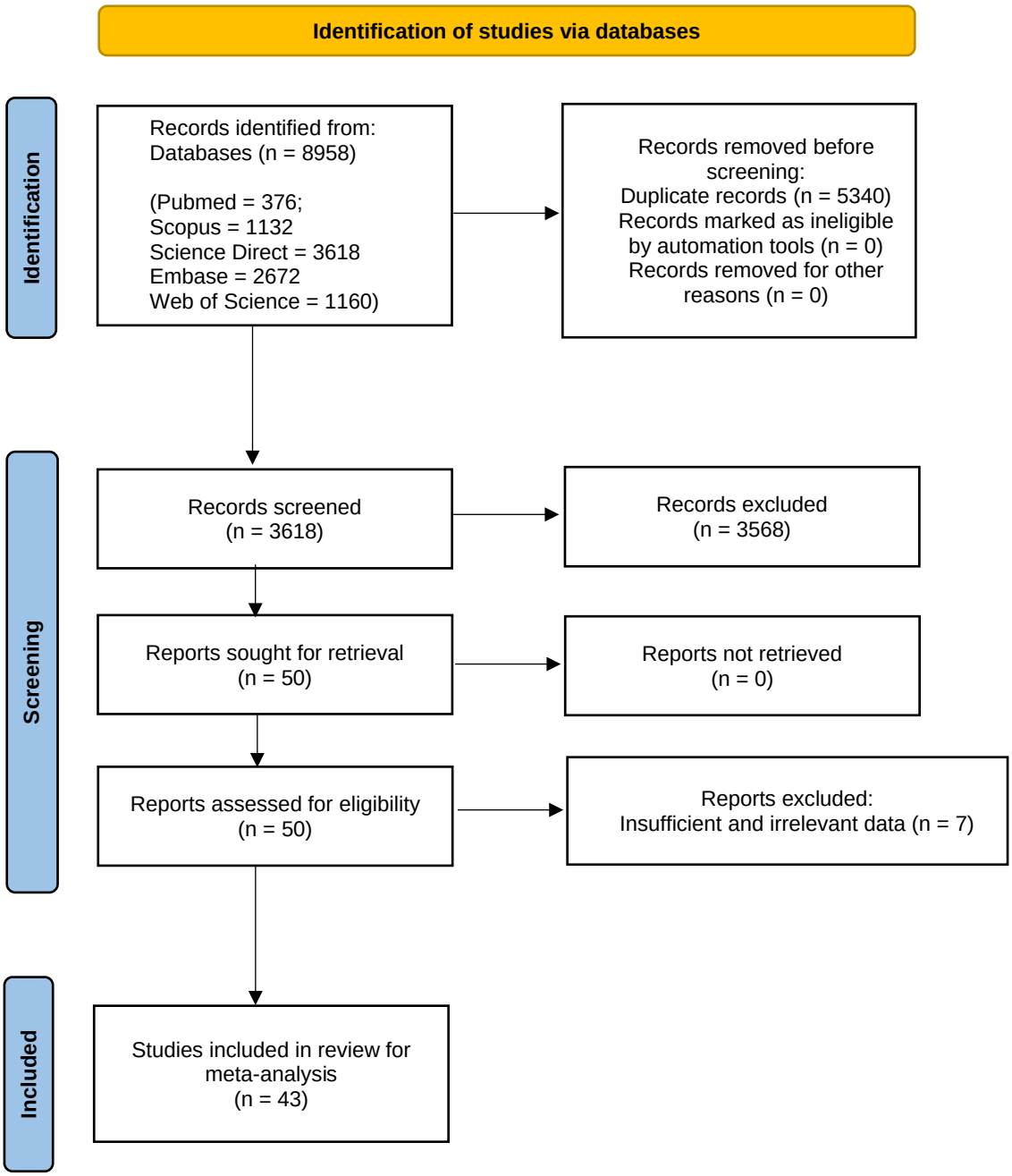

FIGURE 1 | PRISMA 2020 study flow chart showing the number of included and excluded articles for the present meta-analysis.

3.2 | Publication Bias

Meta-analysis can be affected by publication bias, which can compromise the validity and strength of the results. To assess the presence of publication bias in our included studies, we used both Begg's funnel plot and Egger's regression analysis. Our findings indicated that there was a significant publication bias in the comparison of mean Vitamin D levels between SLE patients and healthy controls, as well as in the correlation between Vitamin D and complement C4 (Figure S2). This was supported by Egger's regression analysis, which also demonstrated a significant publication bias in these areas (Table S3). However, we did not observe any publication bias in other correlation meta-analyses involving Vitamin D and SLEDAI scores, C3, and anti-dsDNA, as evidenced by both funnel plots (Figure S2) and Egger's regression analysis (Table S3).

3.3 | Heterogeneity Investigation

In the current meta-analysis, we used the Cochrane Q test and I^2 statistics to assess heterogeneity within the included reports. Notably, we observed significant heterogeneity across all comparison models, with the mean Vitamin D comparison between SLE and HC showing a Q value of 4292.60, $P_{heterogeneity}$ of 0.000, and I^2 of 99.02. Additionally, we found significant heterogeneity while performing the correlations between Vitamin D and SLEDAI scores ($Q=35.32$, $P_{heterogeneity}=0.000$, $I^2=71.69$), Vitamin D and C3 ($Q=14.41$, $P_{heterogeneity}=0.01$, $I^2=65.32$), and Vitamin D and anti-dsDNA ($Q=13.27$, $P_{heterogeneity}=0.004$, $I^2=77.39$). Meanwhile, the Vitamin D and C4 correlation revealed homogeneity among the included reports ($Q=7.41$, $P_{heterogeneity}=0.11$, $I^2=46.08$). Based on these heterogeneity test results, we used a random-effects model (heterogeneity) for the meta-analysis, except for the Vitamin D and C4 comparison (Table S3).

3.4 | SLE Patients Displayed Decreased Vitamin D Serum Levels Than Healthy Controls

Our meta-analysis revealed that SLE patients had significantly lower serum Vitamin D levels compared to healthy controls (mean difference: −10.07 ng/mL, 95% CI: −12.85 to −7.28, $p=0.000$) (Figure 2). Importantly, the high heterogeneity observed for this outcome ($Q=4292.60$, $I^2=99.02$, $P_{heterogeneity}<0.001$) highlights substantial inconsistency across studies.

3.5 | Correlation of Vitamin D With SLEDAI Scores, C3, C4, and Ds DNA

To determine this correlation between Vitamin D and disease severity in SLE patients, we conducted a meta-analysis of 11 previously published reports involving 726 SLE patients. The results shown in Figure 3 indicate a significant inverse correlation between Vitamin D levels and SLEDAI scores (correlation: −0.427, 95% CI: −0.541 to −0.298, $p=0.000$).

We explored the potential association between Vitamin D and complement levels-C3 and C4 levels. Our study included six reports of 349 SLE patients for C3 and five reports of 309 SLE patients for C4. A meta-analysis revealed a positive correlation between Vitamin D levels and both C3 (correlation: 0.268, 95% CI: 0.077–0.440, $p=0.006$) (Figure 4A) and C4 (correlation: 0.299, 95% CI: 0.192–0.400, $p=0.000$) (Figure 4B).

Four reports, comprising 264 cases, explored the possible correlation between Vitamin D and anti-dsDNA levels in patients with SLE. The meta-analysis showed a significant inverse correlation between Vitamin D levels in patients with SLE and anti-dsDNA levels (correlation: −0.397, 95% CI: −0.611 to −0.130, $p=0.004$) (Figure 4C).

3.6 | Sensitivity Analysis

A sensitivity analysis was conducted for each meta-analysis by excluding data from one study at a time. A meta-analysis was then performed, and the results were compared with those of the original meta-analysis. The meta-analysis was considered robust if the sensitivity analysis results were minimally different from the parent meta-analysis results [61]. As shown in Figure S3, sensitivity analysis of the association of Vitamin D with SLE and correlation of Vitamin D with C3, C4, and SLEDAI scores showed minimal variation from the original meta-analysis, indicating the robustness of the meta-analysis. However, slight variation in the sensitivity analysis and meta-analysis results was observed while correlating Vitamin D with anti-dsDNA, suggesting a cautious interpretation of the results.

4 | Discussion

The current meta-analysis of 43 studies [9, 13–51], comprising 2940 SLE cases and 2458 healthy controls, reveals significantly lower Vitamin D levels in SLE patients compared to healthy controls, and it inversely correlates with disease activity scores (SLEDAI). Besides, markers of disease activity, such as anti-dsDNA, correlated negatively, and complement (C3, C4) levels correlated positively with Vitamin D levels. Collectively, the observations of the meta-analysis support the previous observations that Vitamin D has a significant association in modulating disease activity and, perhaps, in the pathogenesis of SLE.

Earlier studies have observed a relationship between low Vitamin D levels and susceptibility to SLE and its severity [62, 63]. However, many case–control studies have revealed elevated Vitamin D levels in patients with SLE compared to healthy controls [14, 17, 31, 32]. This is probably a result of the intake of Vitamin D supplements in these patients. The present meta-analysis revealed that patients with SLE exhibited lower Vitamin D levels, which were inversely associated with disease severity. Previous meta-analyses have demonstrated a similar relationship between Vitamin D levels and SLE [33, 52, 53, 64, 65]. But earlier meta-analyses included a smaller number of studies: (Sahebari et al. [$n=11$], Guan et al. [$n=19$], Bae et al. [$n=18$], Wang et al. [$n=24$], and Islam et al. [$n=34$]) compared to the present analysis ($n=43$). Except for one report [33], none of the earlier studies evaluated the correlation between Vitamin D levels and SLE disease activity scores. The precise mechanism(s) underlying low Vitamin D levels and susceptibility to disease

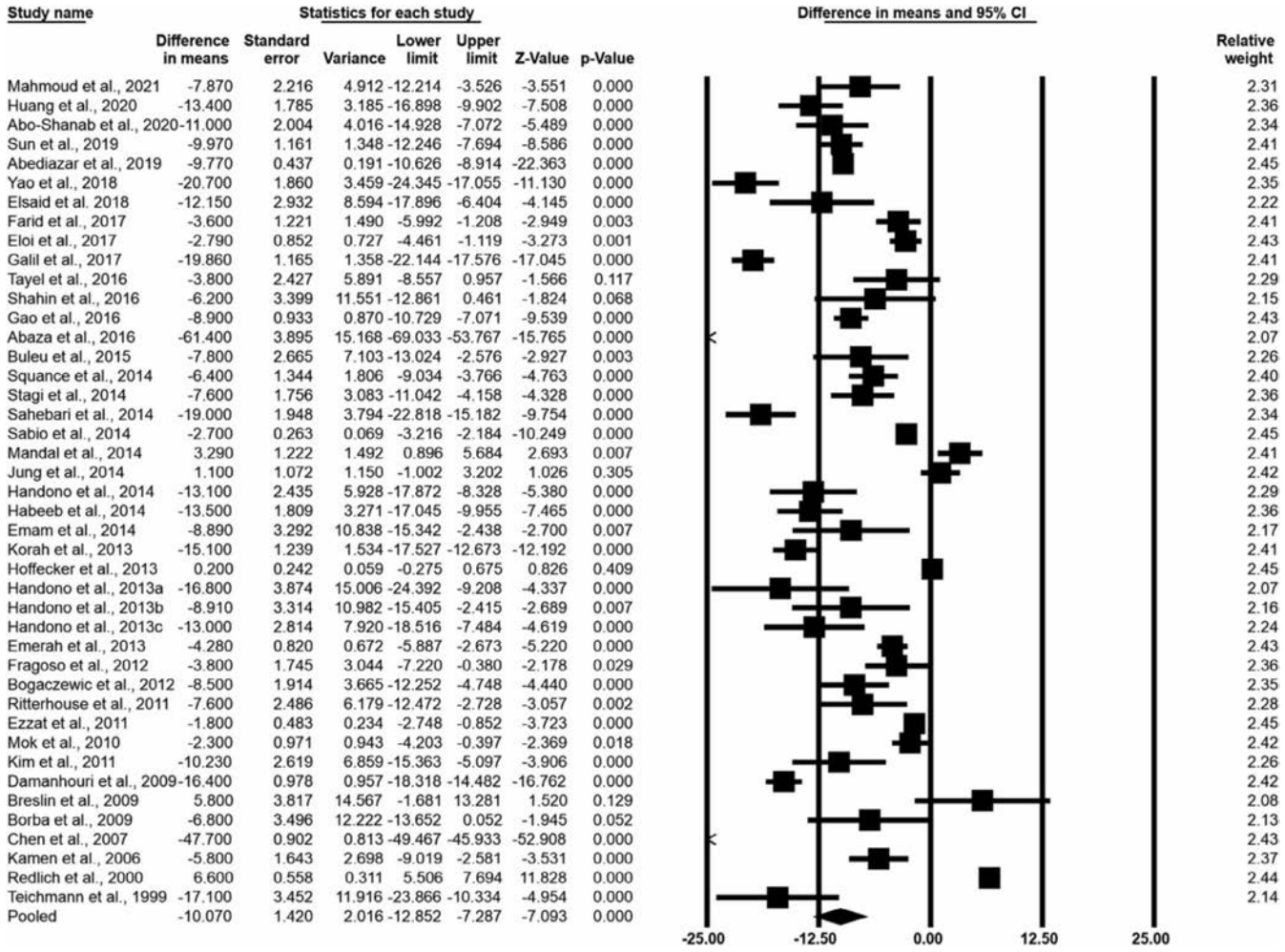

FIGURE 2 | Comparison of mean Vitamin D3 levels among systemic lupus erythematosus patients and healthy controls. The levels of Vitamin D in SLE patients and healthy controls were obtained from articles published earlier in different populations based on inclusion and exclusion criteria. Meta-analysis was performed in the comprehensive meta-analysis software that depicts the standard difference in mean and probability values. A *p* value less than 0.05 was considered significant.

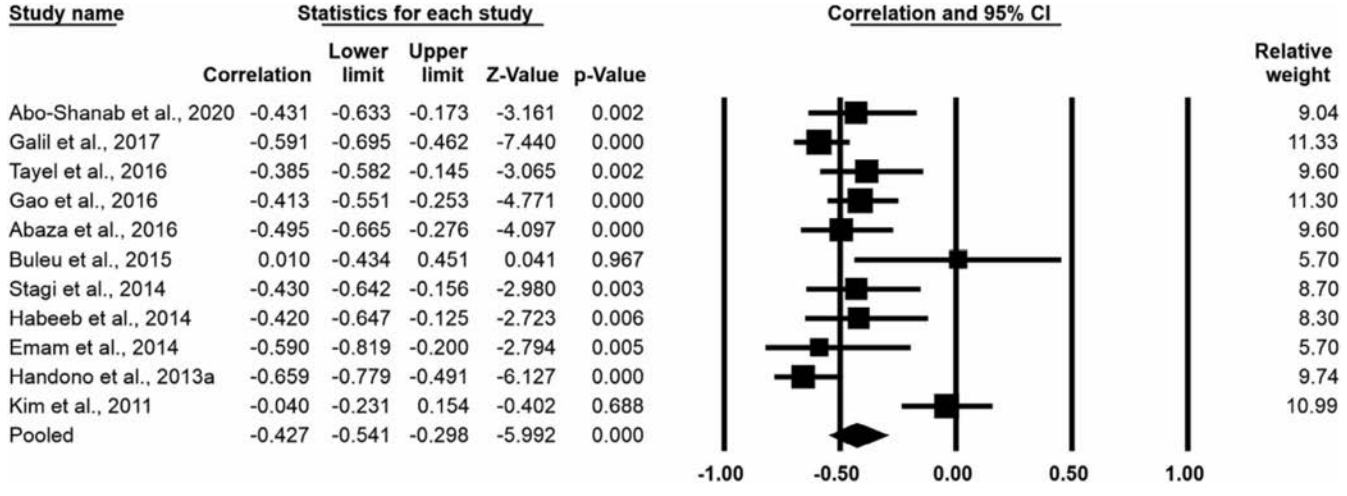

FIGURE 3 | Correlation between plasma Vitamin D levels and SLE disease activity scores. Results from the eligible studies were analyzed for the possible correlation between Vitamin D levels and SLE disease activity scores. The meta-analysis was performed by CMA software. The random effect model was employed for the calculation of the combined correlation and probability value. A *p* value less than 0.05 was taken as significant.

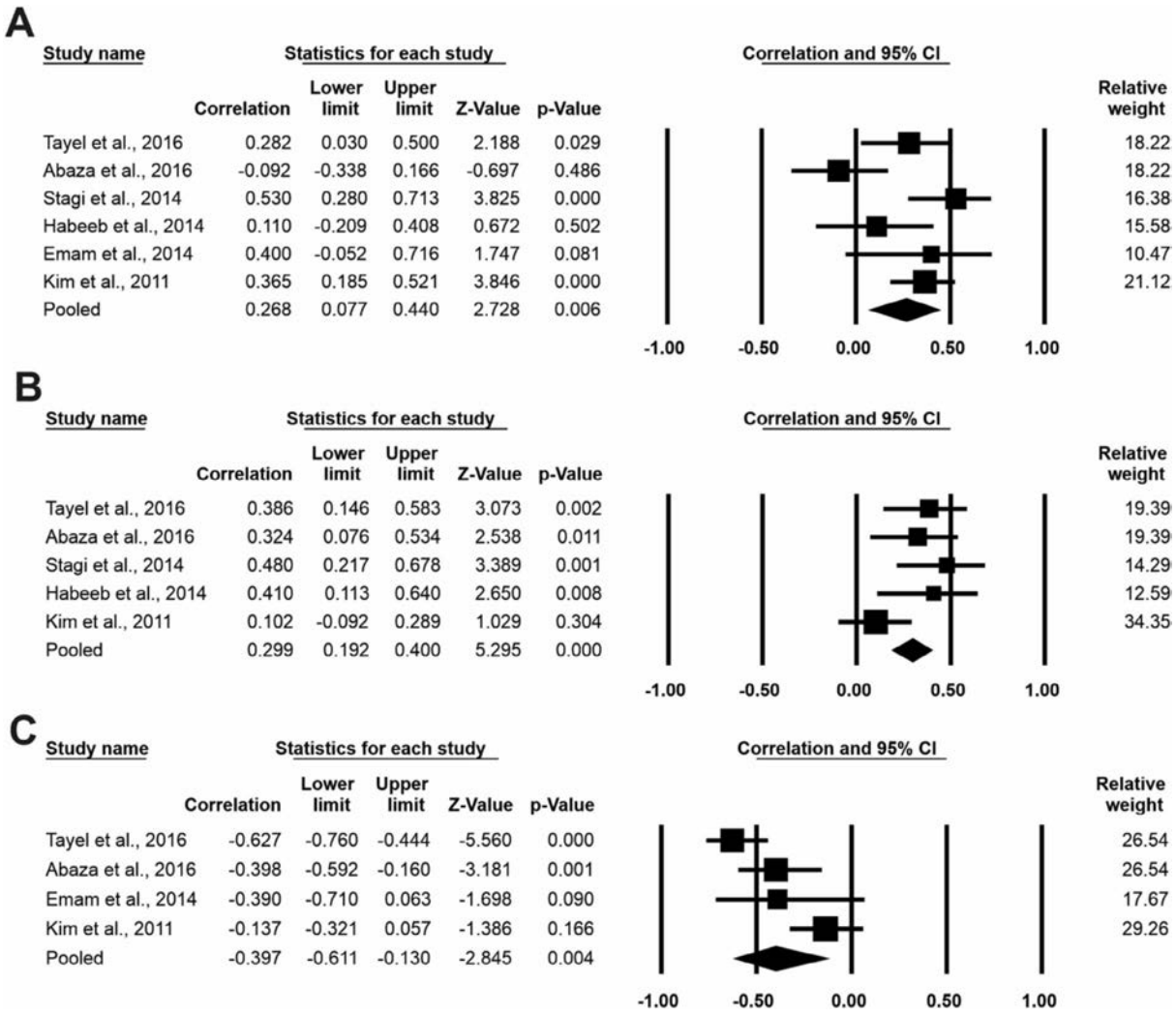

FIGURE 4 | Correlation between plasma Vitamin D levels and complements and anti-ds DNA levels. Results from the eligible studies were analyzed for the possible correlation between Vitamin D levels and C3 (A), C4 (B), and anti-ds DNA (C). The meta-analysis was performed by CMA software. The random effect model was employed for the calculation of the combined correlation and probability value. A p value less than 0.05 was taken as significant.

severity in SLE remain unclear. The multi-pronged immunomodulatory role of Vitamin D could be contributory to this phenomenon. It is unknown what the optimum levels of Vitamin D are that can help reduce the intensity and frequency of SLE flares and lower disease activity scores.

Disease activity scores are based on numerous parameters, such as clinical manifestations and immunological parameters, notably C3, C4, and anti-dsDNA levels. Although the correlation between Vitamin D and complement levels, such as C3 and C4, was not particularly strong, the relationship was statistically significant. The positive correlation between Vitamin D levels and C3/C4 in SLE is noteworthy. To the best of our knowledge, this is the first meta-analysis to investigate the correlation between Vitamin D levels and complement status.

The immunoregulatory role of Vitamin D is multifaceted, as indicated earlier. Vitamin D enhances the function of regulatory T cells [66] and the production of anti-inflammatory cytokines [67], while inhibiting pro-inflammatory cytokines [68] and B-cell activity [69], resulting in a reduction in autoantibody synthesis. Furthermore, Vitamin D modulates gene expression [70] and epigenetic alterations [71], which improve immunological tolerance and reduce autoimmune responses. Its anti-inflammatory and antioxidant capabilities also reduce inflammation and oxidative stress [72], which are essential contributors to the development of SLE. Vitamin D also plays a crucial role in regulating the immune system by suppressing pro-inflammatory Th1 and Th17 cells and promoting regulatory T cells, as indicated earlier, which reduces autoimmunity and inflammation. Furthermore, Vitamin D supports bone health by influencing immune function and reducing systemic inflammation [73]. The combined effects possibly reduce disease activity and, as a consequence, maintain higher levels of C3 and C4.

In the present meta-analysis, it was observed that Vitamin D levels inversely correlated with anti-dsDNA, an important marker of disease activity, and this observation corroborates with previous reports [37, 39]. The relationship between Vitamin D and anti-dsDNA antibodies appears to be influenced by the immune-modulating properties of Vitamin D. As highlighted earlier, Vitamin D regulates T cells, B cells, and reduces the

production of autoantibodies and pro-inflammatory cytokines such as IL-6 and TNF-α [74]. Furthermore, it facilitates the clearance of apoptotic cells [75], which limits the availability of autoantigens and subsequently reduces their production. These multi-functional effects of Vitamin D perhaps play a critical role in maintaining immune tolerance and preventing immune dysregulation in patients with SLE when levels are adequate. The caveat is to determine the optimal levels of Vitamin D that can suppress damaging immune responses and improve immune homeostasis in SLE. This needs to be evaluated by randomized controlled trials to make it an affordable adjunct to standard care in SLE.

Although the current meta-analysis indicated a significant relationship between Vitamin D and SLE, disease activity scores, complements, and anti-dsDNA, this interpretation has several limitations. First, in the present study, publication bias was observed for two meaningful comparisons: the mean Vitamin D levels between SLE and healthy controls, and the correlation of C4 with Vitamin D levels, which could have resulted from the selective publication of positive observations. Second, significant heterogeneity was noted in all comparisons, except for the correlation between Vitamin D and complement C4, which could have been due to the differential study design, clinical categorization, diagnostic criteria, and population type of papers analyzed.

5 | Conclusions

In summary, this study underscores the significant relationship between Vitamin D levels and SLE. SLE patients exhibit lower Vitamin D levels compared to healthy controls. The observed significant inverse correlation between Vitamin D levels and disease severity scores, as well as with anti-dsDNA levels, and a positive correlation with complement C3 and C4, suggests that Vitamin D may have a crucial role in immune modulation in SLE. Larger studies, particularly randomized controlled trials with Vitamin D as an adjunct to standard care, to determine the optimal levels of Vitamin D that can modulate disease severity are essential to establish a causal relationship.

Author Contributions

Shovit Ranjan: methodology, formal analysis, investigation, writing original draft; **Bidyut K. Das:** conceptualization, Writing – Review and Editing, Supervision; **Rina Tripathy:** methodology, formal analysis, investigation, writing original draft; **Sarit S. Pattanaik:** methodology, formal analysis, investigation, writing original draft; **Manoj Parida:** methodology, formal analysis, investigation, writing original draft; **Saumya Ranjan Tripathy:** methodology, formal analysis, investigation, writing original draft; **Aditya K. Panda:** conceptualization, Writing – Review and Editing, Supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** apl70379-sup-0001-DataS1.docx.

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LETTER TO THE EDITOR

Anti-GP210 Antibodies as a Primary Driver of Pruritus: A Hypothetical Two-Hit Model Beyond PBC

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Dear Editor,

GP210, a transmembrane glycoprotein integral to the nuclear pore complex, is crucial in regulating nucleocytoplasmic transport and maintaining cellular homeostasis [1]. This regulation prevents cellular dysfunctions in autoimmune and degenerative settings. In primary biliary cirrhosis (PBC), anti-GP210 antibodies disrupt this transport, potentially leading to bile duct damage and activation of pruritogenic pathways [2]. GP210 is a key autoantigen in PBC due to its structural role in the nuclear pore complex and its interaction with bile duct epithelial cells [3]. Anti-GP210 antibodies disrupt nuclear transport, contributing to bile duct damage and bile acid accumulation, which in turn activates pruritogenic pathways, linking GP210 to the pathophysiology of PBC-associated pruritus. Anti-GP210 antibodies are frequent in PBC patients and are often associated with advanced disease, including progression to fibrosis and cirrhosis [4]. In PBC, the accumulation of bile acids is thought to activate pruritogenic pathways, leading to the characteristic pruritus [5]. We present two cases of patients in whom, in the absence of PBC or associated pathologies, pruritus was associated with the positivity of anti-GP210 antibodies. Treatment with immunomodulators was necessary to reverse the symptoms despite the use of high-dose antihistamines. Two female patients presented with chronic pruritus without significant laboratory abnormalities, particularly liver function markers. Both patients tested positive for GP210 antibodies at intermediate titers, with other autoimmune markers being negative. Anti-GP210 antibodies were detected using the EUROLINE immunoblot system (Euroimmun, Germany), the same platform employed for the extended ANA-23 antigen panel in our institution. Assays were performed according to the manufacturer's protocol. Results are reported in arbitrary units (AU), with values > 20 AU considered positive. The patients had values of 47 and 54 AU, which fall within the low-to-moderate positive range as per the manufacturer's interpretation scale (Table 1). Both patients met the criteria for chronic pruritus (duration > 6 weeks), with predominant involvement of the hands and upper limbs. Pruritus intensity and impact were assessed retrospectively using the 5-D Itch Scale and ItchyQoL questionnaire. Patient 1 improved from 17 to 11 on the 5-D Itch Scale and from 33 to 18 on the ItchyQoL. Patient 2 improved from 16 to 10 and from 30 to 23, respectively. Other pruritus causes (renal, neurologic, psychogenic) were excluded through clinical history, laboratory testing, and follow-up (Table 2). Pruritus was refractory to standard therapies but improved significantly with hydroxychloroquine, suggesting a potential immunological mechanism underlying their symptoms. The presence of GP210 antibodies in the absence of PBC raises intriguing questions about their pathogenic role. We propose a two-hit model to explain the clinical manifestations in these patients. The first hit involves a primary dysfunction of cutaneous nerves, possibly mediated by transient or persistent autoantibody activity directed at the nuclear pore complex (Figure 1). GP210, a key structural and functional protein within the nuclear pore, may influence neuronal signaling when targeted by autoantibodies [6]. Dysfunction in the nuclear pore complex could lead to heightened neuronal sensitivity and activation of pruritogenic pathways, contributing to persistent itching [7]. The second hit involves the subclinical

TABLE 1 | Demographic data and laboratory results of reported patients.

Parameter	Case 1	Case 2
Age (yo)	53	27
Gender	Female	Female
ALP (alkaline phosphatase) (U/L)	44	38
AST (aspartate aminotransferase) (U/L)	22	25
ALT (alanine aminotransferase) (U/L)	10	15
GGT (gamma-glutamyl transferase) (U/L)	17	33
Direct bilirubin (mg/dl)	0.14	0.29
Indirect bilirubin (mg/dl)	0.16	0.25
ANA (antinuclear antibodies) (IFI-Hep-2 cells)	1:80 (AC-4)	1:1280 (AC-6)
C3 complement (mg/dl)	109	114
C4 complement (mg/dl)	22.9	29
Rheumatoid factor (UI/mL)	Negative	Negative
AMA (anti-mitochondrial antibodies) (Inmunoblot)	Negative	Negative
Anti-SLA/LP (soluble liver antigen) (Inmunoblot)	Negative	Negative
Anti-gp210 antibodies (Inmunoblot)	Positive (47)	Positive (54)
Anti-Sp100 antibodies (Inmunoblot)	Negative	Negative
Anti-LKM (liver–kidney microsome) antibodies (Inmunoblot)	Negative	Negative
Anti-F-actin antibodies (Inmunoblot)	Negative	Negative
Anti-Ro/SSA antibodies (Inmunoblot)	Negative	Negative
Anti-La/SSB antibodies (Inmunoblot)	Negative	Negative
Other specific autoantibodies (Inmunoblot)	DFS70 (45)	AntiRNP-SM (14)
C-reactive protein (CRP) (mg/dl)	0.06	0.06
ESR (erythrocyte sedimentation rate) (mm/h)	21	13
Histopathological findings	Not performed	Not performed
Clinical symptoms	Artralgia, pruritus, sicca	Pruritus, sicca, alopecia
Treatment history	Selective H1 blockers	Selective H1 blockers, dapsone
Diagnosis	AntiGP210-associated sine materia pruritus	AntiGP210-associated sine materia pruritus

TABLE 2 | Objective pruritus assessments using 5-D Itch Scale and ItchyQoL.

Patient	5-D Itch Scale (Before → After)	ItchyQoL Score (Before → After)	Improvement (%)[a]
1	17 → 11	33 → 18	35.3% / 45.5%
2	16 → 10	30 → 23	37.5% / 23.3%

[a]Percent of improvement calculated from baseline to post-treatment scores.

accumulation of bile acids, which can further exacerbate pruritus in the context of already affected neurons. Bile acids interact with specific pruritogenic receptors such as TGR5 and activate pathways mediated by autotaxin and lysophosphatidic acid [8]. In the setting of GP210-induced neuronal dysfunction, this interaction becomes more pronounced, leading to amplified itch signaling. This dynamic highlights the synergistic role of structural disruption and biochemical irritants in perpetuating pruritus. In a typical neuronal environment, such subclinical bile acid accumulation would not be sufficient to trigger itching. However, in disrupted neuronal structure due to GP210 autoantibodies, these pruritogenic substances can activate neuronal

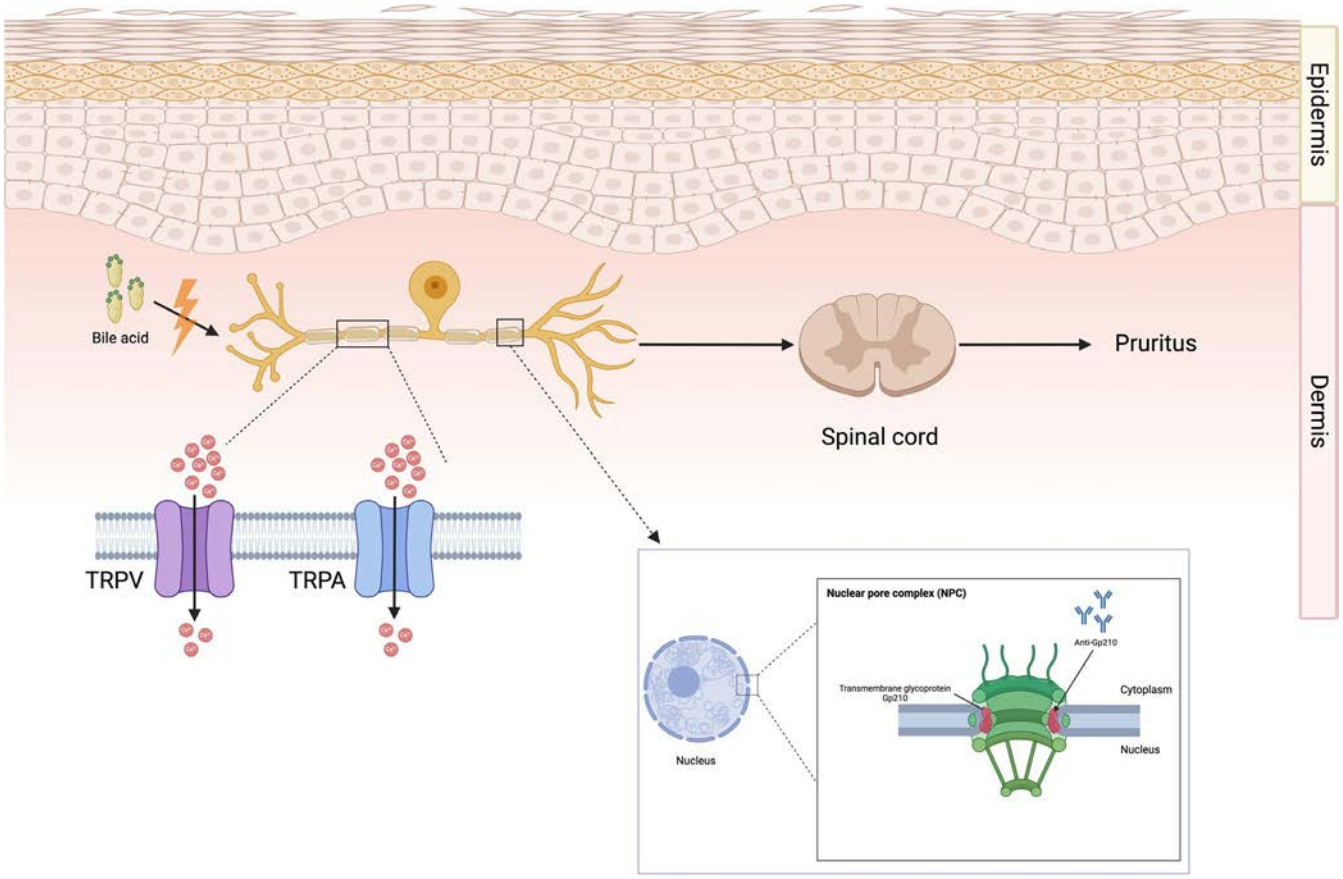

FIGURE 1 | Two-hit model illustrating persistent pruritus: The interplay between the accumulation of pruritogenic substances and inflammatory dysregulation of stimulus transmission highlights the mechanisms underlying persistent pruritus and its responsiveness to immunomodulatory therapies.

itch pathways. This interplay between structural neuronal dysfunction and biochemical irritants creates a self-perpetuating cycle of pruritus. Hydroxychloroquine, as an immunomodulator, may limit the pathogenic role of GP210 autoantibodies by reducing their binding and subsequent neuronal disruption. The proposed two-hit model hypothesizes that anti-GP210 antibodies may disrupt neuronal immune homeostasis, possibly through shared expression of nuclear pore complex proteins in non-hepatic tissues. Although direct neuronal binding has not been demonstrated, analogies with neuroinflammatory conditions support a plausible immunological role [9]. The second hit may involve local accumulation of bile acids, which activate pruriceptive pathways via TGR5 and the autotaxin–lysophosphatidic acid axis. Hydroxychloroquine may exert therapeutic effects by modulating innate immune responses and inhibiting Toll-like receptor signaling, thereby reducing antigen-driven inflammation [10]. Identifying GP210 antibodies in patients with unexplained pruritus could facilitate diagnosis and guide immunological evaluation, potentially reducing the need for invasive procedures. Therapeutically, immunomodulators such as hydroxychloroquine alleviate symptoms and address the underlying immune dysfunction, offering a targeted approach. Further studies in larger cohorts are needed to validate these observations and assess potential therapeutic interventions targeting GP210-associated pathways. Elucidating the mechanisms linking GP210 autoantibodies to peripheral nerve dysfunction could improve diagnostic strategies and lead to targeted therapies for this and related conditions. Therapeutic strategies based on the two-hit model could involve stabilizing neuronal structures affected by GP210 autoantibodies and modulating bile acid accumulation. A combination of immunomodulators (e.g., hydroxychloroquine) and bile acid sequestrants or autotaxin inhibitors might be beneficial. While our findings present an intriguing hypothesis, they are based on anecdotal cases and require further validation. In terms of limitations, this report presents two illustrative cases that support a novel pathophysiological hypothesis. While the small sample size limits generalizability, the consistent findings across both patients and their objective response to immunomodulatory therapy suggest a potential shared mechanism. Further studies involving larger cohorts and appropriate control groups are needed to validate this two-hit model. The presence of anti-GP210 antibodies alone may not be sufficient to induce pruritus, and other contributing factors such as genetic predisposition, environmental triggers, or yet unidentified co-factors could play a role. Additionally, the absence of liver dysfunction was determined based on standard biochemical markers, but more detailed assessments, such as bile acid profiling or liver elastography, were not performed, which may limit exclusion of early cholestatic disease. However, persistently normal liver tests, negative AMA and SLA/LP antibodies, and stable clinical follow-up support the absence of overt biliary pathology. Also, histological nerve studies were not performed; stable clinical course, negative serology, and long-term follow-up mitigate the likelihood of latent cholestatic disease.

These limitations highlight the need for future studies with larger cohorts and more comprehensive diagnostic evaluations.

Author Contributions

C.K., L.H.: conceptualization. L.H., C.K.: data curation. L.H., C.K, K.J.: formal analysis. L.H., C.K.: funding acquisition. C.K., L.H., K.J.: investigation. L.H., C.K., K.J.: resources. L.H., C.K.: supervision. L.H., C.K., K.J.: writing – original draft preparation. L.H., C.K., K.J.: writing – review and editing.

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The authors have nothing to report.

Consent

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

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supporting information.

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LETTER TO THE EDITOR

Case(s) Report: Clinical, Laboratory, and Histopathological Profile of Kikuchi–Fujimoto Disease in Young Adults

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Dear Editor,

Kikuchi–Fujimoto disease, also known as histiocytic necrotizing lymphadenitis, is a rare self-limiting inflammatory disorder [1]. It was first described in Japan by Japanese pathologists Kikuchi and Fujimoto in 1972 [2]. It is a benign disease with uncertain etiology and usually presents as cervical lymphadenopathy, with or without systemic features [3]. It usually affects young adults < 30–40 years of age, but any age group can be affected. It is usually diagnosed retrospectively on doing a biopsy of the diseased lymph node. Treatment mainly involves supportive care, with the occasional use of corticosteroids. Immunosuppressive therapy is reserved for cases with severe or recurrent disease [4]. Patients require close monitoring for resolution of the disease as well as periodic testing for the development of autoimmune diseases (particularly, SLE) once the symptoms have resolved [5].

We present four cases of Kikuchi–Fujimoto disease, observed in our department of Internal Medicine, between July and October 2024. Table 1 enlists the epidemiological and clinical characteristics of these patients. All four patients were young individuals, in the age group of 15–23 years. Three were males and one was female. None of them had any pre-existing illness. All four patients presented with fever, and two of them had generalized weakness with loss of appetite. Two out of four patients had weight loss, and two out of four had headache with dry cough. On examination, all four patients were hemodynamically stable. Right cervical lymphadenopathy was present in three of them, with one patient having left axillary lymphadenopathy as well. Lymph nodes were firm, non-tender, with no signs of inflammation. One patient had hepatosplenomegaly on systemic examination.

Table 2 enlists laboratory, radiological, and histopathological characteristics of all four patients. Routine investigations revealed leucopenia in one patient and elevated liver enzymes in two patients. ESR, CRP, and LDH were elevated in three patients. Ferritin was raised in all four patients, ranging from 340 to 18 285 ng/mL. Triglycerides were raised in one patient. Blood cultures were sterile, and procalcitonin was negative in all four patients. The young female had positive autoimmune markers (ANA 2+ [titre 1:80], PCNA borderline positive in ANA profile). Epstein–Barr virus was positive for one of them (39 150 copies). Contrast-enhanced CT of the thorax and abdomen revealed cervical lymphadenopathy in three patients, accompanied by left axillary lymphadenopathy in one, abdominal lymphadenopathy in two, and hepatosplenomegaly in two patients.

Histopathology in all four patients revealed focal areas of necrosis composed of eosinophilic granular material and karyorrhectic debris (nuclear dust), surrounded by pale areas composed of histiocytes and lymphocytes. Viable areas showed lymphoid aggregates and few reactive mononuclear cells. Neutrophils and plasma cells were not seen. There was no evidence of granuloma, RS cells, or malignant cells. Immuno-histochemistry staining revealed predominant CD3 with CD4 and CD8 positive T-lymphocytes (CD8 > CD4) in the paracortical zones and a CD68-positive histiocyte population. Ziehl–Neelsen (ZN) stain for acid fast bacilli (AFB) and Periodic acid schiff (PAS) stain were negative. A diagnosis of histiocytic necrotizing lymphadenitis/Kikuchi disease was made.

Table 2 further shows the treatment and outcome of our patients. Two patients responded well to NSAIDs, whereas two

TABLE 1 | Epidemiological and clinical characteristics, diagnosis and outcome.

	Case 1	Case 2	Case 3	Case 4
Age	23 years	20 years	15 years	18 years
Gender	Male	Male	Female	Male
Ethnic origin	Indian	Indian	Indian	Indian
Comorbidity	Nil	Nil	Nil	Nil
Presenting illness	Fever upto 102 F, generalized weakness, loss of appetite and weight loss 7 kg for 20 days	Fever upto 100 F, generalized weakness, loss of appetite, headache and dry cough for 7 days	Fever upto 100 F for 1 month and weight loss 3 kg in 1 month	Fever upto 103 F, headache and dry cough for 5 days
Physical examination	Hemodynamically stable Left axillary and right cervical lymphadenopathy (firm, nontender)	Hemodynamically stable Right cervical lymphadenopathy (firm, tender)	Hemodynamically stable Right cervical lymphadenopathy (firm, nontender)	Hemodynamically stable Hepatosplenomegaly

did not respond to NSAIDs alone, and steroids were added. Patients were followed up clinically and with regular monitoring of inflammatory markers. All four patients became asymptomatic, and inflammatory markers returned to baseline over 1–2 months.

Kikuchi–Fujimoto disease has been reported sporadically from various countries, with maximum of cases from Japan (80% of cases) [6]. The disease is more common in females, but as per recent studies in the Asian population, the ratio of male-to-female affection is 1:1. It can affect individuals between 6 and 80 years of age, but the majority of the affected individuals are younger than 40 years [7]. The mean age of participants in our series was 19 years, and three out of four were males.

The etiopathogenesis of Kikuchi disease remains unclear. It is considered to be the result of a local T-cell-mediated hyperresponse to a variety of nonspecific stimuli, mostly viral infections and autoimmune diseases [8]. A number of viruses such as Epstein–Barr virus (EBV), herpes simplex virus (HSV) types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus (HHV-6, 7, and 8), parvovirus B19, human papillomavirus (HPV), hepatitis B virus (HBV) and human T lymphotropic virus 1 (HTLV-1) have been implicated as triggers, but no direct causation has been firmly established [7]. EBV was also found positive in one of our patients. Various bacteria (Brucella, Bartonella, Toxoplasma gondii, *Yersinia enterocolitica*, and Mycobacterium species) have also been suspected as a causative factor, without any definite evidence [7, 9]. Autoimmune disorders including SLE, Sjögren's syndrome, and rheumatoid arthritis have also been implicated as triggers for Kikuchi–Fujimoto disease on certain occasions. Among these, SLE has been found to be most commonly associated with it. Certain human leukocyte antigens (HLA-DPA1 and HLA-DPB1) have been identified in populations with a higher susceptibility to Kikuchi–Fujimoto disease [9]. However, like infective workup, autoimmune workup is also usually negative in Kikuchi disease. A number of other triggers, such as surgery, malignancies, and foreign body implants, have been reported in various case reports [10].

Kikuchi disease has an acute to sub-acute course, with symptoms developing over several weeks. Unilateral cervical lymphadenopathy is the most common presentation, seen in 60%–90% of cases with occasional axillary and supraclavicular lymph node involvement. Lymphadenopathy has been seen in up to 22% of patients [11]. Fever is present in 36% to 77% of cases, often accompanied by weight loss, night sweats, fatigue, headache, arthralgia, sore throat, upper respiratory symptoms, nausea, and vomiting [4]. Hepatosplenomegaly is seen in 5% of cases [7]. Patients with fever $\geq$ 39.0°C, larger lymph nodes, and leucopenia tend to have a more prolonged clinical course. There are no standardized clinical or diagnostic criteria for diagnosing Kikuchi–Fujimoto disease, mainly due to its variable presentation. Once other causes of fever with lymphadenopathy have been ruled out, Kikuchi–Fujimoto disease remains as the diagnosis of exclusion. Total leukocyte counts are normal in the majority of patients, but leucopenia is present in 43% of patients [12]. In our series also, one out of four patients had leucopenia. Inflammatory markers (ESR, CRP, ferritin) are elevated in the majority of patients. Other nonspecific findings include abnormal liver function tests and elevated serum lactate dehydrogenase. The very high ferritin in one of our patients could be explained by the possibility of occult hemophagolymphohistiocytosis, which is supported by high-grade fever, raised triglycerides, and hepatosplenomegaly in that patient. A thorough workup for infections and autoimmune disorders is warranted to exclude alternate causes of lymphadenopathy, such as tuberculosis or malignancy. Definite diagnosis of Kikuchi disease relies on excisional lymph node biopsy. Microscopic examination of the node shows necrotic foci with abundant karyorrhectic nuclear debris and a large number of histiocytes at the edge of necrosis. Neutrophils and eosinophils are typically absent, with that being an important clue [13]. Histiocytes in this disease are positive for lysozyme, myeloperoxidase, CD68, CD163, and CD4. Lymphocytes are mostly CD3-positive T-cells, demonstrating a predominance of CD8 compared with CD4, with very few CD20-positive B-cells [5].

No specific treatment guidelines exist for Kikuchi disease as it usually resolves spontaneously over 1–6 months [14]. Fever

TABLE 2 | Laboratory, radiological, and histopathological characteristics.

	Case 1	Case 2	Case 3	Case 4
Complete blood count				
Hemoglobin (12–15 g/dL)	12.2 g/dL	14.9 g/dL	12.6 g/dL	13 g/dL
Total leucocyte count (4–10 × 10^3/mm^3)	**1.99 × 10^3/mm^3**	7.89 × 10^3/mm^3	4.64 × 10^3/mm^3	4.03 × 10^3/mm^3
Platelet count (1.50–4.50 × 10^5/mm^3)	1.62 × 10^5/mm^3	1.77 × 10^5/mm^3	3.48 × 10^5/mm^3	2.04 × 10^5/mm^3
Kidney function test				
Creatinine (0.57–1.11 mg/dL)	0.67 mg/dL	1.02 mg/dL	0.39 mg/dL	0.83 mg/dL
Sodium/potassium (136–146/3.5–5.1 meq/L)	135/3.6 meq/L	139/4.3 meq/L	139/3.92 meq/L	137/4.17 meq/L
Liver function test				
Total/direct bilirubin (0.2–1.2/0–0.5 mg/dL)	1.15/0.29 mg/dL	0.54/0.28 mg/dL	0.91/0.27 mg/dL	0.98/0.31 mg/dL
Total protein/albumin (6–8.3/3.2/4.6 g/dL)	7.4/3.6 g/dL	6.3/4.2 g/dl	6.8/3.4 g/dL	6.4/3.7 g/dL
SGOT/SGPT (5–34/0–55 IU/L)	41/39 IU/L	**107/117 IU/L**	26/23 IU/L	**72/89 IU/L**
ALP/GGT (40–150/9–36 IU/L)	53/32 IU/L	**181/212 IU/L**	50/35 IU/L	60/38 IU/L
Inflammatory markers (done at the time of admission)				
ESR (< 21 mm/h)	**70 mm/h**	2 mm/h	**40 mm/h**	**65 mm/h**
CRP (< 6 mg/dL)	**58.5 mg/dL**	**37 mg/dL**	< 2 mg/dL	**43.4 mg/dL**
Procalcitonin (< 0.5 ng/mL)	0.2 ng/mL	0.26 ng/mL	0.05 ng/mL	0.11 ng/mL
Ferritin (13–150 ng/mL)	**573 ng/mL**	**980 ng/mL**	**340 ng/ml**	**18 285 ng/mL**
Triglycerides (< 150 mg/dL)	146.4 mg/dL	140 mg/dL	98 mg/dL	**205 mg/dL**
LDH (125–220 IU/L)	**1743 IU/L**	**850 IU/L**	200 IU/L	**995 IU/L**
Blood cultures	Sterile	Sterile	Sterile	Sterile
Antinuclear antibody	Negative	Negative	**2+ (1:80) with borderline PCNA positive. ANA profile and myositis profile: negative, Anti-ds DNA: negative**	Negative
EBV PCR	Negative	**39 150 copies**	Negative	Negative

(Continues)

TABLE 2 | (Continued)

	Case 1	Case 2	Case 3	Case 4
CMV PCR	Negative	Negative	Negative	Negative
HIV by ELISA	Negative	Negative	Negative	Negative
Mantoux test	Negative	Negative	Negative	Negative
TB quantiferon gold	Negative	Negative	Negative	Negative
Radiology				
CECT thorax and abdomen	Axillary and cervical lymphadenopathy	Right cervical lymphadenopathy with subcentimeteric lymph nodes in the mesentery.	Multiple bilateral cervical lymphadenopathy (right > left) with mild hepatosplenomegaly	Hepatosplenomegaly with abdominal lymphadenopathy
Lymph node excision	Left axillary lymph node	Right cervical lymph node	Right cervical lymph node	Right external iliac lymph node
Histopathology	Histiocytic necrotising lymphadenitis	Histiocytic necrotising lymphadenitis	Histiocytic necrotising lymphadenitis	Histiocytic necrotising lymphadenitis
Microbiology (gram stain, KOH stain for fungus, ZN stain and TB Xpert, cultures)	Negative	Negative	Negative	Negative
Diagnosis	Kikuchi–Fujimoto disease	Kikuchi–Fujimoto disease with Epstein barr virus infection	Kikuchi–Fujimoto disease with undifferentiated connective tissue disorder	Kikuchi–Fujimoto disease
Treatment	NSAIDs + oral prednisolone 0.5 mg/kg tapered over 1 month	NSAIDs + supportive	NSAIDs + supportive	NSAIDs + oral prednisolone 1 mg/kg tapered over 2 months
Follow up	Asymptomatic after 2 month of treatment Total leucocyte counts normalized after 1 week of treatment	Asymptomatic after 1 month of treatment EBV PCR became negative after 2 months	Asymptomatic after 1.5 month of treatment	Asymptomatic after 2 months of treatment

Note: Bold values highlight the abnormal values.

Abbreviations: ALP, alkaline phosphatase; CMV, Cytomegalovirus; CPK, creatine phosphokinase; CRP, C reactive protein; EBV, Epstein–Barr virus; ESR, erythrocyte sedimentation rate; GGT, gamma glutamyl transferase; LDH, lactate dehydrogenase; NSAIDs, Nonsteroidal anti-inflammatory drugs; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; TB, Tuberculosis.

usually settles after excision of the affected lymph node as the focus of the inflammatory process is removed. Mild cases respond to NSAIDs and other supportive therapies. Systemic corticosteroids (prednisolone 1–2 mg/kg body weight) are required in patients having prolonged fever, symptoms lasting > 2 weeks, or those having recurrent disease. Hydroxychloroquine, minocycline, or intravenous immunoglobulin are other potential treatment options [11].

It is usually a monophasic disease with a recurrence rate of 3%–4% in adults, but in children, it can be as high as 31%–39% [15]. Patients with Kikuchi–Fujimoto disease require close monitoring to observe for resolution of symptoms and to screen for the development of autoimmune disease (particularly SLE) after the resolution of symptoms. For patients who have a co-diagnosis of Kikuchi–Fujimoto disease and other autoimmune diseases (with SLE being the most common), appropriate treatment for the secondary autoimmune disorder is required [5].

A major limitation of our case series is the small sample size, which restricts the generalizability of our findings. However, our findings contribute to the growing body of literature by underscoring the need for clinicians to maintain a high index of suspicion for KFD in atypical presentations. In our series, follow-up duration was limited, and none of the patients exhibited signs of recurrence or autoimmune disease at last contact. However, given the temporal gap often observed before the onset of such associations, longer-term follow-up is essential to detect late sequelae. We recommend that patients diagnosed with KFD undergo periodic clinical and serological evaluation, particularly if new symptoms arise. Future studies with extended follow-up periods are warranted to better characterize the natural history and autoimmune risk in this patient population.

Kikuchi–Fujimoto disease is one of the important causes of lymphadenopathy in young adults. Lymph node biopsy is required for diagnosis and ruling out conditions like lymphoma, tuberculosis, and lupus adenitis. It usually resolves spontaneously, but may sometimes require steroids. These patients should be on long-term follow-up to monitor the subsequent development of an autoimmune disorder.

Author Contributions

Rahul Kumar: wrote the first draft of the manuscript. **Tanvi Batra:** critical review and revised the manuscript. **Atul Kakar:** critical review, manuscript editing, and finalizing the manuscript.

Ethics Statement

The authors have nothing to report.

Consent

Informed consent was received from all participants.

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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Atul Kakar

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LETTER TO THE EDITOR

Contemporary Knowledge Structure and Research Strand in Antiphospholipid Syndrome

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Dear Editor,

Antiphospholipid syndrome (APS) is a systemic immune disorder characterized by the presence of thrombosis (arterial, venous, or microvascular thrombosis) or obstetric morbidity or nonthrombotic manifestations in patients with persistent positivity of antiphospholipid antibody (aPL) [1]. APS occurs in 1–2 persons per 100 000 population per year, with a prevalence between 40 and 50 per 100 000 [2]. However, APS is clinically significant due to its potential for life-threatening complications and frequent overlap with other autoimmune diseases, most notably systemic lupus erythematosus (SLE) [3].

Bibliometric analysis is a robust analytical and mapping method used to quantitatively evaluate the connections between articles within a specific research field over a designated period [4]. The emergence of literature on APS has sparked significant scholarly interest, but there have been limited attempts through bibliometric analysis. A recent study relied solely on data from the Science Citation Index Expanded of the Web of Science Core Collection (WoSCC) [5]. Another focused on publication output, yet provided limited insight into research hotspots [6]. To better understand the global research output and identify key trends, we conducted a comprehensive bibliometric analysis of APS-related articles.

Data were extracted from the WoSCC between January 2012 and December 2023, including only original English-language articles. Bibliometric networks were constructed using VOSviewer (version 1.6.10), and international collaborations were analyzed via an online platform (https://bibliometric.com/). High-frequency keywords (top 40%) were extracted using BICOMB (version 2.0) to generate a co-word matrix, followed by biclustering with gCLUTO (version 1.0).

A total of 3 877 original research articles were retained after filtering the initial 7 400 APS-related records (Figure 1). The majority of publications originated from America, Western Europe, and Eastern Asia (Figure 2A). Prolific countries were mostly developed, with regional disparities in publications due to various factors, such as research funding, Internet penetration, and researcher interest. To bridge the gap, developing countries should foster collaboration through multinational organizations, patient registries, and multicenter studies. Post-2020, APS publications increased by 59.7% compared to 2012–2019 (Figure S1). The research was widely distributed in 982 journals (Figure S2), led by Lupus (357, 9.2%). Rheumatology (1 024, 26.4%) was the most active subject category. The United States Department of Health and Human Services (199, 5.1%) was the top contributor. Co-authorship analysis revealed APS research across 104 countries (Figure S3). The United States led with 880 publications and 22 728 citations, collaborating most with Italy (Figure 2B). Among 4 515 contributing organizations, the University of Padua ranked highest with 107 articles. Of 19 054 authors, the top was Sciascia (University of Turin) with 2 292 citations.

Li Liu and Yang-Xi Liu contributed equally to this study and share co-first authorship.

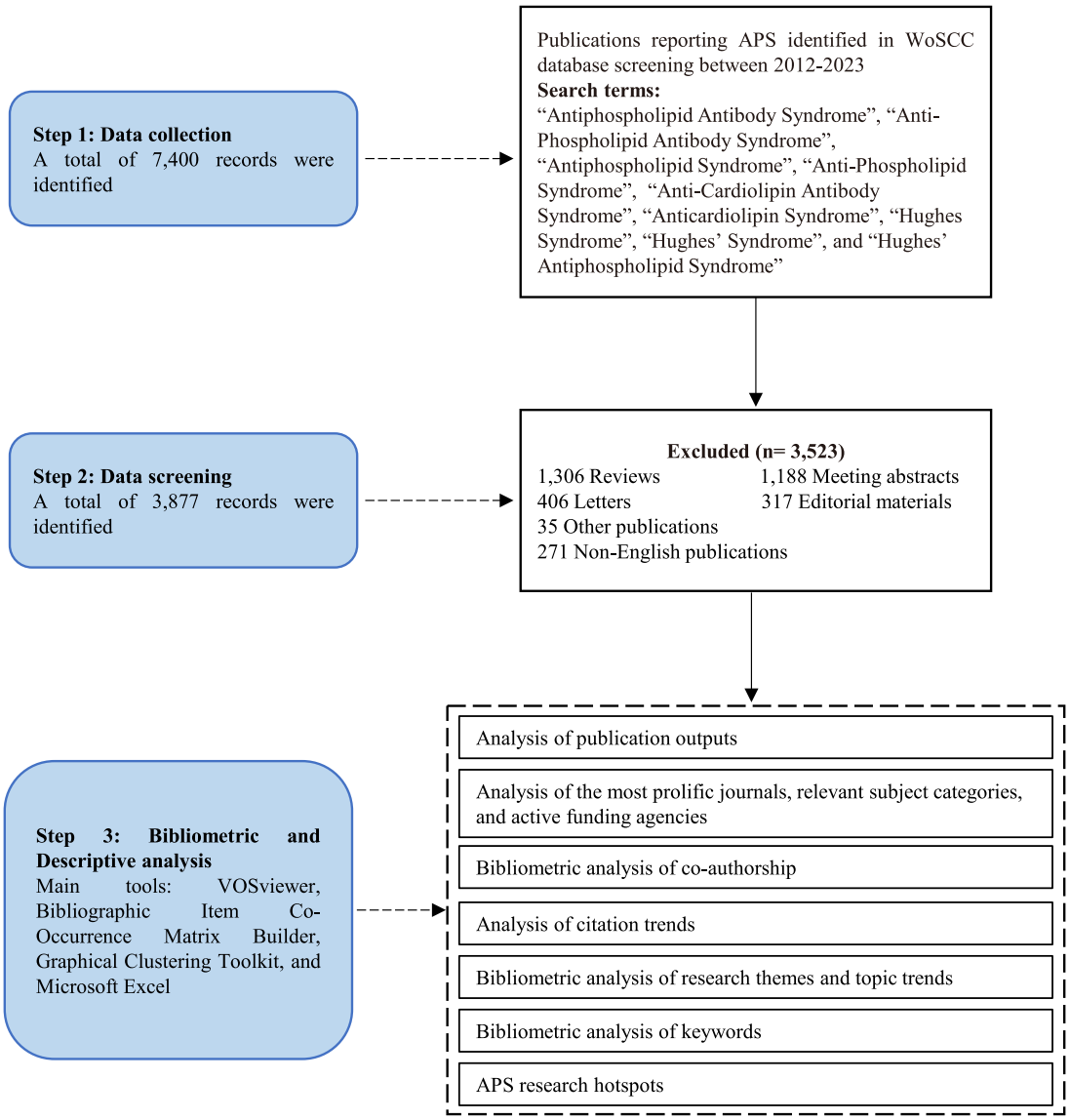
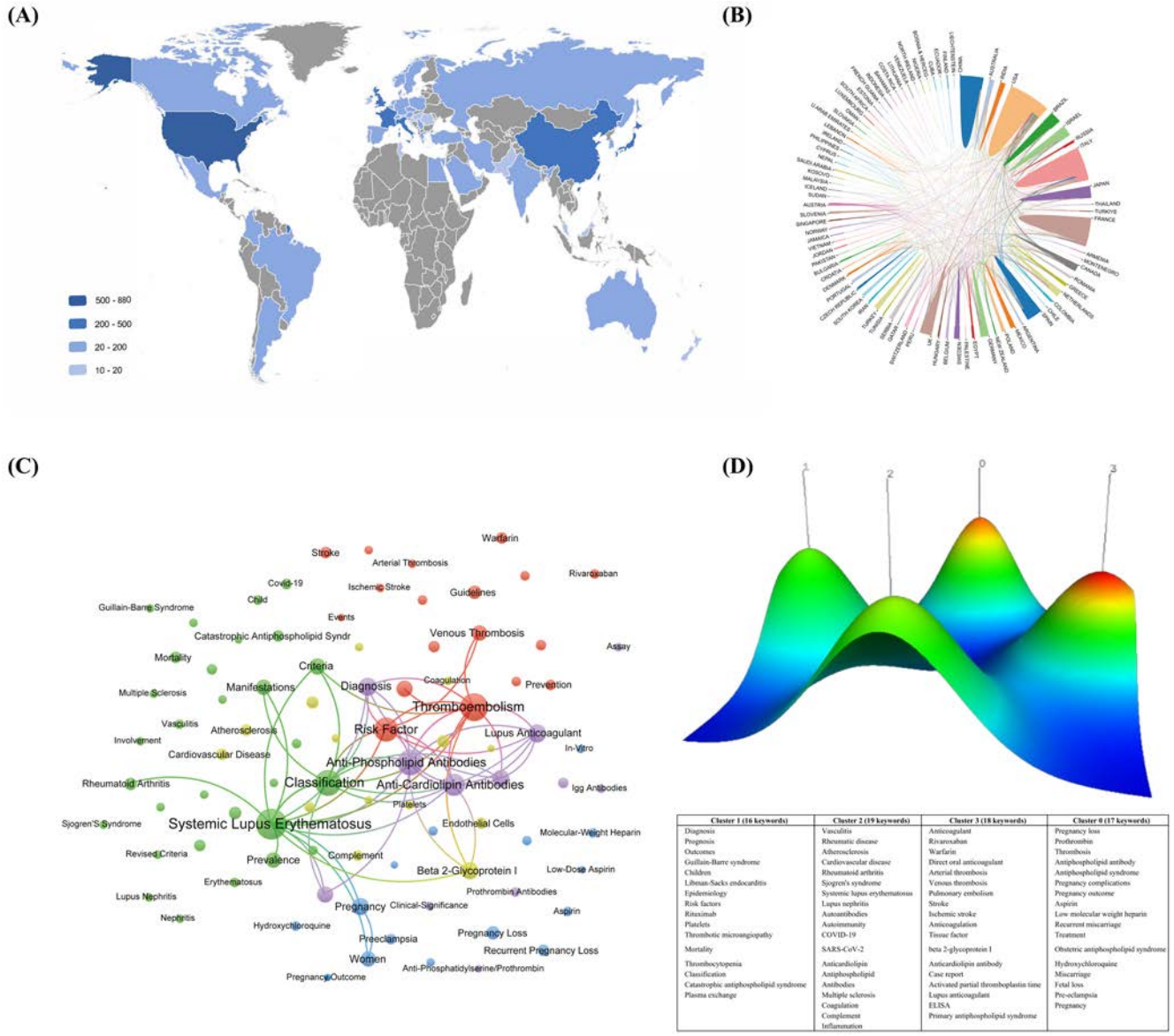


FIGURE 1 | Flow diagram of included publications. APS, antiphospholipid syndrome; WoSCC, web of science core collection.

Table S1 analyzed the top 100 most-cited articles, focusing on authorship, journal impact factor, countries, institutes, and citations per year. The mean and median number of citations in the top 100 cited papers were 218.61 and 124.50, respectively. A strong correlation was found between the number of institutes and citations ($R^2 = 0.1339$, $p = 0.002$) (Figure S4). The strong international collaboration aids publication in high-impact journals and fosters a more equitable global scientific discourse.

The network visualization identified 379 terms (> 50 occurrences), grouped into five theme clusters (Figure S5). The overlay visualization map showed recent studies focused primarily on the classification criterion and prognosis of APS, prevention, and treatment of thromboembolic complications. The hot emerging topics were "covid" (average publication year, 2021.52) and "direct oral anticoagulant" (2019.76). Among 9 698 keywords, 103 keywords related to "Antiphospholipid syndrome" (> 50 occurrences) were further analyzed (Figure 2C). "Systemic lupus erythematosus" (1 124 occurrences), and "Thromboembolism" (894) showed strong links to "Classification" and "Risk factor," respectively. Seventy keywords (top 40% by frequency) were clustered into four groups via mountain visualization (Figure 2D and Table S2). Clusters 0 and 3 (red peaks) showed high internal consistency, reflecting themes of obstetric APS treatment, clinical manifestations, and antithrombotic therapy. Clusters 1 and 2 (green peaks) elucidates the classification and diagnostic approaches, highlighting specific clinical features such as catastrophic APS and thrombocytopenia, as well as APS-related complications.

Thrombotic manifestations in APS are multifactorial, with "antiphospholipid antibodies" playing a pivotal role. Activation of vascular and immune cells by aPL triggers the upregulation of surface adhesion molecules, the release of proinflammatory cytokines, and the generation of procoagulant substances [7]. Additional risk factors include SLE, smoking, hypertension, hypercholesterolemia, inherited thrombophilia, and cancer [8]. Complement activation indicated by elevated sC5b-9 levels, significantly contribute to APS pathogenesis. Eculizumab targeting complement protein C5 have shown promise in catastrophic APS and thrombotic APS refractory to anticoagulation [9].



Cluster 1 (16 keywords)	Cluster 2 (19 keywords)	Cluster 3 (18 keywords)	Cluster 0 (17 keywords)
Diagnosis	Vasculitis	Anticoagulant	Pregnancy loss
Prognosis	Rheumatic disease	Rivaroxaban	Prothrombin
Outcomes	Atherosclerosis	Warfarin	Thrombosis
Guillain-Barre syndrome	Cardiovascular disease	Direct oral anticoagulant	Antiphospholipid antibody
Children	Rheumatoid arthritis	Arterial thrombosis	Antiphospholipid syndrome
Libman-Sacks endocarditis	Sjogren's syndrome	Venous thrombosis	Pregnancy complications
Epidemiology	Systemic lupus erythematosus	Pulmonary embolism	Pregnancy outcome
Risk factors	Lupus nephritis	Stroke	Aspirin
Rituximab	Autoantibodies	Ischemic stroke	Low molecular weight heparin
Platelets	Autoimmunity	Anticoagulation	Recurrent miscarriage
Thrombotic microangiopathy	COVID-19	Tissue factor	Treatment
Mortality	SARS-CoV-2	beta 2-glycoprotein I	Obstetric antiphospholipid syndrome
Thrombocytopenia	Anticardiolipin	Anticardiolipin antibody	Hydroxychloroquine
Classification	Antiphospholipid	Case report	Miscarriage
Catastrophic antiphospholipid syndrome	Antibodies	Activated partial thromboplastin time	Fetal loss
Plasma exchange	Multiple sclerosis	Lupus anticoagulant	Pre-eclampsia
	Coagulation	ELISA	Pregnancy
	Complement	Primary antiphospholipid syndrome	
	Inflammation		

FIGURE 2 | The global map of publication output and bibliometric analysis of the keywords. (A) The global map of publication output. (B) The co-operation of countries. (C) The co-occurrence of highly frequent keywords. The circle size indicated the number of occurrences; the thickness of lines indicated the strength of linkage. (D) Mountain visualization of biclustering of highly frequent keywords of APS. Each peak represented a cluster.

Combining complement inhibition with NETosis suppression represents a novel approach needing further validation.

Regarding anticoagulant for APS, non-vitamin K antagonist oral anticoagulants (NOACs) are not recommended in patients with triple aPL positivity or arterial events. However, NOACs may still be suitable for non-high-risk APS, warranting further study. For obstetric APS, personalized treatment and practical prediction tools, such as the adjusted GAPPS score, are essential but limited, highlighting the need for improved models. Furthermore, current guidelines lack clarity on second-line therapies (e.g., hydroxychloroquine, glucocorticoids, or immunosuppressants) regarding optimal target populations, treatment initiation timelines, and fetal safety profiles, necessitating further research.

The COVID-19 pandemic has prompted scholars to pay attention to the similarities of thrombotic mechanisms between APS and viral infection. The research trend was to detect the persistence of related antibodies and to identify mechanistic links that could further clarify whether a similar aPL was observed in patients with COVID-19 or APS.

Future APS research directions include identifying biomarkers and predictive factors for early diagnosis and targeted interventions. Assays for non-criteria aPL are yet to be standardized, and more evidence is needed on the clinical relevance and therapeutic strategies. The aPL carriers exhibited a high incidence of brain MRI abnormalities, highlighting the need to focus on silent cerebral lesions in patients with persistently positive aPL [10]. Emerging therapeutic approaches like rituximab, sirolimus, belimumab, daratumumab, and obinutuzumab require further investigation.

Our bibliometric assessment provides a comprehensive perspective on APS research. The pathophysiology of thrombus

formation and antithrombotic therapy was the primary hotspot in APS, especially for patients with obstetric APS. Integrating APS-related knowledge structure and research trends can guide further explorations of APS.

Author Contributions

All authors made substantial contributions to the study design, manuscript drafting, critical revision, and final approval. In addition, Li Liu and Yang-Xi Liu performed the data search and drafted the manuscript. Jia Wang developed the analytical software and contributed to methodological writing. Hou-Wen Lin, Jia Li, and Zhi-Chun Gu critically revised the intellectual content. All authors approved the final version and assume responsibility for the work's integrity.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in the study are included in the article/Supporting Information; further inquiries can be directed to the corresponding authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:**

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ORIGINAL ARTICLE

Identification of Causal Effects of Mitochondrial Dysfunction on the Risk of Multiple Autoimmune Disorders: Multi-Omics Mendelian Randomization and Colocalization Analyses

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Keywords: autoimmune diseases | colocalization analyses | Mendelian randomization | mitochondrial dysfunction | multi-omics integration

ABSTRACT

Background: Mitochondrial dysfunction has been implicated in the pathogenesis of autoimmune disorders (AIDs), but its causal role in disease susceptibility and progression remains unclear. This study explores potential causal associations between mitochondrial-related genes and AIDs using integrated multi-omics evidence from Mendelian randomization (MR) and colocalization analyses.

Methods: Summary-level datasets from 10 common AIDs (303 590–456 348 participants) and quantitative trait loci (QTL) at the DNA methylation, gene expression, and protein abundance levels (mQTL, eQTL, and pQTL, respectively; 1 980–35 559 participants), as well as mitochondrial DNA copy number (465 809 participants), were analyzed. Instrumental variables were selected from cis-acting variants near 1136 mitochondrial-related genes with strong associations ($p_{QTL} < 5e\text{-}08$). Summary-data-based MR (SMR) and Bayesian colocalization analyses were applied to identify causal effects, followed by validation assessments integrating multi-omics SMR results.

Results: Four Grade-II mitochondrial genes were causally linked to multiple AIDs. *ATAD3A* (OR: 1.41; 95% CI: 1.13–1.76, $p_{SMR} = 1.64e\text{-}03$) and *TOP1MT* (OR: 1.42; 95% CI: 1.10–1.84, $p_{SMR} = 4.60e\text{-}03$) were strongly associated with multiple myositis, while *SND1* (OR: 1.08; 95% CI: 1.04–1.12, $p_{SMR} = 4.05e\text{-}03$) was linked to osteoarthritis. Notably, TOP1MT expression conferred protective effects against primary Sjögren's syndrome (OR: 0.57; 95% CI: 0.35–0.95, $p_{SMR} = 8.21e\text{-}03$). Crucially, only *UQCRH* was

Abbreviations: AIDs, autoimmune diseases; AOSD, adult-onset Still disease; AS, ankylosing spondylitis; eQTL, expression quantitative trait loci; GWAS, genome-wide association studies; HEIDI, heterogeneity in the dependent instrument; LD, linkage disequilibrium; MM, multiple myositis; mQTL, methylation quantitative trait loci; MR, Mendelian randomization; MS, multiple sclerosis; mtDNA, mitochondrial DNA; OA, osteoarthritis; PheWAS, phenome-wide association study; PMR, polymyalgia rheumatica; pQTL, protein quantitative trait loci; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SMR, summary-data-based MR; SNP, single nucleotide polymorphism; SSc, systemic sclerosis.

Jing Luo, Jingjing Pan, and Guanqun Yao contributed equally to this study.

identified with the same variant (rs41292543) exhibiting inverse effects on multiple myositis, including causal effects through cg11235697 methylation (OR: 1.56; 95% CI: 1.23–1.98, p_{SMR} = 3.02e-04) and protective effects through gene expression (OR: 0.83; 95% CI: 0.72–0.95, p_{SMR} = 2.78e-04).

Conclusions: These findings provide robust evidence of mitochondrial dysfunction's causal role in AIDs and identify potential pharmacological targets for treatment, offering new insights into precision medicine for AIDs.

1 | Introduction

Autoimmune disorders (AIDs) are prevalent chronic systematic diseases characterized by the breakdown of immune self-tolerance and the aberrant activation of self-antigens, ultimately leading to sustained inflammation and the production of diverse autoantibodies [1]. Despite extensive studies into their potential pathophysiological mechanisms, the etiologies of AIDs remain elusive [2]. Emerging evidence implicates mitochondrial dysfunction as a potential contributor to AIDs pathogenesis, including impaired oxidative stress and mitophagy, abnormal mitochondrial energy metabolism, excessive release of reactive oxygen species (ROS), alterations in mitochondrial morphology and dynamics, and variations in mitochondrial DNA (mtDNA) copy numbers [3, 4]. Mild mitochondrial dysfunction may serve as a compensatory mechanism to support energy requirements and preserve cellular physiological function, but severe dysfunction can promote the release of self-antigens, thereby triggering autoimmune responses in AIDs [5]. Understanding the role of mitochondrial dysfunction is thus critical for elucidating its role in the pathogenesis of AIDs. Notably, mtDNA is distinct from nuclear DNA, encoding 13 essential genes involved in oxidative phosphorylation, with mtDNA mutations or deletions increasingly linked to the onset of various AIDs [6]. For instance, the special single nucleotide polymorphism (SNP) nt13708A within the MT-ND5 gene has been reported to increase the genetic susceptibility to multiple sclerosis (MS) and systemic lupus erythematosus (SLE) [7, 8]. Conversely, protective variants within the MT-ND2 gene, such as the MT-ND2a allele, have been shown to reduce ROS production from mitochondrial complex I, thereby mitigating the risk of type-1 diabetes (T1D) [9]. These findings underscore the complex genetic predisposition between mitochondrial dysfunction and AIDs. However, existing genetic studies, including large-scale genome-wide association studies (GWAS), predominantly emphasize isolated genetic variants without fully accounting for confounding factors or the broader landscape of mitochondrial dysfunction. Consequently, there remains an unmet need for comprehensive analyses of all mitochondrial-related genes across multiple AIDs to determine whether mitochondrial dysfunction is a causative or a consequence of AIDs.

Mendelian randomization (MR) is a classical genetic epidemiology approach applied to explore potential causal associations between exposures and outcomes utilizing genetic variants as instrumental variables. Unlike observational studies, MR analysis leverages the random allocation of alleles, thereby reducing potential biases from confounders, including various ethological and environmental factors, as well as reverse causality [10]. Previous large-scale GWAS and various quantitative trait loci (QTL) datasets offer an intriguing avenue to investigate and validate these susceptible associations involving mitochondrial dysfunction-related genes and AIDs. Several MR studies have already demonstrated the causal effect of mtDNA copy number on various diseases, such as Crohn's disease [11], stroke prognosis [12], cardiometabolic diseases [13] and so on. However, conventional two-sample MR analysis can't be applied to integrated studies involving multiple QTLs. Therefore, the summary-data-based MR (SMR) method was developed to prioritize potential causal effects based on independent GWAS data and multiple QTL datasets [14]. Through the heterogeneity in the dependent instrument (HEIDI) test, the SMR algorithm effectively eliminates potential causal associations from linkage disequilibrium (LD). To our knowledge, integrated SMR analyses combining GWAS datasets with gene expression, DNA methylation, and protein abundance of QTLs (eQTLs, mQTLs, and pQTLs respectively) have only been applied to investigate the causal relationships between mitochondrial dysfunction and the risks of several complex diseases, including osteoarthritis [15], type-2 diabetes [16], Alzheimer's disease [17], various cancers [18] and inflammatory bowel disease (IBD) [19]. Nevertheless, the comprehensive evaluation of causal relationships between mitochondrial dysfunction and the risks of AIDs remains unexplored.

In this study, we performed SMR analysis at the levels of eQTLs, mQTLs, and pQTLs to investigate potential causal relationships between mitochondrial dysfunction and the risk of multiple AIDs. Furthermore, the HEIDI test and colocalization analysis were combined to identify significant QTL-GWAS regulatory loci, and a series of validation assessments were implemented to strengthen the evidence of causality, including sensitivity analysis, phenome-wide association study (PheWAS) analysis, and bidirectional MR analysis of mtDNA copy number and AIDs. Finally, we integrated multi-omics SMR results and re-conducted SMR analysis across multiple QTLs to further ascertain and validate the potential causal effects of genetic predisposition to mitochondrial dysfunction on AIDs outcomes.

2 | Methods

2.1 | Study Design

Figure 1 illustrates the overall workflow of this study. To investigate the genetic predisposition to mitochondrial dysfunction in autoimmune diseases (AIDs), we first curated 1136 mitochondrial-related genes from the MitoCarta database (v3.0) [20]. SMR analyses were then conducted to assess causal relationships between mitochondrial gene regulation and AIDs using publicly available genome-wide association study (GWAS) datasets, including FinnGen v10 [21],

Summary

- Integrated multi-omics SMR analyses identified potential causal associations between four mitochondrial dysfunction-related genes and multiple autoimmune diseases (AIDs).
- Strong causal effects of *ATAD3A*'s and *TOP1MT*'s eQTLs on Myositis and *SND's* eQTLs on Osteoarthritis.
- mQTLs of mitochondrial genes displayed complex causal or protective effects on diverse AIDs.
- *UQCRH* showed dual effects on multiple myositis by rs41292543, including causal effects via cg11235697 methylation and protective effects via eQTLs.
- These findings provide valuable clues to potentially pharmacological targets for AIDs.

NHGRI-EBI GWAS Catalog [22], and other large-scale GWAS studies (details provided in Table S1). T A series of validation procedures were implemented to reinforce causal inference, including HEIDI testing, sensitivity analyses, Bayesian colocalization, PheWAS, bidirectional MR of mtDNA copy number, and multi-omics integration of SMR results. All datasets were restricted to individuals of European ancestry to mitigate population stratification bias.

2.2 | Dataset Source of eQTL, mQTL, and pQTL Instruments for Mitochondrial Genes

To comprehensively explore mitochondrial dysfunction across molecular layers, we integrated cis-acting genetic instruments (within 1 Mb of coding regions) from multi-omics quantitative trait loci (QTL) datasets. Cis-expression QTLs (eQTLs) in whole blood were derived from the eQTLGen Consortium ($n = 31\,684$), yielding 6 822 795 SNPs associated with 1012 mitochondrial transcripts (https://www.eqtlgen.org/cis-eqtls.html) [23]. Methylation QTLs (mQTLs) were obtained from McRae et al. ($n = 1980$) [24], identifying 925 899 SNPs associated with 2565 CpG sites linked to mitochondrial genes. Protein QTLs (pQTLs) were sourced from a large proteomic GWAS of 4907 proteins in 35 559 Icelandic individuals [25], from which 194 SNPs corresponding to 89 mitochondrial proteins were retained ($p_{QTL} < 1e\text{-}5$).

2.3 | GWAS Summary Statistic Datasets of AIDs

GWAS summary datasets for various AID outcomes were primarily retrieved from the GWAS Catalog database (https://www.ebi.ac.uk/gwas/home) and the FinnGen cohorts (v10, https://finngen.gitbook.io/documentation). This study encompassed 10 common AIDs, with all participants being of European ancestry. Those datasets included 122 cases and 456 226 controls for SLE (trait ID: GCST90044512), 8561 cases and 295 029 controls for rheumatoid arthritis (RA) (trait ID: GCST90081504), 99 cases and 456 249 controls for primary Sjogren's syndrome (pSS) (trait ID: GCST90044534), 3554 cases and 369 098 controls for multiple myositis (MM) (trait ID: AB1_Dermatophytosis), 619 cases and 365 533 controls for systemic sclerosis (SSc) (trait ID: M13_SYSTSLCE), 186 cases and 456 162 controls for ankylosing spondylitis (AS) (trait ID: GCST90044542), 8439 cases and 447 909 controls for osteoarthritis (OA) (trait ID: GCST90044589), 375 cases and 455 973 controls for Gout (trait ID: GCST90043662), 113 cases and 341 455 controls for Adult-onset Still disease (AOSD) (trait ID: STILL_ADULT), and 3501 cases and 365 533 controls for polymyalgia rheumatica (PMR) (trait ID: M13_Polymyalgia). Importantly, there was no sample overlap among these datasets, ensuring the integrity of the comparative analysis. To avoid bias caused by limited samples, we re-collected the latest GWAS summary datasets from FinnGen cohorts (version 11) to replace small scale patients, including SLE, pSS, Gout, AS, and RA (details provided in Table S1). For more information about the bioinformatic analysis, validation procedures, and statistical analyses, see online Supporting Information S1.

3 | Results

3.1 | SMR Analysis of Mitochondrial Cis-eQTLs and AIDs Outcomes

Through SMR analysis, we identified 52 significant associations involving 55 genetic loci across 10 diverse AIDs (adjusted $p_{SMR} < 0.05$ and $p_{HEIDI} > 0.01$, Table S2). To eliminate the influence of LD, we performed the colocalization analysis and further identified 10 unique genetic loci for 4 AIDs (AS, MM, OA and pSS, respectively), including 6 genes conferring causal effects and 4 genes exhibiting protective effects on AIDs (Figure 2). For AS, reduced *OCIAD2* expression was associated with a 37% lower disease risk (OR: 0.63, $p_{SMR} = 1.96e\text{-}03$; PP.H4 = 1.00). In MM, we detected strong causal effects of *ATAD3A* (OR: 1.41, $p_{SMR} = 1.64e\text{-}03$; PP.H4 = 0.92) and *TOP1MT* expression (OR: 1.42; $p_{SMR} = 4.60e\text{-}03$; PP.H4 = 0.92), along with significant protective roles of *UQCRH* expression (OR: 0.83, $p_{SMR} = 2.78e\text{-}04$; PP.H4 = 0.96). In addition, we also detected several causal associations between the expression of *SND1* (OR: 1.08; $p_{SMR} = 4.05e\text{-}03$; PP.H4 = 0.94) and *OCAID2* (OR: 1.10; $p_{SMR} = 9.75e\text{-}03$; PP.H4 = 1.00), and OA traits. Moreover, genetically predicted higher expression levels of *NDUFS4* (OR: 1.52; $p_{SMR} = 1.65e\text{-}03$; PP.H4 = 0.86) and *YRDC* (OR: 1.64; $p_{SMR} = 3.81e\text{-}03$; PP.H4 = 0.86) were positively associated with the risks of AOSD. Conversely, higher expression levels of *VARS2* (OR: 0.76; $p_{SMR} = 1.89e\text{-}03$; PP.H4 = 1.00) and *TOP1MT* (OR: 0.57; $p_{SMR} = 8.21e\text{-}03$; PP.H4 = 0.94) were negatively linked to the risks of pSS. However, no significant genetic loci were identified for Gout, PMR, RA, SLE, and SSc (PP.H4 < 0.8). Notably, several loci exhibited opposite effects across different diseases: e.g., the expression levels of *OCIAD2*, upregulated by the rs7673965 variant, possessed causal effects on the risks of OA while showing protective effects on AS traits. Similarly, the expression levels of *TOP1MT*, upregulated by the rs62524728, exhibited causal effects on MM risks while showing protective effects on pSS risks (Figure 2). These associations were validated in independent datasets and confirmed through sensitivity analyses (Tables S5 and S15).

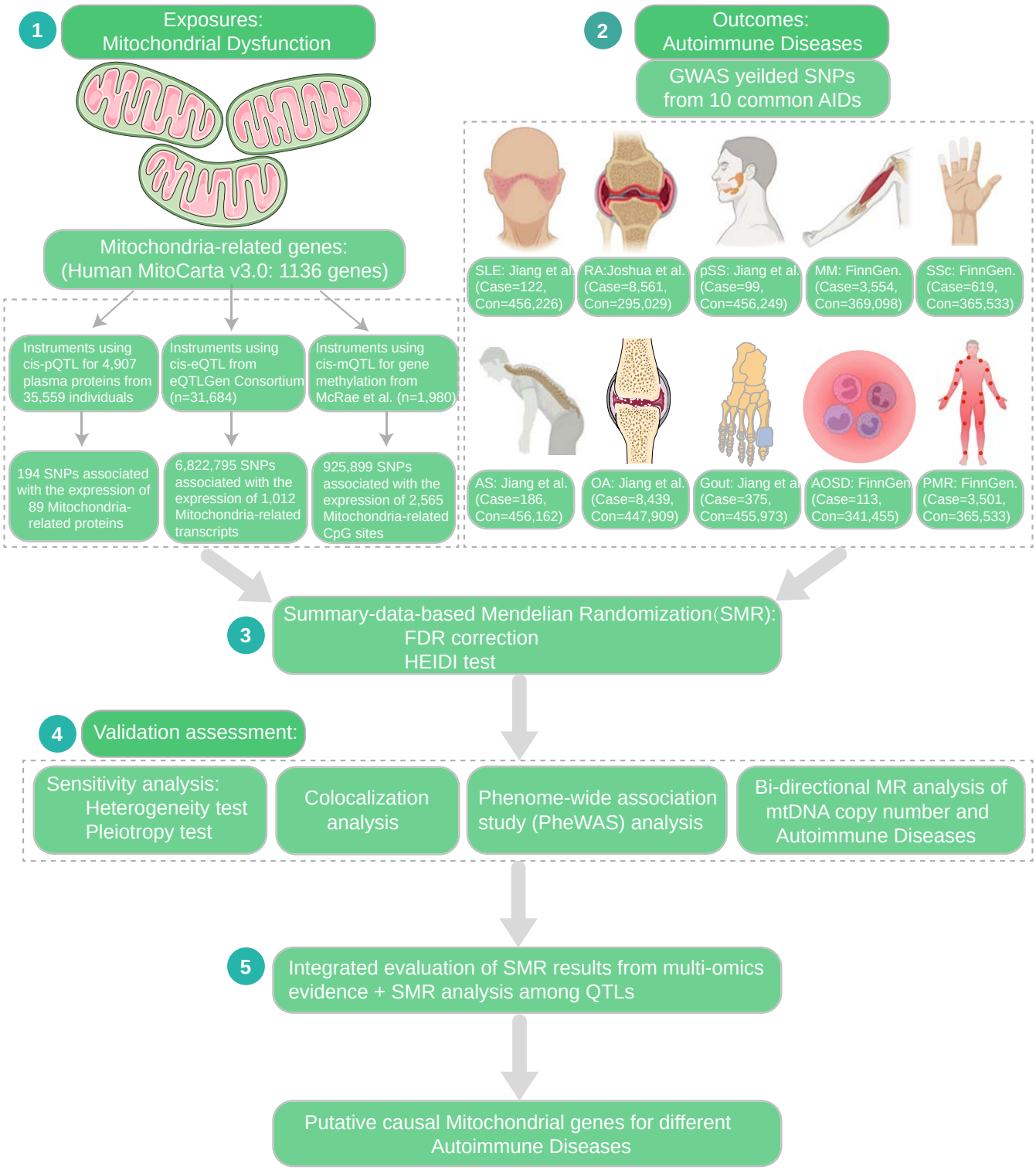
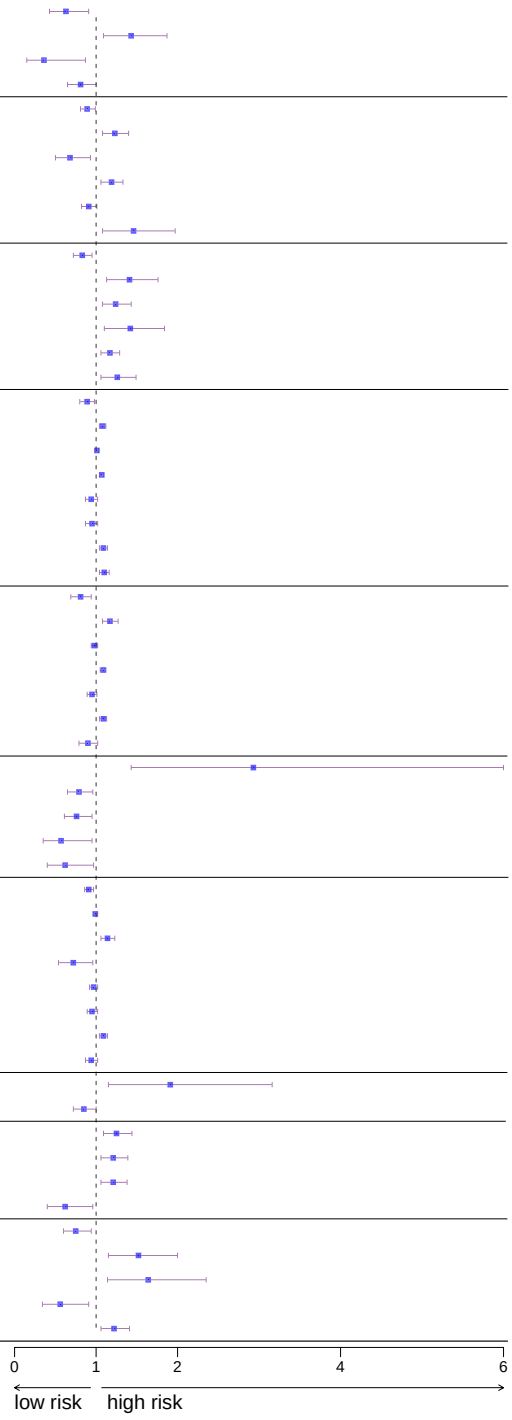


FIGURE 1 | The flowchart of the analytical framework performed in this study. AIDs, autoimmune diseases; AOSD, adult-onset Still disease; AS, ankylosing spondylitis; eQTL, expression quantitative trait loci; GWAS, genome-wide association study; HEIDI, heterogeneity in the dependent instrument; MM, multiple myositis; mQTL, methylation quantitative trait loci; mtDNA, mitochondrial DNA; OA, osteoarthritis; PMR, polymyalgia rheumatica; pQTL, protein quantitative trait loci; pSS, primary Sjogren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphisms; SSc, systemic sclerosis.

3.2 | SMR Analysis of Mitochondrial Cis-mQTLs and AIDs Outcomes

We next investigated the associations between mitochondrial DNA methylation and AID risk using mQTL-based SMR (Figure 3). After applying quality control thresholds (adjusted $p_{SMR} > 0.05$ or $p_{HEIDI} < 0.01$), 183 CpG sites across 115 genes demonstrated statistical significance (Table S3). After colocalization analysis correction, we ultimately identified 23 CpG sites across 12 unique genes associated with the

Disease	Gene	Instrumental variant	eQTL size effect	β coefficient	OR (95%CI)	*P* SMR Value	*P* HEIDI Value	Colocalization
AS	OCIAD2	rs7673965	0.12	−0.47	0.63 [0.43−0.91]	1.96e−03	0.330	1.00
	IBA57	rs10916277	0.16	0.36	1.43 [1.09−1.87]	6.23e−03	0.046	<0.001
	TMEM186	rs9939787	−0.07	−1.01	0.36 [0.15−0.87]	6.53e−03	0.298	<0.001
	SLC25A46	rs72773149	−0.34	−0.22	0.81 [0.65−1.00]	7.13e−03	0.637	<0.001
Gout	NDUFA7	rs561	−0.37	−0.11	0.89 [0.81−0.99]	8.58e−04	0.649	<0.001
	HINT1	rs3864285	−0.69	0.21	1.23 [1.08−1.40]	2.04e−03	0.245	<0.001
	PHB1	rs6917	−0.13	−0.38	0.68 [0.50−0.93]	2.10e−03	0.351	<0.001
	HAGH	rs171162	0.38	0.17	1.19 [1.06−1.33]	5.16e−03	0.275	<0.001
	ATP5F1D	rs12983363	−0.32	−0.09	0.91 [0.82−1.01]	5.71e−03	0.972	<0.001
	VDAC2	rs11543	0.10	0.38	1.46 [1.08−1.97]	9.97e−03	0.595	<0.001
MM	UQCRH*	rs41292543	−0.33	−0.19	0.83 [0.72−0.95]	2.78e−04	0.986	0.96
	ATAD3A*	rs3766170	−0.13	0.34	1.41 [1.13−1.76]	1.64e−03	0.879	0.92
	GFM2	rs17647023	−0.22	0.22	1.24 [1.08−1.43]	3.59e−03	0.568	<0.001
	TOP1MT*	rs62524728	0.15	0.35	1.42 [1.10−1.84]	4.60e−03	0.419	0.92
	MTPAP	rs3802622	−0.27	0.16	1.17 [1.06−1.29]	4.83e−03	0.236	<0.001
	PUSL1	rs3889966	−0.25	0.23	1.26 [1.06−1.49]	9.18e−03	0.444	<0.001
OA	BAD	rs947939	0.09	−0.12	0.89 [0.80−0.98]	4.88e−04	0.471	<0.001
	SND1*	rs12706818	−0.20	0.08	1.08 [1.04−1.12]	4.05e−03	0.641	0.94
	ECH1	rs7248208	−0.31	0.01	1.01 [0.99−1.03]	4.75e−03	0.848	<0.001
	MTHFD1L	rs13201018	0.45	0.06	1.07 [1.04−1.09]	5.80e−03	0.686	<0.001
	SLC25A40	rs7796247	−0.26	−0.06	0.94 [0.87−1.02]	7.76e−03	0.369	<0.001
	REXO2	rs2365626	−0.10	−0.06	0.95 [0.87−1.02]	7.78e−03	0.019	<0.001
	RAB5IF	rs77914630	−0.23	0.09	1.09 [1.04−1.14]	9.34e−03	0.309	<0.001
	OCIAD2	rs7673965	0.12	0.10	1.10 [1.04−1.16]	9.75e−03	0.168	1.00
PMR	MRPS30	rs6875287	0.07	−0.22	0.81 [0.69−0.94]	2.19e−04	0.332	<0.001
	BPHL	rs9405613	−0.15	0.16	1.17 [1.08−1.27]	3.86e−04	0.030	<0.001
	UQCC2	rs2296330	−0.26	−0.02	0.98 [0.94−1.02]	1.03e−03	0.281	<0.001
	COQ6	rs35897291	−0.40	0.08	1.09 [1.05−1.12]	1.22e−03	0.367	0.14
	VWA8	rs12866839	−0.20	−0.05	0.95 [0.89−1.01]	2.62e−03	0.562	<0.001
	ACAA2	rs75116816	0.26	0.08	1.09 [1.04−1.13]	5.59e−03	0.859	<0.001
	ACAD10	rs10774634	0.10	−0.11	0.90 [0.79−1.02]	8.64e−03	0.381	<0.001
pSS	ACSS3	rs12810791	0.21	1.08	2.93 [1.43−6.00]	9.14e−04	0.635	<0.001
	MTX1	rs370545	−0.30	−0.24	0.79 [0.65−0.96]	1.25e−03	0.138	<0.001
	VARS2	rs2523594	−0.26	−0.27	0.76 [0.61−0.95]	1.89e−03	0.068	1.00
	TOP1MT*	rs62524728	0.15	−0.55	0.57 [0.35−0.95]	8.21e−03	0.119	0.94
	THNSL1	rs10764517	−0.14	−0.47	0.62 [0.40−0.97]	8.94e−03	0.263	<0.001
RA	UQCC2	rs2296330	−0.26	−0.09	0.91 [0.86−0.97]	8.69e−07	0.233	<0.001
	PIGBOS1	rs28685005	−0.55	−0.01	0.99 [0.97−1.02]	4.66e−05	0.523	<0.001
	COA5	rs3769698	0.19	0.13	1.14 [1.06−1.23]	3.76e−03	0.062	<0.001
	CHDH	rs2680671	−0.05	−0.33	0.72 [0.54−0.96]	3.88e−03	0.286	<0.001
	FTH1	rs111605165	−0.60	−0.03	0.97 [0.92−1.02]	5.76e−03	0.245	<0.001
	BCS1L	rs36085075	0.52	−0.05	0.95 [0.89−1.02]	7.28e−03	0.119	<0.001
	RMDN3	rs4924501	0.28	0.09	1.09 [1.04−1.14]	7.72e−03	0.786	<0.001
	IDH2	rs184860993	−1.11	−0.06	0.94 [0.87−1.02]	8.75e−03	0.298	<0.001
SLE	ALDH1B1	rs77054814	0.20	0.64	1.91 [1.15−3.16]	5.94e−03	0.862	<0.001
	TOMM5	rs73441369	0.42	−0.17	0.85 [0.72−1.00]	6.13e−03	0.152	0.61
SSc	MRPS33	rs801155	−0.36	0.22	1.25 [1.09−1.44]	2.14e−03	0.109	<0.001
	AGPAT5	rs2911977	0.17	0.19	1.21 [1.06−1.39]	6.47e−03	0.328	<0.001
	NDUFAF7	rs2372993	0.17	0.19	1.21 [1.06−1.38]	6.51e−03	0.949	<0.001
	TRMT10C	rs9825208	−0.06	−0.48	0.62 [0.40−0.96]	7.90e−03	0.686	<0.001
AOSD	SDHAF1	rs7251575	−0.24	−0.29	0.75 [0.60−0.94]	1.14e−03	0.947	<0.001
	NDUFS4	rs4147744	0.21	0.42	1.52 [1.15−2.00]	1.65e−03	0.865	0.86
	YRDC	rs9701812	0.13	0.49	1.64 [1.14−2.35]	3.81e−03	0.560	0.86
	ECSIT	rs34803265	0.22	−0.58	0.56 [0.34−0.91]	4.40e−03	0.145	<0.001
	CPT1B	rs8137478	−0.90	0.20	1.22 [1.06−1.41]	7.90e−03	0.338	<0.001



* means Grade-II genes

FIGURE 2 | The SMR results for the association between eQTLs of mitochondrial genes and the risks of AIDs. The eQTL size effect represents β value of a variant on the expression of mRNA. The significant variants were identified as $p_{SMR} < 0.05$, $p_{HEIDI} > 0.01$, and colocalization/PP.H4 > 0.8. CI, confidence interval; OR, odds ratio; PPH4, posterior probability of H4.

risks of 7 AIDs (PP.H4 > 0.8). These association signals included *TARS2* (cg04165759) for Gout, *ATAD3A* (cg26863172), *TOP1MT* (cg15707110, cg15845333) and *UQCRH* (cg11235697) for MM, *FIS1* (cg24687253, cg05770091, cg24809529), *GCAT* (cg22275857), *MSRA* (cg08841257, cg05753693, cg06620390, cg11044290, cg11548083), and *SND1* (cg00893242, cg13969327, cg27269962) for OA, GLS2 (cg00377653, cg10306450) and *VARS2* (cg02306936) for PMR, *STX17* (cg14153061) and *TOP1MT* (cg15845333, cg15707110) for pSS, *NDUSF4* (cg18899355) for RA, and SND1 (cg23439280) for SLE (Figure 3). The sensitivity analysis also removed the heterogeneity and horizontal pleiotropy among SNPs, supporting similar causal associations on corresponding AIDs traits (Table S6).

Interestingly, CpG sites within the same gene often showed divergent effects. For instance, one SD increase of *GLS2* methylation

Disease	Gene	Methylation probe	Instrumental variant	mQTL size effect	β coefficient	OR (95.% CI)	*P* SMR value	*P* HEIDI value	Colocalization
Gout	TARS2	cg04165759	rs12023277	−0.74	−0.35	0.70 (0.58−0.86)	6.98e−04	0.744	0.97
MM	ATAD3A*	cg26863172	rs6694994	0.32	−0.61	0.54 (0.35−0.85)	7.67e−03	0.345	0.96
	TOP1MT*	cg15707110	rs7002559	0.58	0.49	1.63 (1.17−2.29)	4.33e−03	0.806	0.91
		cg15845333	rs2931710	0.46	0.57	1.77 (1.15−2.72)	9.04e−03	0.544	0.94
	UQCRH*	cg11235697	rs41292543	0.96	0.44	1.56 (1.23−1.98)	3.02e−04	0.630	0.96
OA	FIS1	cg24687253	rs10244139	0.98	0.10	1.11 (1.03−1.19)	6.40e−03	0.263	0.84
		cg05770091	rs10270498	−0.41	−0.26	0.77 (0.63−0.93)	7.44e−03	0.303	0.81
		cg24809529	rs12334190	0.62	0.15	1.17 (1.04−1.31)	8.55e−03	0.183	0.82
	GCAT	cg22275857	rs5995500	−0.30	−0.25	0.78 (0.69−0.88)	9.20e−05	0.043	0.82
	MSRA	cg08841257	rs11774474	−0.52	0.11	1.12 (1.05−1.19)	6.85e−04	0.412	0.99
		cg05753693	rs11779480	−0.35	0.16	1.17 (1.06−1.29)	1.43e−03	0.425	0.96
		cg06620390	rs6982308	0.35	0.14	1.15 (1.05−1.26)	2.86e−03	0.651	0.94
		cg11044290	rs6601427	−0.17	−0.30	0.74 (0.60−0.92)	5.70e−03	0.769	0.92
		cg11548083	rs11249992	0.54	0.08	1.08 (1.02−1.15)	9.29e−03	0.692	0.91
	SND1*	cg00893242	rs3808093	0.57	−0.08	0.92 (0.87−0.98)	5.20e−03	0.911	0.85
		cg13969327	rs117151498	1.14	−0.13	0.88 (0.79−0.96)	7.13e−03	0.885	0.91
		cg27269962	rs55660321	−0.27	0.18	1.20 (1.05−1.37)	7.68e−03	0.538	0.87
PMR	GLS2	cg00377653	rs2657909	0.57	−0.16	0.85 (0.78−0.93)	4.64e−04	0.860	0.84
		cg10306450	rs12316930	−0.20	0.47	1.60 (1.19−2.16)	2.09e−03	0.996	0.88
	VARS2	cg02306936	rs142282930	−0.79	0.75	2.11 (1.23−3.64)	6.84e−03	0.786	0.92
pSS	STX17	cg14153061	rs10739792	0.95	0.58	1.79 (1.30−2.47)	3.44e−04	0.924	1.00
	TOP1MT*	cg15845333	rs2931710	0.46	−1.21	0.30 (0.13−0.70)	5.50e−03	0.172	0.95
		cg15707110	rs7002559	0.58	−0.90	0.41 (0.21−0.79)	7.94e−03	0.393	0.93
RA	NDUFS4	cg18899355	rs256092	−0.34	−0.29	0.75 (0.60−0.93)	9.02e−03	0.639	0.92
SLE	SND1*	cg23439280	rs322772	0.24	1.54	4.65 (1.46−14.75)	9.18e−03	0.169	0.94

* means Grade-II genes

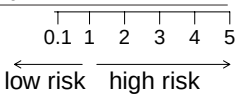


FIGURE 3 | The SMR results for the association between mQTLs of mitochondrial genes and the risks of AIDs. The mQTL size effect represents β value of a variant on DNA methylation. The significant CpG sites were identified as $p_{SMR} < 0.05$, $p_{HEIDI} > 0.01$, and colocalization/PP.H4 > 0.8. CI, confidence interval; OR, odds ratio; PPH4, posterior probability of H4.

(cg10306450) by rs12316930 was linked to a 60% higher risk of PMR (OR: 1.60, p_{SMR} = 2.09e-03; PP.H4 = 0.88), while conversely, one SD increase of *GLS2* methylation (cg00377653) by rs2657909 was associated with a 15% lower risk of PMR (OR: 0.85, p_{SMR} = 4.64e-04; PP.H4 = 0.84). Additionally, methylation levels of *FIS1* at cg24687253 (OR: 1.11, p_{SMR} = 6.40e-03; PP.H4 = 0.84) and cg24809529 (OR: 1.17, p_{SMR} = 8.55e-03; PP.H4 = 0.82) were linked to increased risks of OA, while methylation at cg05770091 (OR: 0.77, p_{SMR} = 7.44e-03; PP.H4 = 0.81) was associated with decreased risks. Similarly to cis-eQTL, the same CpG sites were also found to exhibit opposed effects on different AIDs. The methylation levels of *TOP1MT* at cg15707110 and cg15845333 exhibited strong causal effects on MM traits (OR: 1.63, p_{SMR} = 4.33e-03, PP.H4 = 0.91; and OR: 1.77, p_{SMR} = 9.04e-03, PP.H4 = 0.94, respectively), while showing significant protective effects on pSS (OR: 0.30, p_{SMR} = 5.50e-03, PP.H4 = 0.95; and OR: 0.41, p_{SMR} = 7.94e-03, PP.H4 = 0.93, respectively) (Figure 3). These findings support the potential causal relationships between the methylation of mitochondrial dysfunction and the risk of various AIDs.

3.3 | SMR Analysis of Mitochondrial Cis-pQTLs and AIDs Outcomes

A total of 15 specific genetic loci across 15 unique mitochondria-related proteins were identified as causal associations with 9 AIDs (except AOSD, adjusted $p_{SMR} < 0.05$ and $p_{HEIDI} > 0.01$, Table S4). Interestingly, genetic variants of the same proteins also displayed different potential causal effects on distinct AIDs. For instance, genetically predicted higher abundance of *HTATIP2* protein (rs56361406) was positively associated with the risks of SLE (OR: 3.92, p_{SMR} = 1.80e-04), while negatively associated with the risks of PMR (OR: 0.87, p_{SMR} = 4.40e-02). Additionally, a decrease of one SD alteration in the protein abundance of *ECI2* (rs7756630) was associated with a 62% lower risk of pSS (OR: 0.38, p_{SMR} = 4.22e-02), while associated with a 27% higher risk of RA (OR: 1.27, p_{SMR} = 2.93e-02) (Figure 4). However, no causal association at cis-pQTL levels was identified through the colocalization correction (PP.H4 < 0.001), suggesting that the identification of genetic variants robustly linked to protein levels remains to be further investigated (Table S4).

3.4 | PheWAS Analysis of Identified Effective SNPs

To further exclude potential pleiotropy of investigated AIDs, we performed a PheWAS analysis using the identified effective SNPs, based on the PheWAS Catalog database and IEU website's PheWAS tool. For the cis-eQTLs and cis-mQTLs, the majority of effective SNPs were not associated with rheumatic diseases, and only a few SNPs were associated with available secondary traits, but not with their corresponding AIDs traits. For example, the SNP

Disease	Gene	Instrumental variant	pQTL size effect	β coefficient	OR (95.% CI)	*P* SMR value	*P* HEIDI value	Colocalization
AS	TSTD1	rs10908821	0.49	0.83	2.28 (1.21−4.29)	1.05e−02	0.556	<0.001
Gout	CRYZ	rs696703	0.32	0.50	1.65 (1.04−2.61)	3.25e−02	0.056	<0.001
	NUDT9	rs28529046	−0.14	1.76	5.83 (1.24−27.34)	2.55e−02	0.017	<0.001
MM	FHIT	rs2687899	−0.08	2.27	9.71 (1.30−72.33)	2.65e−02	0.177	<0.001
	GRHPR	rs151297784	−0.13	−3.75	0.02 (0.00−0.54)	1.93e−02	0.094	<0.001
	CAT	rs769218	−0.21	−0.85	0.43 (0.19−0.97)	4.08e−02	0.718	<0.001
OA	GRHPR	rs151297784	−0.13	−0.83	0.44 (0.20−0.96)	3.90e−02	0.234	<0.001
PMR	ACAA1	rs2229528	−0.60	−0.24	0.78 (0.62−0.99)	4.37e−02	0.388	<0.001
	NUDT2	rs10814111	0.18	0.63	1.87 (1.02−3.42)	4.23e−02	0.045	<0.001
	HTATIP2	rs56361406	0.39	−0.14	0.87 (0.76−1.00)	4.40e−02	0.851	<0.001
	ALDH6A1	rs8013751	0.11	−0.89	0.41 (0.22−0.77)	5.62e−03	0.016	<0.001
pSS	ECI2	rs7756630	−0.32	−0.98	0.38 (0.15−0.97)	4.22e−02	0.446	<0.001
	SDSL	rs71465896	0.41	−1.79	0.17 (0.04−0.77)	2.23e−02	0.212	<0.001
RA	DTYMK	rs144303868	0.16	−1.28	0.28 (0.09−0.82)	2.02e−02	0.335	<0.001
	QDPR	rs3822233	−0.39	−0.20	0.82 (0.68−0.99)	4.28e−02	0.366	<0.001
	ECI2	rs7756630	−0.32	0.24	1.27 (1.02−1.57)	2.93e−02	0.689	<0.001
SLE	HTATIP2	rs56361406	0.39	1.37	3.92 (1.92−8.01)	1.80e−04	0.932	<0.001
SSc	NME4	rs169955	−0.06	−2.92	0.05 (0.00−0.75)	2.94e−02	0.017	<0.001

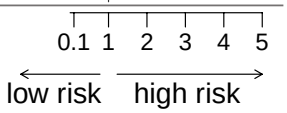


FIGURE 4 | The SMR results for the association between pQTLs of mitochondrial genes and the risks of AIDs. The pQTL size effect represents β value of a variant on protein's abundance. The significant variants were identified as $p_{SMR} < 0.05$, $p_{HEIDI} > 0.01$, and colocalization/PP.H4 > 0.8. CI, confidence interval; OR, odds ratio; PPH4, posterior probability of H4.

rs2523594 (*VARS2* expression-related loci for pSS) was linked to secondary traits such as AS, spondyloarthritis, and RA. Similarly, the SNP rs9701812 (*YRDC* expression-related loci for AOSD) was associated with other AIDs traits (such as RA and TID) (Table S7). Additionally, the rs2096706 (*MACROD1* methylation-related loci for OA) and rs13214874 (*UQCC2* methylation-related loci for PMR) were also linked to secondary traits such as IBD, Crohn's disease, and RA (Table S8). These results underscore the specificity of these pivotal variants in the causal relationships with homologous AIDs.

3.5 | Bidirectional MR Analysis of mtDNA Copy Number and AIDs

To further validate the causality between mitochondrial dysfunction and AIDs, we performed the bi-directional MR analysis between mtDNA copy number and AIDs across both discovery and replication datasets. The results revealed that the directions of causal effects varied specifically with each AID. In the discovery dataset, the mtDNA copy number variants were casually associated with MM, pSS, OA, and SLE, while RA, PMR, and pSS showed significant causal effects on mtDNA copy number variants (Tables S9 and S10). Consistently, in the validation dataset, the mtDNA copy number variants also exhibited significant causal associations with MM, pSS, OA, and SLE, while OA, PMR, RA, and pSS were casually associated with mtDNA copy number variants (Tables S11 and S12).

3.6 | Integrating SMR Results From Multi-Omics Evidence

Although no genetic variant of cis-pQTL passed the correction of colocalization analysis, the regulatory roles of four Grade-II mitochondria-related genes were identified for several AIDs at both mQTL and eQTL levels, including *ATAD3A* and *UQCRH* for MM traits, *TOP1MT* for MM and pSS traits, and *SND1* for OA and SLE traits (Table 1, Table S13). Noteworthy, these regulatory genes exhibited either coincident or inverse causal effects even on the same AIDs at mQTL and eQTL levels, implying the complex causal associations between mitochondrial dysfunction and the risk of AIDs. The consistent associations between gene's methylation and expression were observed in *TOP1MT* (cg15707110 and cg15845333), which were consistent with the causal role for MM and protective effects on pSS respectively. Similarly, the inverse mQTL-eQTL associations were also detected in *ATAD3A* (cg26863172) for MM, and *SND1* (cg00893242) for OA. Moreover, the colocalization analysis further demonstrated significant positive correlation between both mQTL and eQTL with GWAS loci, including 10 variants in mQTL-related and 5 variants eQTL-related abnormalities (Figures S1 and S2). Crucially, *UQCRH* was the only gene identified with the same SNP (rs41292543) exhibiting inverse effects on MM, including the pathogenic effects via cg11235697 methylation and protective effects via UQCRH expression (Table 1). The colocalization analysis not only demonstrated significant causal associations between mQTL/eQTL regulation and SNP rs41292543, but also

TABLE 1 | Integrating SMR results from multi-omics evidence (cis-mQTL and cis-eQTL).

		Cis-mQTL				Cis-eQTL		
Disease	**Gene**	**CpG sites**	**Instrumental variant**	**OR [95% CI]**	***FDR*$_{SMR}$**	**Instrumental variant**	**OR [95% CI]**	***FDR*$_{SMR}$**
MM	*ATAD3A*	cg26863172	rs6694994	0.54 [0.35–0.85]	4.78E-03	rs3766170	1.41 [1.13–1.76]	4.10E-03
	TOP1MT	cg15707110	rs7002559	1.63 [1.17–2.29]	4.03E-03	rs62524728	1.42 [1.10–1.84]	5.75E-03
	TOP1MT	cg15845333	rs2931710	1.77 [1.15–2.72]	4.78E-03	—	—	—
	UQCRH[a]	cg11235697	**rs41292543**	1.56 [1.23–1.98]	7.77E-03	**rs41292543**	0.83 [0.72–0.95]	1.39E-03
OA	*SND1*	cg00893242	rs3808093	0.92 [0.87–0.98]	4.30E-03	rs12706818	1.08 [1.04–1.12]	5.75E-03
	SND1	cg13969327	rs117151498	0.88 [0.79–0.96]	4.47E-03	—	—	—
	SND1	cg27269962	rs55660321	1.20 [1.05–1.37]	4.49E-03	—	—	—
SLE	*SND1*	cg23439280	rs322772	4.65[1.46–14.75]	4.86E-03	—	—	—
pSS	*TOP1MT*	cg15845333	rs2931710	0.30 [0.13–0.70]	4.99E-03	rs62524728	0.57 [0.35–0.95]	8.21E-03
	TOP1MT	cg15707110	rs7002559	0.41 [0.21–0.79]	4.99E-03	—	—	—

Note: The common regulatory genes were identified with significant causal effects on same AIDs (FDR adjusted $p_{SMR}<0.05$, $p_{HEIDI}>0.01$, and PP.H4 > 0.8).

Abbreviations: CI, confidence interval; MM, multiple myositis; OA, osteoarthritis; OR, odds ratio; pSS, primary Sjogren's syndrome; SLE, systemic lupus erythematosus; SMR, summary-data-based Mendelian randomization.

[a]Bold formatting applied to UQCRH and rs41292543 explicitly to indicate that UQCRH is the regulatory gene with the same instrumental variant (rs41292543).

indicated significant positive correlation between cg11235697 methylation and UQCRH expression (Figure 5). Through the SMR and colocalization analyses, we successfully validated the causal effects between the methylation and gene expression of *ATAD3A*, *TOP1MT*, and *UQCRH* ($p_{SMR}<1.95e\text{-}05$ [Bonferroni correction, $p<0.05/2565$], $p_{HEIDI}>0.01$, and PP.H4 > 0.8) (Tables S14 and S15).

4 | Discussion

In this study, we confirmed that genetic predisposition related to mitochondrial dysfunction was causally associated with the susceptibility of multiple AIDs, and further identified significant putative causal genes through integrated SMR and colocalization analyses, including: [1] *ATAD3A* for MM, [2] *TOP1MT* for MM and pSS, [3] *SND1* for OA and SLE, [4] *VARS2* for pSS and PMR, [5] *NDUFS4* for AOSD and RA, and [6] *UQCRH* for MM. These findings underscore potential genetic connections between mitochondrial dysfunction and AIDs in a disease-specific manner, and provide promising insights into the underlying mitochondrial mechanisms linking DNA methylation, gene expression, and protein abundance with the development of multiple AIDs.

As the member of the mitochondrial ATPase family, *ATAD3A* plays a crucial role in regulating dynamic interactions between mitochondrial outer and inner membrane proteins, primarily maintaining essential mitochondrial functions and organization through mitochondrial dynamics, homeostasis, and metabolism [26]. Previous studies have indicated that mutations in *ATAD3A* caused phenotypes for SSc with the activation of interferon-stimulated genes (ISG), and the knockdown of *ATAD3A* in THP-1 cell lines also resulted in the up-regulation of ISG's expression induced by activated cGAS-STING signaling [27]. Mutations in *ATAD3A* have been shown to induce mitophagy and autophagy, disrupt mitochondrial cristae morphology, and reduce the expression or activity of multiple mitochondrial respiratory complexes. These mitochondrial impairments have been implicated in the pathogenesis of various neurological and inflammatory diseases [28, 29]. Consistently, our results also demonstrated that the CpG site (cg26863172) and SNP (rs3766170) related to *ATAD3A* were linked to decreased risks of MM. Moreover, our PheWAS analysis further excluded the possibility of causal associations influenced by horizontal pleiotropy, supporting the potential protective effect of *ATAD3A* on MM. The impairment in mitochondrial dynamics caused by *ATAD3A* can contribute to cellular dysfunction, highlighting its role in both mitochondrial integrity and AID's development.

TOP1MT is a mitochondria-specific type IB topoisomerase that is involved in altering the topological structure of mtDNA as well as regulating mtDNA replication and transcription through transiently breaking and re-joining the phosphodiester backbone [30]. Notably, mitochondrial *TOP1MT* has been identified as an autoantigen targeted by several autoantibodies in AIDs, such as anti-Scl-70 for SSc [31] and a specific autoantibody for SLE [32]. However, the causal association between methylation and expression of *TOP1MT* and multiple AIDs remains unclear. In this study, our SMR analysis

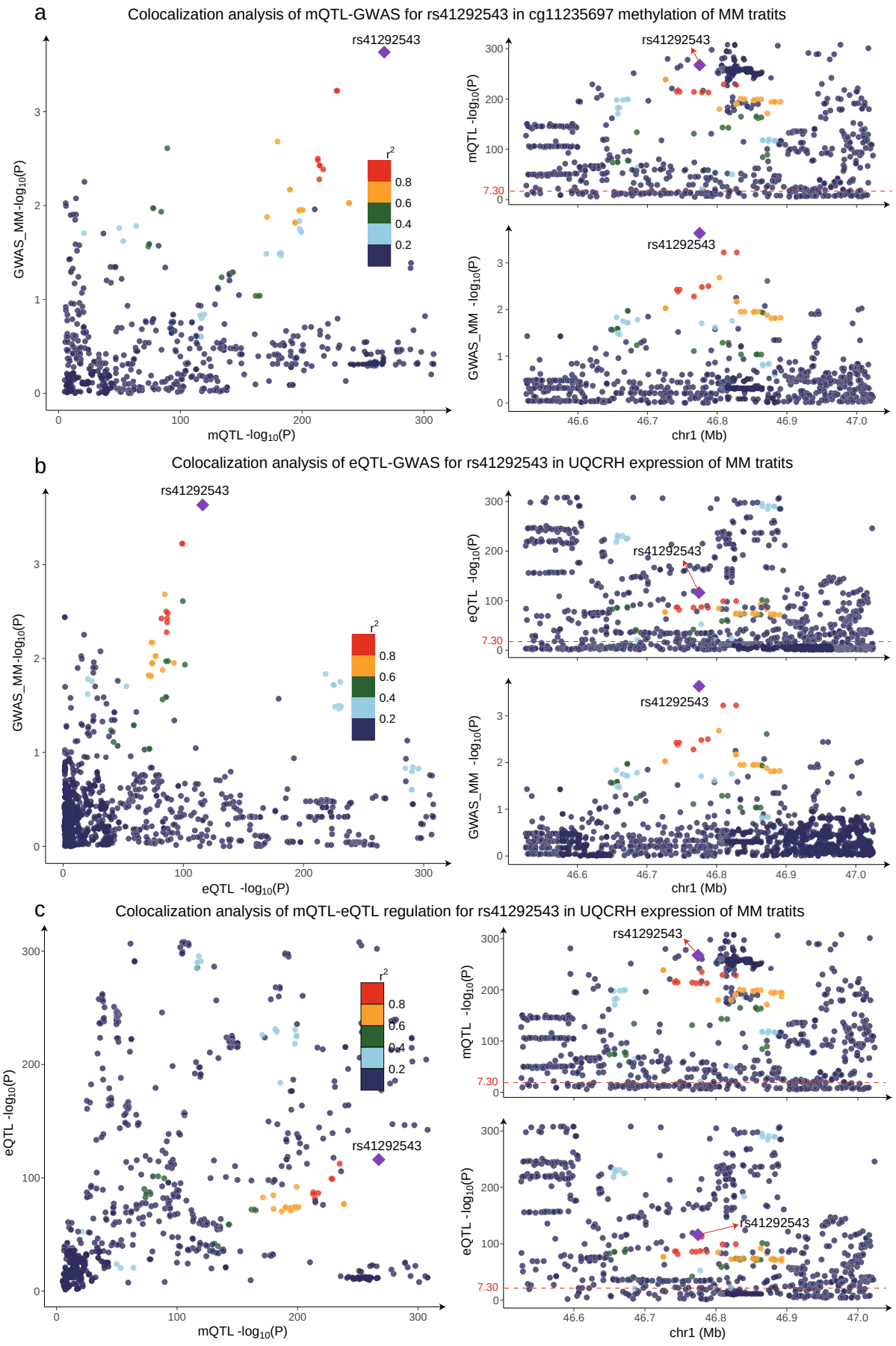


FIGURE 5 | Legend on next page.

FIGURE 5 | The colocalization results of shared SNPs between mQTLs and eQTLs. Colocalization analysis shows significant positive correlation between GWAS and mQTLs (a) and eQTLs (b) in rs41292543 for MM traits. Colocalization analysis indicates significant mQTL-eQTL regulation for rs41292543 in UQCRH expression of MM traits (c).

revealed that gene expression and methylation of *TOP1MT* exhibited a causal effect on MM while exerting a protective effect on pSS. Additionally, the deficiency of *TOP1MT* might cause the release of mtDNA into cytoplasm and further activate the cGAS-STING innate immune signaling pathway, thereby participating in the pathogenesis of SLE and other AIDs [33, 34]. Through performing cellular function experiments, Khatib et al. [33] further identified a specific variant in *TOP1MT* (called P193L) with the ability to rescue several functions of *TOP1MT* in SLE. These functions included repletion of mtDNA replication and transcription, as well as maintenance of mitochondrial morphology, suggesting that *TOP1MT* might serve as a potential drug target for SLE.

As a transcriptional co-activator, Staphylococcal Nuclease and Tudor Domain Containing 1 (*SND1*) has been reported to interact with multiple transcriptional factors and regulate pre-mRNA splicing [35]. Elevated levels of *SND1* have been observed in various tumors, including breast cancer, intestinal cancer, and hepatocellular carcinomas, suggesting its stimulative role in carcinogenesis [36, 37]. However, in infectious diseases, *SND1* exhibited a significant capability of host defense against intracellular infections by promoting helper T cell 1/17 (Th1/17) immunity and inhibiting the expansion of regulatory T (Treg) cells [38]. In our study, the rs12706818 variant related to *SND1* expression and specific CpG sites (cg27269962 and cg23439280) associated with *SND1* methylation were linked to increased risks of OA and SLE traits. However, methylation-related sites (cg00893242 and cg13969327) of *SND1* exhibited associated protective effects on OA. These findings imply that the role of *SND1* is complex and multifaceted across diverse diseases.

Valyl-tRNA synthetase 2 (*VARS2*) plays an essential role in mitochondrial protein synthesis, and mutations in this gene have been implicated in multiple genetic diseases, including cardiomyopathy [39], epilepsy [40], and increased risks of breast and lung cancer [41, 42]. Interestingly, another SMR analysis revealed that *VARS2* methylation exhibited significant protective effects on lung cancer, with various CpG sites implicated. Here, our results showed that the cg02306936 of *VARS2* methylation had a causal effect on PMR, while the rs2523594 variant of *VARS2* expression was linked to decreased risks for pSS. The potential causal effects of *VARS2* in different AIDs need to be further explored by experimental studies.

Unlike other pivotal genes related to mitochondrial dysfunction, ubiquinol cytochrome c reductase hinge (*UQCRH*) is the exclusive gene with the same SNP (rs41292543) displaying inverse effects on MM, including the causal effects via cg11235697 methylation and protective effects via *UQCRH* expression. Notably, the production of ROS and mitochondrial damage induced by IFN-β has been reported to contribute to the impairment of muscles and activation of inflammatory cytokines in dermatomyositis [43]. As an important protein located in the mitochondrial membrane, *UQCRH* induces the production of intracellular ROS, thus exhibiting its potential ability to trigger autoimmune responses for AIDs [44]. Additionally, mutations in UQCRH can cause mitochondrial complex III deficiency, contributing to neurodegenerative disorders such as psychomotor retardation and encephalopathy [45]. These findings highlight UQCRH as a potential therapeutic target for the treatment of inflammatory diseases. Our SMR results indicated that the methylation at cg11235697 might inhibit the expression of *UQCRH*, thereby upregulating the risks of MM. Further functional research needs to be conducted to interpret and validate the role of *UQCRH* in MM.

One advantage of this study is that we performed a comprehensive SMR analysis and integrated results from multi-omics evidence to evaluate causal effects of mitochondrial dysfunction on multiple AIDs. This approach incorporated data on DNA methylation, gene expression, and protein abundance levels of all known mitochondrial-related genes. The SMR design effectively minimized biases stemming from potential confounders and reverse causality, thereby strengthening the robustness of causal effects. Furthermore, the colocalization analysis further eliminated potential biases induced by LD. Secondly, this study included large-scale GWAS summary statistics of various QTLs, mtDNA copy number, and outcomes for 10 diverse AIDs, which not only increased the statistical power but also provided us with a sufficient understanding of causal relationships for several AIDs. Thirdly, the consistency of our results rigorously assessed by a series of validated analyses, including sensitivity analysis, colocalization analysis, PheWAS analysis, and bi-directional MR analysis, supports the robustness of these findings. Finally, only individuals of European descent were enrolled in this study, thereby minimizing the potential ethnic biases and enhancing the generalizability of our results within this population.

However, several limitations need to be acknowledged in this study. Firstly, although we integrated SMR results from multi-omics QTLs evidence, no genetic variant of cis-pQTL passed the threshold of colocalization correction, which might be associated with a limited number of proteins related to mitochondrial dysfunction in pQTL datasets. Additionally, we must acknowledge that all individuals of single European ancestry restrict the reproducibility and trans-ethnic generalizability of the findings, and these findings are promisingly to be further assessed and validated in other races. Moreover, genetic variants from various QTLs datasets mainly located on the mitochondrial-related nuclear genome, lacking information from the sex chromosome and mitochondrial genome because the corresponding GWAS datasets have not yet been developed. Hence, further studies are needed to explore potential causal relationships between the mitochondrial genome itself and the risks of AIDs. Additionally, unlike the total effect estimated by standard univariable MR, multivariable MR analysis offers a significant advantage in evaluating the direct causal effect of a risk exposure on the outcome [46]. However, in our study, the genetic exposure related to mitochondrial dysfunction was retrieved from QTLs datasets, thereby unable to perform the multivariable MR analysis.

Finally, this study has certain limitations, including the absence of experimental functional validation, potential pleiotropic effects, and limited exploration of the underlying biological mechanisms. These constraints warrant further investigation, which will be systematically addressed in future studies.

5 | Conclusions

In conclusion, this study utilized SMR to evaluate the potential causal effects of DNA methylation, gene expression, and protein abundance related to mitochondrial dysfunction on multiple AIDs, demonstrating the significance of mitochondrial dysfunction in the genetic predisposition to several AIDs. These findings offer promising insights into the underlying mitochondrial mechanisms involved in AIDs and provide valuable clues of potentially pharmacological targets for the treatment of AIDs.

Author Contributions

Jing Luo: conceptualization, data curation, formal analysis, visualization, and writing – review and editing. **Jingjing Pan:** formal analysis, methodology, visualization, and writing – original draft preparation. **Guanqun Yao:** data curation, methodology, software, and visualization. **Zenghao Xu:** investigation and methodology. **Shan Jiang:** data curation and investigation. **Jian Peng:** validation. **Xiaopeng Shang:** investigation, visualization, validation, and writing – review and editing. **Huaxiang Wu:** conceptualization, validation, and writing – review and editing. **Xin Liao:** conceptualization, resources, supervision, and writing – review and editing.

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Ethics Statement

All GWAS summary statistics used in this study were derived from previously published studies, which received approval from corresponding ethical committees and obtained the informed consent forms from all participants.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Datasets used in our study are available from the corresponding studies and summarized in Table S1. The GWAS summary statistics are publicly downloaded according to Supporting Information S1. The statistical codes are available upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** apl70383-sup-0001-DataS1.docx.

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ORIGINAL ARTICLE

Ubiquitin Ligase MDM2 Regulates Chondrocyte Damage, Ferroptosis, and Oxidative Stress by Modulating the Ubiquitination Level of CDKN1A

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Keywords: CDKN1A | ferroptosis | MDM2 | OA | ubiquitination

ABSTRACT

Background: Osteoarthritis (OA) is a common degenerative disease involving pathological changes in joint tissues, which seriously affects the quality of life of patients. It was reported that both Cyclin dependent kinase inhibitor 1A (CDKN1A) and ubiquitinating enzyme MDM2 exhibited abnormal expression in OA. However, it is currently unclear whether there is a specific regulatory mechanism between the two.

Methods: Firstly, C28/I2 cells were treated with IL-1β to construct an in vitro cell model of OA, while qRT-PCR and western blot were used to detect the mRNA and protein levels of CDKN1A and MDM2. C28/I2 cell viability, apoptosis, and the release of inflammatory factors were measured by CCK-8, flow cytometry, and ELISA kits. In addition, the levels of Fe^{2+}, glutathione (GSH), reactive oxygen species (ROS), and Malondialdehyde (MDA) were evaluated by corresponding kits. Subsequently, the relationship between MDM2 and CDKN1A was predicted by the UbiBrowser website, and the ubiquitination level of CDKN1A and the interaction between them were verified by western blot and Co-IP technology. Cycloheximide (CHX) exposure was used to assess mRNA stability.

Results: CDKN1A was downregulated in OA cartilage tissues and IL-1β induced C28/I2 cells, while overexpression of CDKN1A enhanced C28/I2 cell activity and inhibited cell apoptosis. Meanwhile, the release of IL-6 and TNF-α as well as ferroptosis and oxidative stress, were also hindered by CDKN1A overexpression. MDM2 was highly expressed in OA patients and in IL-1β induced C28/I2 cells and mediated the ubiquitination modification of CDKN1A. Most importantly, MDM2 knockdown alleviated IL-1β-induced chondrocyte damage via upregulating CDKN1A.

Conclusion: MDM2 downregulated the level of CDKN1A in chondrocytes by mediating the ubiquitination modification of CDKN1A, leading to impaired chondrocyte viability and inducing ferroptosis and oxidative stress.

1 | Introduction

Osteoarthritis (OA) is a chronic joint disorder characterized by the degeneration and damage of articular cartilage as its central pathology, extending to affect bone, synovial membrane, joint capsule, and other joint structures [1, 2]. It ranks among the primary causes of disability globally, particularly in the elderly population, and significantly undermines the quality of life for those affected [3, 4]. The pathogenesis of OA is multifaceted and encompasses a multitude of contributing factors.

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Chondrocyte injury constitutes a pivotal aspect of the disease process. Under normal physiological conditions, chondrocytes play an indispensable role in preserving the structural integrity and functional capacity of articular cartilage [5, 6]. However, in the course of OA development, the metabolic equilibrium of chondrocytes is disrupted, resulting in an imbalance between the synthesis and degradation of extracellular matrix components, which ultimately leads to the progressive attrition and deterioration of cartilage tissue [7]. In addition, the inflammatory response contributes significantly to the progression of OA [8]. The liberation of inflammatory mediators further exacerbates chondrocyte damage, thereby perpetuating a deleterious cycle of cartilage degradation [9, 10].

Cyclin dependent kinase inhibitor 1A (CDKN1A) stands as a pivotal cell cycle regulatory protein. The protein encoded by the CDKN1A gene possesses the capacity to bind to cyclin dependent kinase complexes, effectively inhibiting their enzymatic activity [11]. This regulatory mechanism modulates cell cycle progression during the G1 phase, acting as a critical checkpoint to prevent cells from transitioning into the S phase [12]. In recent years, significant strides have been made in understanding the role of CDKN1A in OA. Specifically, research has unveiled that CDKN1A is aberrantly downregulated in the cartilage tissues of OA patients. Subsequent investigations have demonstrated that inhibiting CDKN1A expression markedly impedes the differentiation and proliferation of OA chondrocytes. Concurrently, CDKN1A activated the AKT signaling pathway, prompting MAPK8 to participate in the inactivation of MAPK signaling, consequently mitigating chondrocyte apoptosis effectively [13, 14]. As a distinct mode of programmed cell death, ferroptosis is marked by the iron-dependent accumulation of lipid peroxides. This process is characterized by substantial glutathione (GSH) depletion, lipid metabolism disorders induced by excess ferrous ions, heightened reactive oxygen species (ROS) generation, and cell death mediated by excessive oxidation of polyunsaturated fatty acids (PUFA) [15, 16]. Through bioinformatics analysis, recent investigations have uncovered a notable link between the CDKN1A gene and ferroptosis regulation in the pathogenesis of OA [17, 18]. Nevertheless, the precise molecular mechanism by which CDKN1A governs ferroptosis in chondrocytes remains to be systematically elucidated.

Ubiquitination, a fundamental protein post-translational modification mechanism, entails the intricate process whereby ubiquitin molecules, under the orchestration of specialized enzyme systems, precisely categorize intracellular proteins, identify target proteins, and execute specific modifications. The central hallmark of the ubiquitination process lies in a meticulously coordinated cascade involving three pivotal enzymes [19, 20]. Firstly, the E1 ubiquitin-activating enzyme plays a crucial role by activating ubiquitin molecules predominantly through an ATP-dependent mechanism, leading to the formation of high-energy thioester bond intermediates [21]. Secondly, the E2 ubiquitin-conjugating enzyme binds to the ubiquitin-E2 thioester complex, which is initially formed by the E1 enzyme, and subsequently transfers ubiquitin molecules to their conserved cysteine residues [22]. Lastly, E3 ubiquitin ligases execute the final step by specifically recognizing substrate proteins and binding ubiquitin molecules, which are carried by E2 enzymes, to specific lysine residues of the substrates, thereby forming isopeptide bonds [23, 24]. This highly specific enzymatic cascade empowers cells to precisely modulate the stability, activity, and subcellular localization of proteins, ultimately safeguarding the homeostasis of the intracellular environment. Ubiquitination is related to cancer [25]. As a pivotal E3 ubiquitin ligase, MDM2 holds a critical position in the cellular protein regulatory machinery. Prior investigations have demonstrated a marked elevation in MDM2 expression levels within the cartilage tissue of OA-afflicted rats when juxtaposed with healthy counterparts and chondrocytes. Notably, curbing MDM2 overexpression has been corroborated to elicit a pronounced anti-inflammatory response, thereby positioning MDM2 as a promising therapeutic target for OA intervention [26].

Based on the aforementioned research context, we put forth the following hypothesis: MDM2 may orchestrate the ferroptotic process in chondrocytes by mediating the ubiquitination of CDKN1A and subsequently triggering its proteasomal degradation pathway, thereby expediting the pathological progression of OA. To substantiate this scientific hypothesis, our study constructed an in vitro OA chondrocyte model to systematically dissect the regulatory mechanism underlying the MDM2/CDKN1A signaling axis in the pathogenesis and development of OA.

2 | Materials and Methods

2.1 | Collection of Samples

A total of 22 pairs of human knee joint cartilage samples were adopted in this study, all of which were collected from patients who underwent total knee arthroplasty due to OA. Specifically, the lesioned areas of the cartilage were defined as OA samples, while the corresponding non-lesioned areas served as healthy control samples. The collection of all samples strictly adhered to ethical guidelines and received formal approval from the Huanggang Central Hospital Ethics Committee, with written informed consent obtained from each patient.

2.2 | Cell Culture and Treatment

Using C28/I2 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, China) as a research tool, they were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, USA) containing 10% fetal bovine serum (Genetimes, Shanghai, China) and penicillin/streptomycin (50 μg/mL) (Solarbio, Beijing, China) in an incubator at 37°C with 5% CO_2. C28/I2 cells were seeded. Once the confluency reached 50%, 10 ng/mL of IL-1β was added to stimulate the C28/I2 cells, simulating the inflammatory environment of OA chondrocytes [27].

2.3 | Cell Transfection

To achieve overexpression of CDKN1A in C28/I2 cells, the sequence of CDKN1A was cloned into the pcDNA3.1 vector (Invitrogen), using an empty pcDNA3.1 plasmid as a control,

to construct the pcDNA3.1-CDKN1A overexpression vector (oeCDKN1A). For loss-of-function treatment of MDM2 and CDKN1A, shRNAs targeting MDM2 (shMDM2) and CDKN1A (shCDKN1A) were designed and synthesized by GenePharma (Shanghai, China). The above overexpression vector and shRNAs were transfected into C28/I2 cells with Lipofectamine 2000 (Invitrogen) to achieve specific gene editing.

2.4 | Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Initially, total cellular RNA was extracted using the TriQuick Reagent (Solarbio) and subsequently reverse-transcribed into cDNA. Following this, the cDNA was amplified utilizing SYBR Premix Ex Taq II (TaKaRa, Dalian, China) under the direction of specific primers. Ultimately, the mRNA expression level was quantified via the $2^{-\Delta\Delta Ct}$ method, based on the calculated Ct values. The primer sequences are shown in Table 1.

2.5 | Western Blot Assay

C28/I2 cells were lysed to extract total proteins. The protein concentration was determined using the BCA Protein Assay Kit (Beyotime, Shanghai, China), and 30 μg of total proteins were loaded for SDS-PAGE electrophoresis, followed by transfer onto a PVDF membrane (Millipore, Billerica, MA, USA). After blocking with 5% skim milk (Solarbio), the PVDF membrane was incubated with the Anti-p21 (CDKN1A) antibody (1:1000, ab109520, Abcam, Cambridge, UK), Anti-MDM2 antibody (1:1000, ab259265, Abcam), Anti-p53 antibody (1:1000, ab26, Abcam), Anti-xCT/SLC7A11 antibody (1:1000, ab307601, Abcam), Anti-Glutathione Peroxidase 4 antibody (1:1000, ab125066, Abcam), and Anti-GAPDH antibody (1:10000, ab181602, Abcam) overnight at 4°C. Then, the PVDF membrane was incubated with Goat Anti-Rabbit IgG H&L (HRP) (1:5000, ab6721, Abcam) and immersed at room temperature for 2 h. Finally, the proteins were visualized using the BeyoECL Plus Kit (Beyotime).

2.6 | Cell Counting Kit 8 (CCK-8)

C28/I2 cells were evenly seeded into a 96-well plate after corresponding treatments, and 10 μL/well of CCK-8 reagent (Yeasen, Shanghai, China) was added 24 h later. After a 3-h reaction at 37°C, the optical density value at 450 nm was measured using a microplate reader to analyze cell viability.

TABLE 1 | Primer sequences for RT-qPCR.

Name		Primers for PCR (5′ −3′)
CDKN1A	Forward	AGTCAGTTCCTTGTGGAGCC
	Reverse	CATTAGCGCATCACAGTCGC
MDM2	Forward	GGTGCTGTAACCACCTCACA
	Reverse	TGGCACGCCAAACAAATCTC
GAPDH	Forward	AATGGGCAGCCGTTAGGAAA
	Reverse	GCGCCCAATACGACCAAATC

2.7 | Detection of Cell Apoptosis

Cell apoptosis was evaluated by the Annexin V-FITC/PIApoptosis Detection Kit (Yeasen). The treated C28/I2 cells were collected and re-suspended in 100 μL of 1× binding buffer, and they were stained using 5 μL Annexin V-FITC and 10 μL propidium iodide and reacted in the dark for 15 min at room temperature. 400 μL of 1× Binding Buffer was added, and apoptotic cells were counted with the aid of flow cytometry after mixing.

2.8 | Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of IL-6 and TNF-α were monitored with the help of the Human IL-6 ELISA Kit (Yeasen) and the Human TNF-α ELISA Kit (Yeasen); the procedure was performed in strict accordance with the instructions for the use of the kit.

2.9 | Determination of Ferroptosis Indicators

When cells experience ferroptosis, they typically exhibit a series of distinctive alterations, encompassing a marked elevation in ferrous ion (Fe^{2+}), lipid peroxide, and ROS levels, accompanied by a corresponding reduction in GSH levels [15, 28]. Employing specialized kits to quantify the levels of these markers enables a more precise assessment of the progression of ferroptosis within C28/I2 cells. In this study, Fe^{2+} and GSH levels were measured using the Ferrous Ion Content Detection Kit (Solarbio) and Reduced Glutathione Content Determination Kit (Beyotime). ROS levels were determined by the Reactive Oxygen Species Detection Kit (Yeasen) using DCFH-DA probe. Furthermore, the Lipid Oxidation [29] Assay Kit (Beyotime) was employed to measure the concentration of malondialdehyde [29].

2.10 | Bioinformatics Analysis

The UbiBrowser platform (http://ubibrowser.bio-it.cn/ubibrowser/home/index) was employed to forecast the binding interaction between CDKN1A and MDM2.

2.11 | Determination of Ubiquitination Levels

Immunoprecipitation (IP) [30] and immunoblotting (IB) techniques were employed to evaluate the specific effects of MDM2 knockdown on the ubiquitination level of CDKN1A. The chondrocytes that had been transfected with sh-NC/sh-MDM2 or treated with MG132 (Beyotime) were respectively digested and lysed. The CDKN1A antibody (ab102013, Abcam) was incubated with the lysate overnight at 4°C. Subsequently, pre-rinsed Protein A/G magnetic beads (Beyotime) were introduced to the antibody-protein complex to enhance the process of antibody adsorption. Following the elution of the conjugates from the

beads, Anti-HA tag antibody (ab236632, Abcam), CDKN1A antibody, and MDM2 antibody (ab259265, Abcam) were employed for western blot analysis to ascertain the extent of ubiquitination and protein expression.

2.12 | GST Pull-Down

MDM2 gene or CDKN1A gene was cloned into GST fusion expression vector (Beyotime) and transformed into *E. coli*. *E. coli* cells expressing GST-MDM2 or GST-CDKN1A fusion proteins were collected and sonicated to obtain GST-MDM2/CDKN1A fusion proteins. The GST-MDM2/CDKN1A fusion protein was bound to a GST affinity resin. Cell lysates containing CDKN1A/MDM2 proteins were then added to the reaction system containing GST-MDM2/CDKN1A-resin complex for incubation. The protein complexes bound to the resin were subsequently eluted using an eluent. Western blot was performed with antibodies specific for CDKN1A and MDM2. Partial cell lysates were used as output samples, GST fusion vector was used as negative control, and GST-CDKN1A and GST-MDM2 were used as experimental groups. If a band of MDM2 or CDKN1A protein was observed, it indicated that CDKN1A protein was able to be pulled down by MDM2, and MDM2 protein was able to be pulled down by CDKN1A, confirming that there was an interaction between the two.

2.13 | Co-Immunoprecipitation (Co-IP)

A Co-IP assay was employed to validate the interaction between MDM2 and CDKN1A. C28/I2 cells were harvested and lysed, followed by the removal of cell debris via centrifugation. A fraction of the collected supernatant was set aside as an input sample, while the remaining supernatant was subjected to overnight incubation with IgG (ab172730, Abcam) or MDM2 antibodies (ab259265, Abcam) at 4°C. Then, protein A/G agarose beads (Beyotime) were added to the supernatant and incubated for 2 h at 4°C, enabling efficient capture of the protein complexes. Following this, the eluted proteins were subjected to analysis via western blot to assess protein expression, thereby verifying protein interactions.

2.14 | Protein Stability Assay

Cycloheximide (CHX) is a unique inhibitor of protein synthesis that functions by binding to the E-site of the 60S ribosomal subunit, thereby inhibiting translation elongation [31]. This property allows CHX to be employed as a tool for monitoring alterations in protein degradation over time. C28/I2 cells, transfected with either shNC or shMDM2, were subjected to CHX treatment and subsequently harvested at 0, 30, 60, and 90 min post-treatment for western blot analysis.

2.15 | Statistical Analysis

Statistical analysis of the data was performed using GraphPad Prism 8.0.1 software. The measurement data followed a normal distribution and were expressed as mean ± standard deviation. Student's *t*-test was used for pairwise comparisons between groups; one-way or two-way ANOVA was applied for

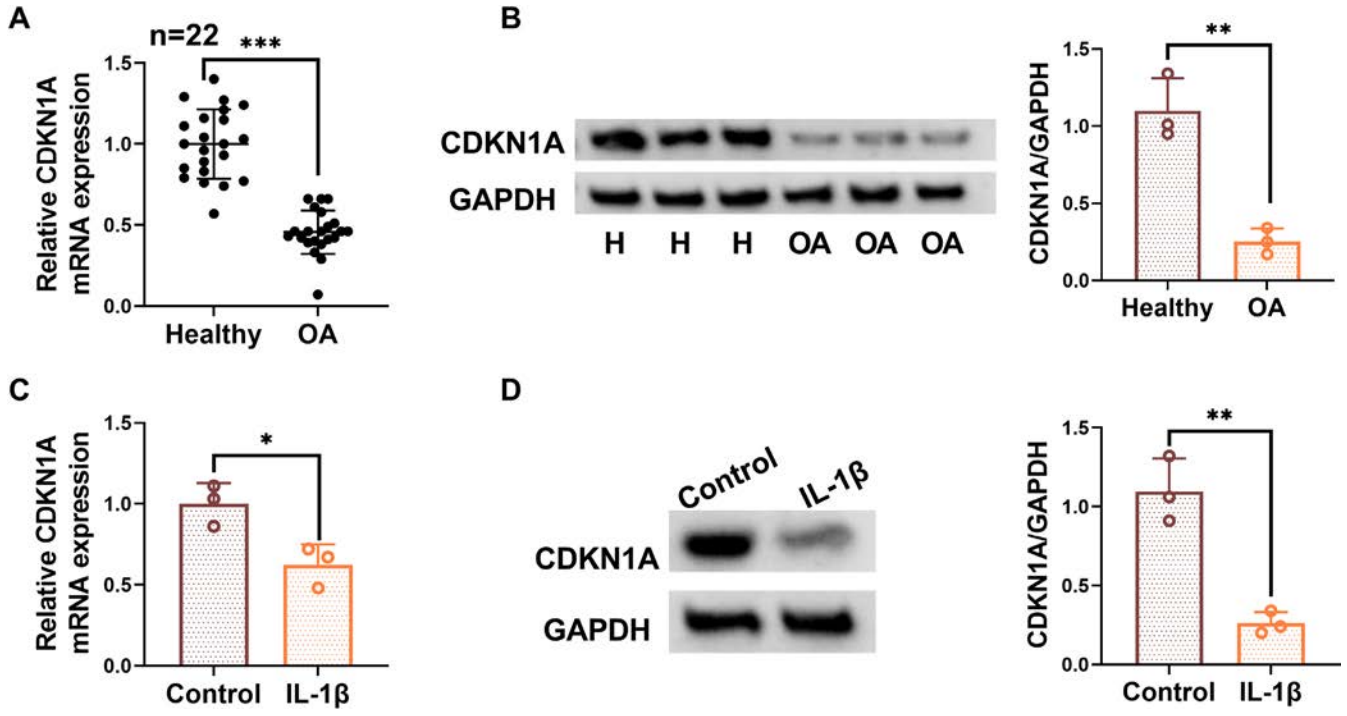

FIGURE 1 | Expression levels of CDKN1A in cartilage tissue and IL-1β-induced C28/I2 cells. (A) qRT-PCR was used to detect the mRNA levels of CDKN1A in healthy ($n = 22$) and diseased cartilage tissues ($n = 22$) of OA patients. (B) Western blot determination of CDKN1A protein levels in healthy ($n = 3$) and diseased cartilage tissues ($n = 3$) of OA patients. (C, D) C28/I2 cells were divided into the Control group and the IL-1β group after treatment with IL-1β. CDKN1A mRNA and protein levels in C28/I2 cells were monitored by qRT-PCR and western blot experiments. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

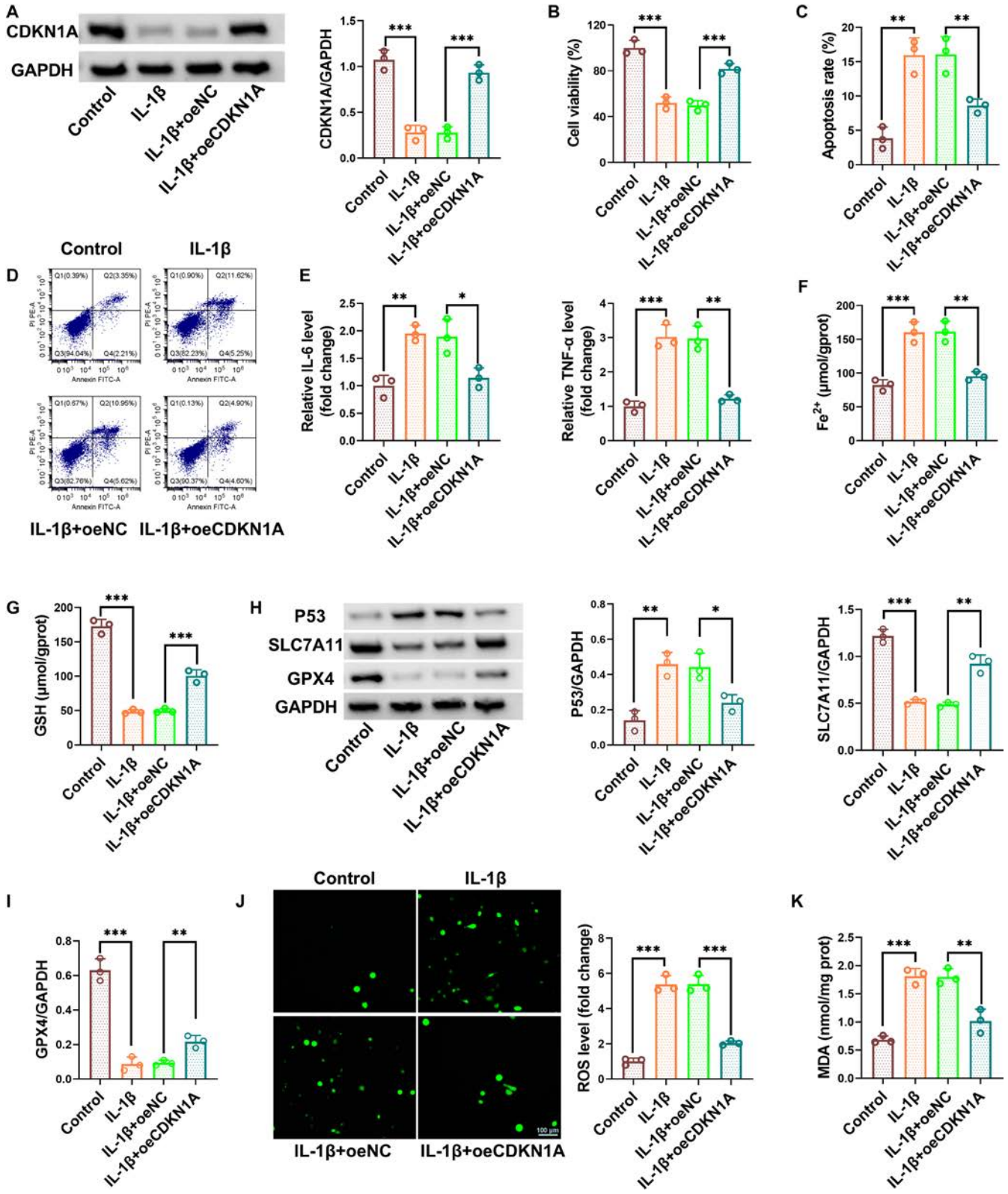

FIGURE 2 | Effect of overexpression of CDKN1A on IL-1β-exposed C28/I2 cells. (A–K) C28/I2 cells were divided into the Control group, the IL-1β group, the IL-1β + oeNC group, and the IL-1β + oeCDKN1A group. (A) Western blot analysis of CDKN1A protein levels in C28/I2 cells. (B) The viability of C28/I2 cells was detected by the CCK-8 kit. (C–D) The apoptosis of C28/I2 cells was analyzed by flow cytometry. (E) The corresponding ELISA kits were performed to measure the levels of IL-6 and TNF-α. (F, G) Fe^{2+} and GSH levels in C28/I2 cells were examined by ferrous colorimetric assay kit and reduced glutathione colorimetric assay kit. (H, I) Determination of protein levels of p53, SLC7A11, and GPX4 by western blot. (J) DCFH-DA probe was utilized to detect the level of ROS. (K) The level of MDA in C28/I2 cells was detected by MDA assay kit. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

comparisons among multiple groups. A p value <0.05 was considered statistically significant.

3 | Results

3.1 | CDKN1A was Lowly Expressed in OA Patients and IL-1β Induced C28/I2 Cells

By performing qRT-PCR and western blot on tissues collected from healthy and diseased areas of cartilage from OA patients, CDKN1A expression was found to be significantly lower in the OA group compared with the healthy group (Figure 1A,B). Similarly, both mRNA and protein levels of CDKN1A were also remarkably lower in IL-1β-treated C28/I2 cells than in the Control group (Figure 1C,D). In other words, CDKN1A was consistently maintained at a low expression level in OA.

3.2 | Overexpression of CDKN1A Enhanced C28/I2 Cell Viability and Suppressed Apoptosis, Inflammation, Ferroptosis and Oxidative Stress

To explore the role of CDKN1A in OA, CDKN1A was overexpressed. In Figure 2A, it could be seen that when IL-1β-exposed C28/I2 cells were transfected with oeCDKN1A, the protein level of CDKN1A was obviously upregulated in comparison with the IL-1β group and the IL-1β + oeNC group. CCK-8 assay showed that when C28/I2 cells were exposed to IL-1β, the cell viability was evidently decreased, while when CDKN1A was overexpressed, the cell viability was restored (Figure 2B). In the assay of apoptosis, it was demonstrated that IL-1β could promote the apoptosis of C28/I2 cells, while overexpression of CDKN1A reduced the apoptosis rate of C28/I2 cells (Figure 2C,D). Subsequently, ELISA results displayed that IL-1β treatment stimulated the release of IL-6 and TNF-α in C28/I2 cells. On the contrary, when the expression of

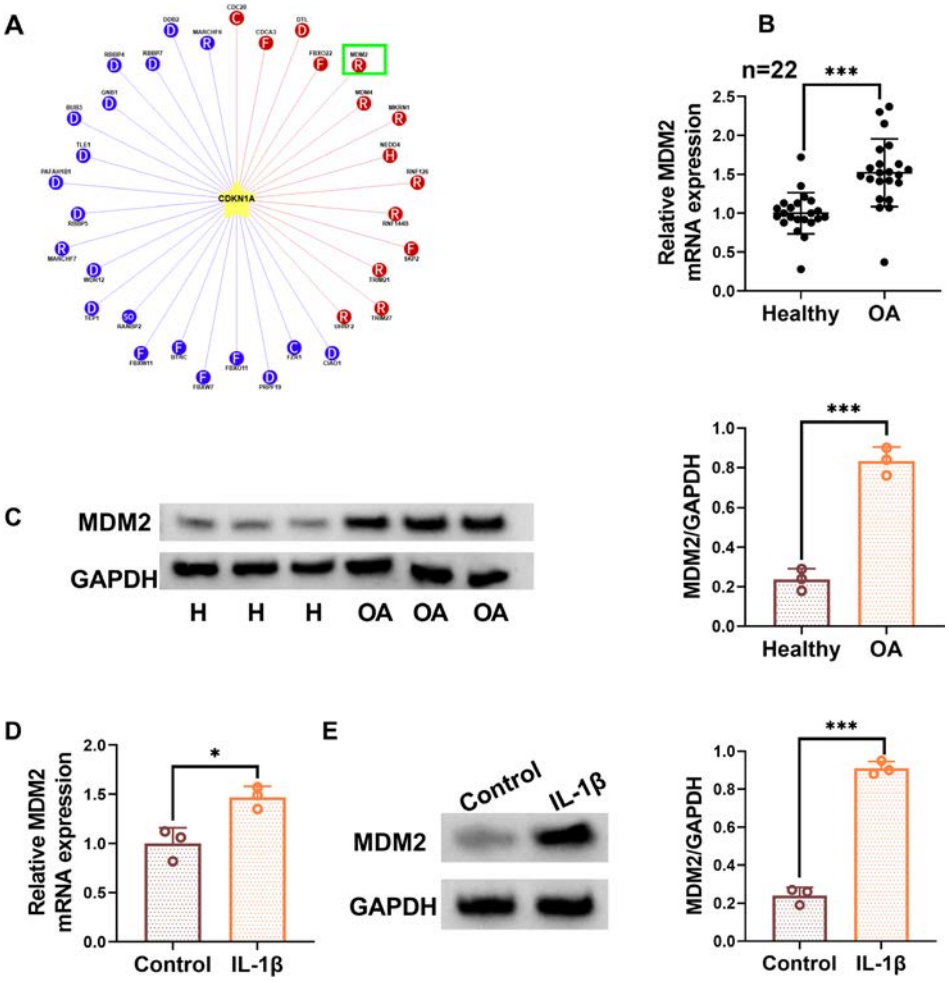

FIGURE 3 | Expression levels of MDM2 in cartilage tissue and IL-1β induced C28/I2 cells. (A) Prediction of CDKN1A binding to MDM2 by the UbiBrowser website. The letters in the circle all belong to the E3 family classification. H represents the Homologous to E6AP Carboxy Terminus (HECT) family, R represents the RING family, C represents the family related to CDC20, F represents the F-box family, D represents the DDB1-binding WD-repeat domain (DWD) family, and SO represents SINGLE other. (B) qRT-PCR was used to detect the mRNA levels of MDM2 in healthy ($n=22$) and diseased cartilage tissues ($n=22$) of OA patients. (C) Western blot determination of MDM2 protein levels in healthy ($n=3$) and diseased cartilage tissues ($n=3$) of OA patients. (D, E) C28/I2 cells were divided into the Control group and the IL-1β group after treatment with IL-1β. MDM2 mRNA and protein levels in C28/I2 cells were analyzed by qRT-PCR and western blot experiments. *$p<0.05$; ***$p<0.001$.

CDKN1A was upregulated, the levels of IL-6 and TNF-α were greatly decreased (Figure 2E). More importantly, IL-1β increased the level of Fe^{2+} but decreased the level of GSH in C28/I2 cells, and the levels of both were reversed when CDKN1A was overexpressed (Figure 2F,G). Interestingly, it was also found that IL-1β could increase the protein level of p53 and inhibit the expression of SLC7A11 and GPX4. However, the presence of oeCDKN1A nullified the effect of IL-1β on the expression of p53, SLC7A11, and GPX4 (Figure 2H,I). Furthermore, monitoring of ROS and MDA levels revealed that following IL-1β induction, C28/I2 cells exhibited a significant increase in ROS and MDA levels. However, when cells were transfected with oeCDKN1A, the levels of ROS and MDA were retarded (Figure 2J,K). In general, the effects of IL-1β on C28/I2 cell viability, apoptosis, inflammation, ferroptosis, and oxidative stress could be rescued by overexpression of CDKN1A.

3.3 | MDM2 was Highly Expressed in OA Patients and IL-1β Induced C28/I2 Cells

Interestingly, the UbiBrowser website showed that CDKN1A could bind to MDM2, and the predicted results were shown in Figure 3A. Then, qRT-PCR and western blot results indicated that MDM2 was expressed at high levels both in the OA group and the IL-1β group (Figure 3B–E). This suggested that MDM2 was upregulated in OA and might interact with CDKN1A.

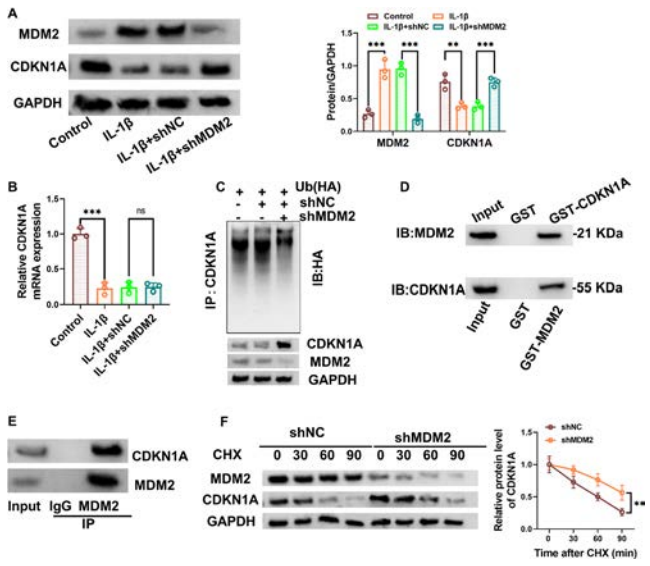

FIGURE 4 | Regulation of CDKN1A by MDM2. (A, B) C28/I2 cells were divided into the Control group, the IL-1β group, the IL-1β + shNC group, and the IL-1β + shMDM2 group after transfection with shNC and shMDM2. Western blot and qRT-PCR were used to examine MDM2 and CDKN1A protein and mRNA levels after the knockdown of MDM2. (C) Western blot was employed to detect the protein levels of MDM2 and CDKN1A, and the ubiquitination level of CDKN1A. (D) The binding of MDM2 and CDKN1A was verified by GST pull-down assay. (E) The binding of MDM2 and CDKN1A was verified by Co-IP assay. (F) The protein levels of MDM2 and CDKN1A were detected by western blot at 0, 30, 60, and 90 min after CHX treatment in C28/I2 cells. $^{**}p < 0.01$, $^{***}p < 0.001$.

3.4 | MDM2 Mediated Ubiquitination of CDKN1A and Reduced Its mRNA Stability

MDM2 was inactivated to explore its regulation on CDKN1A and its role in OA. Western blot results elucidated that when IL-1β induced C28/I2 cells were transfected with shMDM2, the protein expression of MDM2 was effectively blocked, while the protein level of CDKN1A showed an upward trend (Figure 4A). It should be noted that knocking down MDM2 had no significant effect on the mRNA level of CDKN1A after the qRT-PCR assay was carried out (Figure 4B). Based on this, the ubiquitination status of CDKN1A was analyzed by western blot, with the results shown in Figure 4C. When MDM2 was silenced, a significant reduction in the ubiquitination modification of CDKN1A was observed, accompanied by an upregulation of CDKN1A protein expression levels. At this point, if treated with MG132, the content of the ubiquitinated form of CDKN1A did not change (Figure S1). The GST pull-down experiment was carried out to confirm that CDKN1A can directly bind to MDM2 (Figure 4D). At the same time, the Co-IP assay further confirmed that MDM2 could bind to CDKN1A (Figure 4E). After that, C28/I2 cells were treated with CHX, and the mRNA levels of MDM2 and CDKN1A were determined by qRT-PCR. For MDM2, the level of MDM2 mRNA was markedly lower in the shMDM2 group than in the shNC group, and the level of MDM2 mRNA became progressively lower with increasing CHX treatment time. Regarding CDKN1A, CDKN1A mRNA levels in both the shNC group and the shMDM2 group were downregulated with the increase of CHX treatment time, but the downward trend of CDKN1A protein level in the shMDM2 group was notably less than that in the shNC group, indicating that the stability of CDKN1A protein was enhanced when MDM2 was silenced (Figure 4F). Collectively, the knockdown of MDM2 affected the expression of CDKN1A protein. Specifically, MDM2 mediated the ubiquitination of CDKN1A, thereby reducing its protein stability.

3.5 | Knockdown of CDKN1A Abolished the Effect of MDM2 Silencing on IL-1β-Induced C28/I2 Cells

In order to further clarify the regulatory role of MDM2/CDKN1A on OA progression, a reversion experiment was performed in which CDKN1A was knocked down in addition to MDM2 silencing. WB analysis of CDKN1A protein showed that the upregulated CDKN1A protein level due to MDM2 silencing was downregulated again after shCDKN1A transfection (Figure 5A). Silencing of CDKN1A reversed the recovery of cell viability and apoptosis induced by MDM2 knockdown (Figure 5B–D). Simultaneously, when the expression of CDKN1A was confined, the originally decreased levels of IL-6 and TNF-α showed an upward trend (Figure 5E). Likewise, the impact of MDM2 knockdown on Fe^{2+}, GSH, p53, SLC7A11, GPX4, ROS, and MDA levels in IL-1β treated C28/I2 cells was entirely abrogated upon CDKN1A silencing (Figure 5F–K). In other words, MDM2 and CDKN1A collaboratively modulated chondrocyte damage in OA progression.

4 | Discussion

Although current treatment approaches for OA can alleviate patients' symptoms to some degree, they are not without

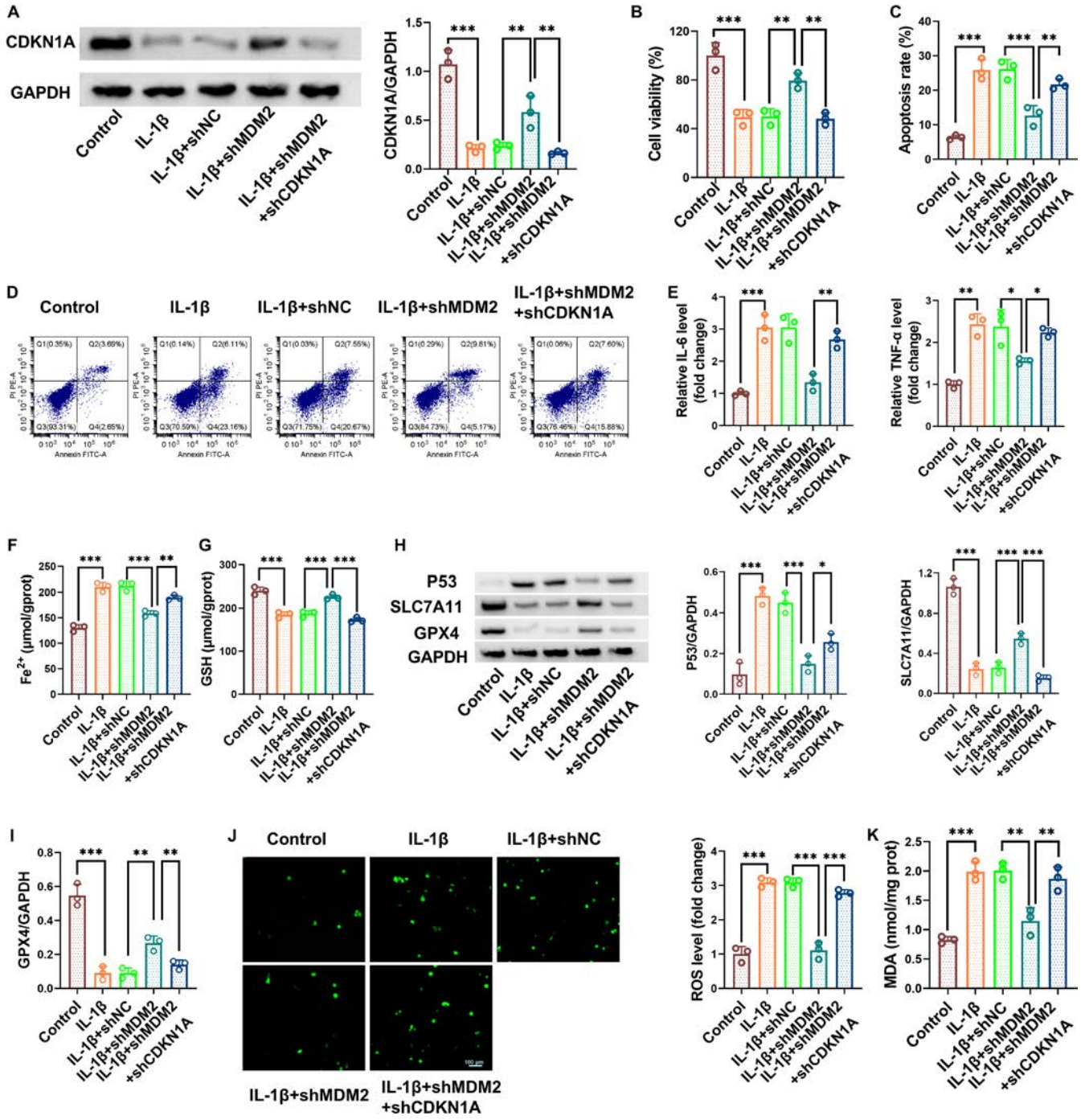

FIGURE 5 | Effects of silencing CDKN1A after knockdown of MDM2 on C28/I2 cells. (A–K) C28/I2 cells were divided into the Control group, the IL-1β group, the IL-1β + shNC group, the IL-1β + shMDM2 group, and the IL-1β + shMDM2 + shCDKN1A group. (A) Western blot analysis of CDKN1A protein levels in C28/I2 cells. (B) Assay of C28/I2 cell viability by CCK-8 kit. (C–D) Analysis of apoptosis in C28/I2 cells by flow cytometry. (E) The levels of IL-6 and TNF-α were determined by the corresponding ELISA kits. (F, G) Fe^{2+} and GSH levels were measured by the corresponding kits. (H, I) Determination of protein levels of p53, SLC7A11, and GPX4 by western blot. (J, K) ROS and MDA levels in C28/I2 cells were measured by the corresponding kits. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

significant limitations, particularly in reversing established pathological changes [30, 32]. Similarly, while surgical intervention may prove effective in specific cases, it carries considerable risks and is unsuitable for all patient demographics. More critically, given the intricate pathogenesis of OA, prevailing treatment strategies frequently employ a generalized "one-size-fits-all" model, which fails to provide tailored interventions based on individual pathophysiological traits [29]. Given this context, the exploration and identification of biomarkers for OA assume paramount significance. It not only facilitates precise diagnosis during the disease's nascent stages or even prior to symptom onset, enabling the implementation of timely intervention strategies, but also serves as a pivotal target or efficacy benchmark in the realm of novel drug discovery and development, ultimately enhancing the efficacy of targeted OA therapies.

According to existing studies, CDKN1A expression is relatively low in OA, and its expression characteristics are closely related to the infiltration of immune cells in OA cartilage [33, 34]. Consistent with the conclusions of previous studies, this study experimentally confirmed that CDKN1A exhibited significantly low expression characteristics in the cartilage lesion areas of OA patients and the in vitro constructed C28/I2 cell model. The pathogenesis of osteoarthritis is intricately linked to diverse cell death modalities, encompassing apoptosis, necrosis, pyroptosis, ferroptosis, and autophagy. Emerging evidence underscores the pivotal role of CDKN1A in modulating autophagy, wherein alterations in its expression profile may significantly influence chondrocyte autophagy, thereby dictating cellular viability and survival [35]. In the present investigation, the upregulation of CDKN1A expression was observed to revitalize the viability of OA chondrocytes and impede cell apoptosis. The inflammation associated with OA does not stem from infectious agents; instead, it is elicited by a cascade of events including damage, ongoing wear and tear, or the natural deterioration of articular cartilage. Upon cartilage shedding, the debris incites the synovial membrane, initiating a sterile inflammatory response. During this process, inflammatory mediators such as prostaglandins and interleukins are released, and their presence further exacerbates the extent of cartilage destruction [36, 37]. Accordingly, the concentrations of IL-6 and TNF-α were closely monitored, revealing that upon overexpression of CDKN1A, there was a marked decrease in the secretion of these inflammatory factors. It has also been confirmed that CDKN1A is associated with ferroptosis in OA [18]. The overexpression of CDKN1A led to a reduction in Fe^{2+} accumulation and p53 protein and an elevation in GSH, SLC7A11, and GPX4 levels. Concurrently, there was a notable suppression of ROS and MDA levels within the cells. These findings implicitly indicated that CDKN1A played a regulatory role in chondrocyte injury, while also promoting inflammation and ferroptosis in the context of OA.

In recent years, numerous studies have validated that ubiquitination exerts a pivotal regulatory influence across diverse pathological processes, particularly in the pathogenesis of degenerative diseases like OA [38, 39]. Liao et al. demonstrated that HECTD1 orchestrated the autophagic pathway in chondrocytes by mediating Rubicon ubiquitination and facilitating its proteasomal turnover, ultimately fostering the pathological progression of OA [40]. Another study has revealed that the SKP2-mediated ubiquitination modification of KLF11 and its subsequent proteasomal degradation process promote the pathological progression of osteoarthritis by regulating the JMJD3/NOTCH1 signaling axis [41]. By utilizing the bioinformatics analysis tool UbiBrowser platform, we systematically screened out potential binding proteins that have interaction relationships with CDKN1A. Further literature review revealed that MDM2 exhibited significantly high expression characteristics in OA rats and possessed biological functions that inhibited the proliferation of chondrocytes [26]. Indeed, in our results, it was confirmed that MDM2 expression was upregulated at the site of cartilage lesions in OA patients as well as in IL-1β-induced C28/I2 cells. Intriguingly, CDKN1A expression was reinstated following MDM2 silencing, which implied that there must be some regulatory relationship between MDM2 and CDKN1A. Experiments have validated that MDM2 can interact with CDKN1A, induce its ubiquitination modification, facilitate CDKN1A degradation via the proteasome pathway, and subsequently markedly decrease the protein expression level of CDKN1A within chondrocytes. Interestingly, although it has been confirmed that MDM2 can affect the degradation of the CDKN1A protein, we found that in the cartilage tissue of patients with osteoarthritis and in C28/I2 cells induced by IL-1β, the mRNA expression of CDKN1A also decreases. MDM2, as an E3 ubiquitin ligase, mainly inhibits the activity of p53 by binding to it and promoting its degradation. However, p53 is the transcriptional activator of CDKN1A (p21), and when the activity of MDM2 increases, the level of p53 decreases, which may lead to a reduction in the transcription of CDKN1A [42]. Besides, IL-1β can induce the activation of transcription factors such as NF-κB, which may inhibit the transcription of CDKN1A by competing for binding to the promoter region of CDKN1A or recruiting inhibitory factors [43, 44]. In addition, molecules such as ROS or nitric oxide (NO) in the inflammatory environment may also silence the CDKN1A gene through epigenetic modifications (such as DNA methylation or histone deacetylation). Consequently, does the regulatory influence of MDM2 on CDKN1A exert an impact on the progression of OA? Through conducting reversion experiments, we revealed that the MDM2/CDKN1A axis cooperatively enhanced proinflammatory cytokine secretion and triggered ferroptosis by restraining cell proliferation and initiating apoptotic pathways within the IL-1β-stimulated C28/I2 chondrocyte model.

In summary, the current study has comprehensively elucidated the pivotal role of the MDM2-CDKN1A signaling axis in the pathological regulation of OA. These discoveries not only enhance our comprehensive understanding of the molecular pathogenesis underlying OA but also underscore its potential significance in the realm of translational medicine. The dissection of the intricate interaction network between MDM2 and CDKN1A offers a novel paradigm for the identification of highly sensitive early diagnostic biomarkers, prognostic assessment tools, and innovative therapeutic targets. However, existing studies are confined to in vitro cellular models, and the efficacy of therapeutic interventions targeting the MDM2/CDKN1A axis remains unverified within complex biological systems. To comprehensively evaluate the clinical translation potential of this therapeutic target, future investigations must establish disease-relevant animal models and systematically dissect the in vivo dose–response relationship and underlying mechanisms through integrated pharmacokinetic–pharmacodynamic (PK–PD) analysis, tissue-specific distribution profiling, and multifaceted safety assessments.

Author Contributions

Kaihong Gui and Xiaosong Li conducted the experiments and drafted the manuscript. Junlai Song collected and analyzed the data. Ao Li contributed the methodology. Jun Fan operated the software and edited the manuscript. Lin Huang designed and supervised the 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 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. **FIGURE S1.** The influence of MDM2 on the ubiquitination level of CDKN1A. The ubiquitination level of CDKN1A was detected by western blot after treatment with MG132 or knockdown of MDM2.

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ORIGINAL ARTICLE

A Potential CD8$^+$ T-Cell-Related Biomarker *IFIT3* for Rheumatoid Arthritis

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ABSTRACT

Objective: The aim is to pinpoint crucial genes linked to CD8$^+$ T cells in rheumatoid arthritis (RA) for aiding in diagnosis, predicting disease progression, and ultimately discovering potential drug targets.

Methods: This study utilized datasets from the Gene Expression Omnibus (GEO) database to analyze gene expression profiles in RA patients. Weighted Gene Coexpression Network Analysis (WGCNA) was conducted to identify gene modules associated with the diseases, followed by differential gene expression analysis. Functional enrichment and protein–protein interaction (PPI) network analysis were employed. Gene Set Enrichment Analysis (GSEA) and Disease Ontology (DO) analysis were applied to understand their potential pathways. Transcription factors (TFs) correlated with target gene expression were screened, and TF binding sites were predicted. The proportion of immune cell infiltration was calculated using CIBERSORT.

Results: The study identified 58 candidate genes associated with CD8$^+$ T cells in RA. The top five genes, *RSAD2, IFIT3, OAS1, IFIT2*, and *SAMD9L*, were found to be upregulated in RA and other autoimmune diseases. *IFIT3* showed potential diagnostic value in RA, with significant expression differences in RA vs. OA samples. TF *BCL11B* bound to the *IFIT3* promoter. GSEA analysis indicated *IFIT3*'s influence on pathways like the cell cycle and TNF signaling. Immune landscape analysis showed *IFIT3*'s correlation with immune cell infiltration such as B cells and plasma cells. Drug prediction analysis suggested naringin's treatment potential for RA via targeting *IFIT3*.

Conclusion: This study identifies key CD8$^+$ T-cell genes in RA, with *IFIT3* as a potential diagnostic and therapeutic target, revealing *BCL11B*'s regulatory role.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease caused by multiple factors that primarily affects the joints, leading to inflammation, pain, joint damage, and other organs including lung, heart, kidney, eye, skin, etc. [1]. The exact cause of RA remains elusive, but genetic predisposition, environmental factors, immune system dysfunction, microbiota, and diet are believed to play a role in its development [2]. RA is the most prevalent systemic inflammatory rheumatic disease [3], limited evidence from randomized controlled trials is available in this field, resulting in a therapeutic strategy primarily reliant on empirical approaches grounded in small case series and retrospective studies [4]. Early diagnosis and effective management strategies, including medications and lifestyle modifications, are crucial for

minimizing joint damage and improving the quality of life for individuals living with RA.

Cytotoxic T lymphocytes, or $CD8^+$ T cells, play a pivotal role in the immune system by recognizing and eliminating infected or abnormal cells [5]. In autoimmune diseases, autoreactive $CD8^+$ T cells targeting and killing specific cells can release a multitude of autoantigens, triggering excessive production of autoantibodies and ultimately culminating in the demise of self-cells. Modifications of activation and suppression to $CD8^+$ T cells occur simultaneously in autoimmune diseases [6]. Increasing evidence indicates that the homeostasis of $CD8^+$ T-cell populations is disrupted in human autoimmune diseases, including RA [7]. In RA, $CD8^+$ T cells have been proven to be elevated in the synovial tissue and play a significant role in the immune-mediated inflammatory response that characterizes the disease by infiltrating the synovial tissue and releasing proinflammatory cytokines such as interferon (IFN)-γ that further exacerbate the inflammatory milieu [8]. Their interactions with other immune cells and cytokines in the joint microenvironment contribute to the perpetuation of chronic inflammation and tissue destruction observed in RA. Unlike osteoarthritis (OA), which is largely a result of wear and tear on the joints over time, RA involves the immune system mistakenly attacking healthy joint tissues. Differentially expressed genes (DEGs) between RA and OA are enriched on gene sets related to $CD8^+$ T-cell pathways, which are positively correlated to RA [9].

Understanding the complex involvement of $CD8^+$ T cells in the intricate immune response of RA is crucial for developing targeted therapeutic strategies to slow disease progression and alleviate symptoms. Here, we aim to identify key genes associated with $CD8^+$ T cells in RA to assist in diagnosis and predict disease progression, with the hope of finding useful drug targets.

2 | Materials and Methods

2.1 | Data Collection

Datasets with accession numbers GSE93272, GSE15258, GSE17755, GSE55235, GSE39340, and GSE93776 were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). GSE93272, GSE15258, and GSE17755 consisted of blood samples. GSE93272 comprised 232 RA samples and 43 control samples. GSE15258 included 86 RA samples. GSE17755 contained 112 RA samples and 53 control samples. GSE55235 had 10 RA synovial tissue samples and 10 control samples. GSE39340 comprised 10 RA synovial tissue samples and seven OA synovial tissue samples. GSE93776 contained data on various immune cell types, with a total of 83 RA blood samples and 90 control samples.

Additional datasets for other autoimmune diseases were downloaded: GSE154851 (38 systemic lupus erythematosus, SLE, blood samples and 32 controls), GSE73754 (52 ankylosing spondylitis, AS, blood samples and 20 controls), GSE84844 (30 primary Sjogren's syndrome, PSS, blood samples and 30 controls), and GSE48556 (106 OA, peripheral blood mononuclear cell [PBMC] samples and 33 controls).

The GSE224330 dataset (10 samples from healthy patients, six samples from MTX-, five samples from abatacept-, five samples from anti-TNFalpha-, and five samples from tocilizumab-treated patients) was analyzed for gene differences between RA patients receiving drug treatments and healthy controls. GSE54629 (90 Response and 48 Non_Response samples) and GSE129705 (65 Response and 63 Non_Response samples) were used to analyze gene differences between rituximab and tumor necrosis factor inhibitors (TNFi) responders and nonresponders in RA patients.

Moreover, all dataset information was summarized in Table 1. The principal component analysis (PCA) was conducted on various cohorts to further confirm the matrix quality (Figure S1).

2.2 | Weighted Gene Coexpression Network Analysis (WGCNA)

WGCNA analysis was conducted using the "WGCNA" R package (version1.72-1) [10], based on gene expression values. The initial step involved the selection of the top 25% of genes based on variance analysis. Subsequently, Pearson correlation coefficients were computed between each gene, and a suitable soft threshold (β) was determined to ensure that the constructed network adhered to the scale-free network criterion. Following this, a network was established utilizing a one-step method, and the adjacency matrix underwent transformation into a Topological Overlap Matrix (TOM). A hierarchical clustering tree for genes was then generated through hierarchical clustering.

2.3 | Differential Gene Expression and Functional Enrichment Analysis

The identification of DEGs was conducted utilizing the "limma" package (version 3.52.4) [11], employing criteria of Log_2FC absolute value greater than 0.5 and adj.p-value < 0.05.

For the exploration of enriched biological processes (BPs) and pathways associated with DEGs, enrichment analysis was performed using the "clusterProfiler" package (version 4.7.1.2) [12] in R. Functional annotation primarily relied on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Selected GO terms, including BP, Molecular Function (MF), and Cellular Component (CC), as well as KEGG pathways exhibited statistically significant enrichment (p.adjust < 0.05).

2.4 | Protein–Protein Interaction (PPI) Network

The STRING database was used for analyzing and predicting functional associations and interactions among proteins. We

TABLE 1 | Baseline information of samples from different datasets.

GEO accession	Sample type	Sample	Platform	Age			Sex			
				RA	Control	*p*		RA (%)	Control (%)	*p*
GSE93272	blood	RA:Control = 232:43	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	57.2 (±14.7)	42.4 (±9.3)	0.001	Female	205 (88.4)	38 (88.4)	1
							Male	27 (11.6)	5 (11.6)	
GSE15258	blood	RA = 86	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array				/			
GSE17755	blood	RA:Control = 112:53	GPL1291 Hitachisoft AceGene Human Oligo Chip 30 K 1 Chip Version	51.9 (±11.1)	31.7 (±12.9)	0.001	Female	93 (83)	24 (45.3)	0.001
							Male	19 (17)	29 (54.7)	
GSE55235	synovial tissue	RA:Control = 10:10	GPL96 [HG-U133A] Affymetrix Human Genome U133A Array				/			
GSE39340	synovial tissue	RA:OA = 10:7	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip	51.7 (±15.2)	54 (±6.3)	0.675	Female	7 (70)	5 (71.4)	1
							Male	3 (30)	2 (28.6)	
GSE93776	blood	RA:Control = 83:90	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	52.9 (±18)	43.8 (±6.7)	0.001	Female	54 (65.1)	90 (100)	0.001
							Male	29 (34.9)	0 (0)	
GSE154851	blood	SLE:Control = 38:32	GPL16699 Agilent-039494 SurePrint G3 Human GE v2 8 × 60K Microarray 039381 (Feature Number version)				/			
GSE73754	blood	AS:Control = 52:20	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip				/			
GSE84844	blood	PSS:Control = 30:30	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array				/			
GSE48556	PBMC	OA:Control = 106:33	GPL6947 Illumina HumanHT-12 V3.0 expression beadchip				/			

(Continues)

TABLE 1 | (Continued)

GEO accession	Sample type	Sample	Platform	Age			Sex		
				RA	Control	*p*	RA (%)	Control (%)	*p*
GSE224330	monocytes	RA:Control = 21:10	GPL21185 Agilent-072363 SurePrint G3 Human GE v3 8 × 60K Microarray 039494 [Probe Name Version]				/		
GSE54629	blood	RA = 138 (Response:Non_ Response = 90:48)	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0st Array [transcript (gene) version]				/		
GSE129705	blood	RA = 128 (Response:Non_ Response = 65:63)	GPL16791 Illumina HiSeq 2500 (*Homo sapiens*)				/		

utilized STRING [13] (https://string-db.org/, version 11.0) to analyze functional associations and protein interactions. The resulting network was visualized using Cytoscape [14] (version 3.7.2). Additionally, the cytoHubba plugin within the Cytoscape software, based on the MNC algorithm, was employed to further filter key genes within the PPI network.

2.5 | Gene Set Enrichment Analysis (GSEA) and Disease Ontology (DO) Analysis

Based on the median gene expression levels and as a grouping criterion, samples with expression levels higher than the median were categorized into the high-expression group, while those with levels lower than the median were categorized into the low-expression group. GSEA analysis was then conducted between these groups; pathways with a p-value < 0.05 were considered significantly enriched.

Simultaneously, differential gene expression analysis was performed using the "limma" function package in R. For the analysis of differential gene expression, the "DOSE" package (version 3.26.1) [15] in R was employed to conduct DO analysis on the identified DEGs.

2.6 | Screening Transcription Factors (TFs) Correlated With the Expression Levels of Target Genes

In the mRNA dataset of GSE93272, based on the results of differential expression analysis between RA and control samples, significant differentially expressed TFs were selected using the criteria of $|\text{Log}_2\text{FC}| > 0.5$ and p.adjust < 0.05. The TFs for selection were obtained from [16].

Pearson correlation coefficients were then calculated to assess the correlation between the selected TFs and the expression levels of the target gene. TFs showing a significant correlation with the expression levels of the target gene, based on a p-value < 0.05, were filtered.

2.7 | Predicting TFs Binding Sites

First, the upstream 3000 bp sequence file was downloaded from the UCSC (http://genome.ucsc.edu/). The motif files corresponding to TFs were obtained from the JASPAR database (https://jaspar.genereg.net/). The online tool FIMO (https://meme-suite.org/meme/tools/fimo) was used to predict whether TF binding motifs existed in the upstream region of gene promoters.

2.8 | Calculation of Immune Cell Infiltration Proportion

Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) software [17] was utilized to calculate the relative proportion of 22 types of immune cells in the samples.

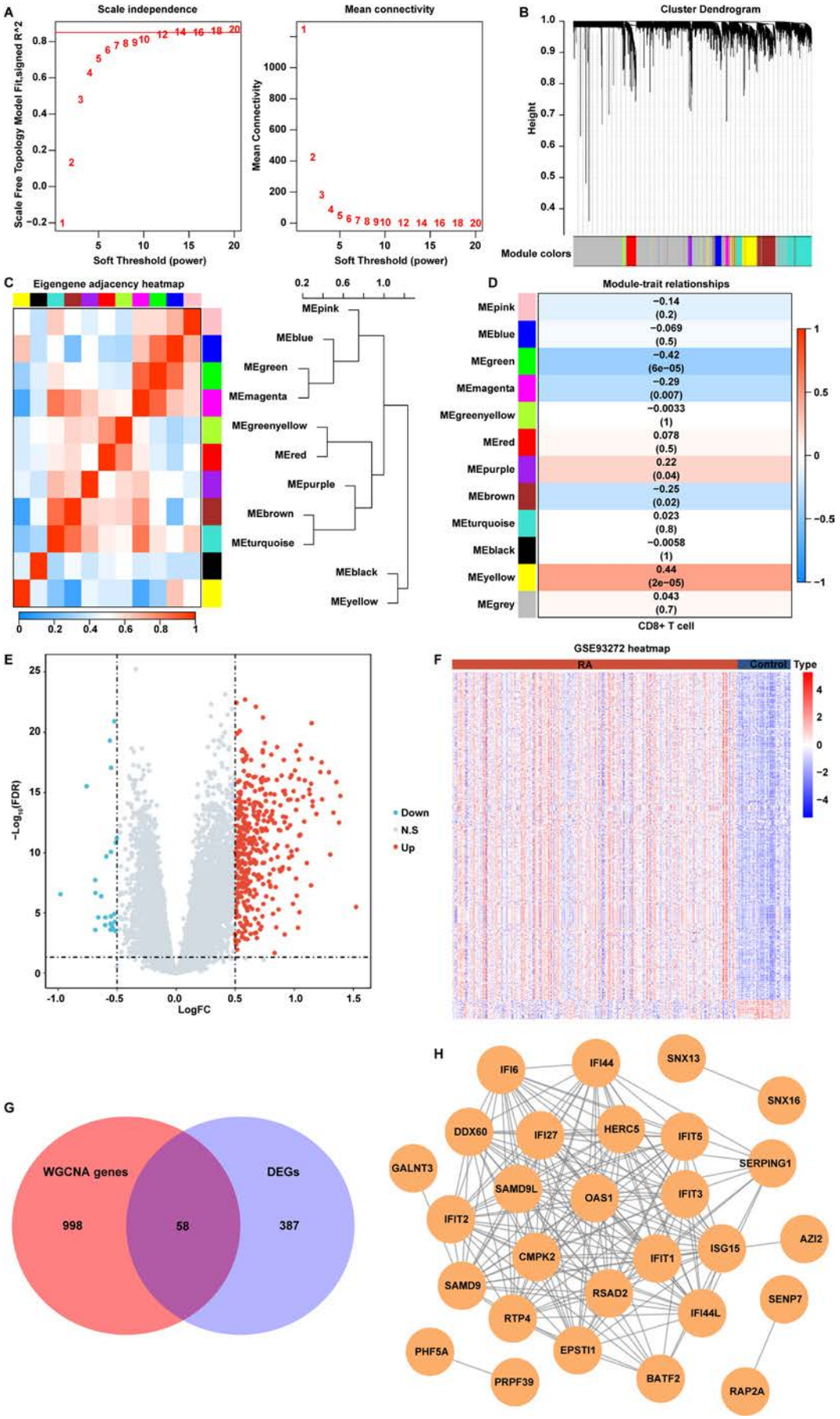

FIGURE 1 | Legend on next page.

FIGURE 1 | Determination of candidate genes by WGCNA and differential gene expression analysis. (A) Determination of soft threshold in WGCNA. (B) Clustering of gene modules in WGCNA. The upper half of the figure is a hierarchical clustering dendrogram of genes, and the lower half represents gene modules, where the module colors indicate the color of each module. The gray module represents a collection of genes that could not be grouped with any other module. (C) Heatmap of eigengene adjacency in WGCNA. (D) Correlation between each gene module and trait of $CD8^+$ T-cell level. (E) Volcano plot of differential gene expression analysis. (F) Heatmap of differential gene expression analysis. (G) Intersection of the 1056 WGCNA module-related genes and the 445 DEGs. (H) PPI network of 58 candidate genes.

2.9 | Drug Sensitivity Analysis

The potential correlation between hub gene and drugs was predicted using the Connectivity Map (CMAP) (https://clue.io) database, using default settings [18]. Then the drugs with binding scores > 90 were screened as crucial drugs.

2.10 | Molecular Docking

Subsequently, after focusing on the crucial molecular naringin, the three-dimensional (3D) structure of naringin was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The crystal structure of hub gene IFIT3 was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) [19]. The naringin and IFIT3 were taken as ligand and receptor, respectively. The molecular docking between IFIT3 and naringin was conducted employing the CB-Dock2 tool (https://cadd.labshare.cn/cb-dock2/php/index.php). The active cavity structure was searched within the receptor structure, and the Vina score was calculated. Finally, Discovery Studio 4.5 was used for the 2D and 3D visualization of the complex.

2.11 | Molecular Dynamics Simulation

We then performed molecular dynamics simulations using Gromacs (2024.4) to assess protein–ligand binding. Ligand and receptor topologies were generated using the CHARMM36 force field and TIP3P water model. The system was partitioned into ligand/receptor and water/ion groups. Following energy minimization, NVT and NPT simulation was conducted at 300 K and 1 bar. Subsequently, a 100 ns molecular dynamics simulation was run. Trajectory analysis included root mean square deviation (RMSD, structural stability), root mean square fluctuation (RMSF, residue flexibility), radius of gyration (Rg, protein compactness), and hydrogen bond analysis (Hbond, protein–ligand interactions) [20, 21].

2.12 | Other Statistical Analyses

The Wilcoxon rank-sum test was employed to compare the differences in gene expression and immune cell infiltration between different groups. Pearson correlation analysis was conducted using the "cor" function in the R language. ROC curves were generated using the "pROC" package (version 1.18.4) [22] in R to assess whether the target genes had diagnostic value. Differences were considered statistically significant when $p < 0.05$. All statistical analyses were performed using R software version 4.3.1.

3 | Results

3.1 | Screening of $CD8^+$ T-Cell-Related Candidate Genes

In this work, a total of 13 GEO cohorts were included, which were summarized in Table 1. We noticed that in most cohorts, the RA patients were older than non-RA participants, and significantly higher proportions of female patients were found among RA patients (Table 1). Next, GSE15258 was used for WGCNA analysis, and a soft threshold (β) of 14 was chosen (Figure 1A) to construct a gene network, resulting in 12 gene modules (Figure 1B). The CIBERSORT algorithm was utilized to calculate the relative abundance of 22 immune cell types in each sample. The score for $CD8^+$ T cells was selected as the trait data for WGCNA. The correlation between each gene module and $CD8^+$ T cells was calculated (Figure 1C,D). Gene modules with a p-value less than 0.05 and correlated with $CD8^+$ T cells (green, magenta, purple, brown, and yellow modules) were identified, encompassing a total of 1056 genes (Table S1).

GSE93272 was then used for differential gene expression analysis in RA vs. Control. A total of 445 DEGs were identified, including 420 upregulated genes and 25 downregulated genes in RA samples compared to normal samples (Figure 1E,F). The intersection of the 1056 WGCNA module-related genes and the 445 DEGs resulted in 58 shared genes, which were considered candidate genes (Figure 1G, Table S2).

The STRING database was used to construct a PPI network for the 58 candidate genes, with a minimum required interaction score > 0.4 as the threshold for selecting interacting pairs. The Cytoscape software was employed for visualizing this PPI network (Figure 1H), revealing a total of 28 nodes and 176 edges; each node represents a gene, and edges represent the interactions between them.

3.2 | Functional Enrichment Analysis of the 58 Candidate Genes

To determine the functions of the 58 candidate genes, GO and KEGG enrichment analyses were conducted on these genes. There were seven significantly enriched KEGG pathways ($p < 0.05$) including O-glycan biosynthesis, RIG-I-like receptor signaling pathway, biosynthesis of cofactors, etc. (Figure S2A); 150 significantly enriched BP terms such as defense response and viral genome replication, 20 significantly enriched CC terms such as spliceosomal complex, and 23 significantly enriched MF terms like enzyme inhibitor activity. The top 10 enriched GO

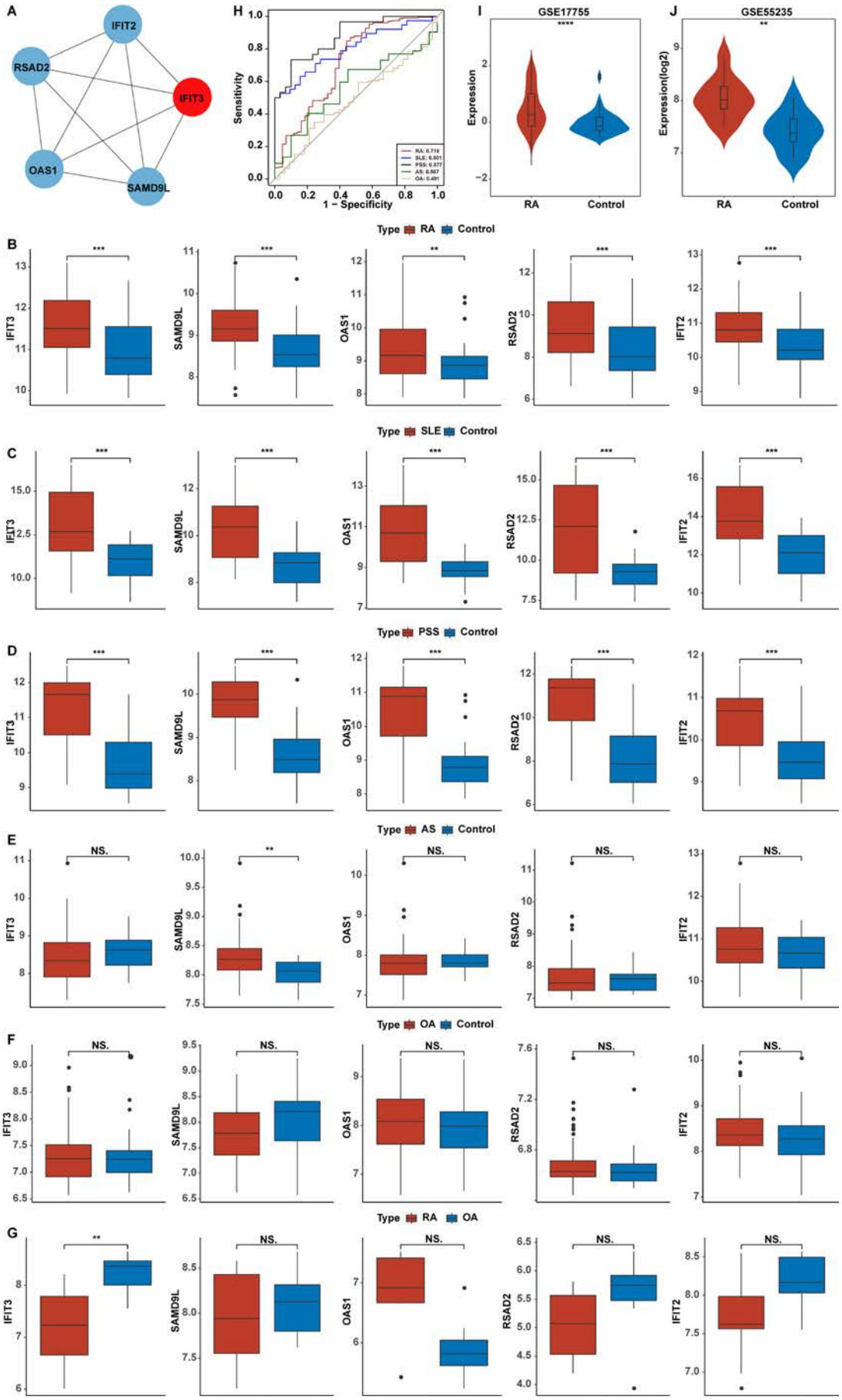

FIGURE 2 | Legend on next page.

FIGURE 2 | Expression of crucial genes in RA and other diseases. (A) Top 5 crucial genes PPI network based on MNC algorithm. (B–F) Expression levels of *IFIT3*, SAMD9L, OAS1, RSAD2, and IFIT2 in RA, SLE, PSS, AS, and OA, respectively. (G) Expression levels of five crucial genes between RA and OA. (H) ROC curves of RA, SLE, PSS, AS, and OA. (I, J) Expression of *IFIT3* in RA blood samples and synovial tissue samples, respectively.

terms were displayed in Figure S2B, with detailed functional enrichment results available in Table S3.

3.3 | Expression Levels of Crucial Genes in RA and Other Diseases

After the determination of candidate genes, the Maximum Neighborhood Component (MNC) algorithm was used to score the importance of each node in the network, to further select crucial genes for RA. The top five key node genes identified by score (Figure 2A, Table S4) were *RSAD2*, *IFIT3*, *OAS1, IFIT2*, and *SAMD9L*. Gene expression analysis of these genes in RA samples vs. normal samples (GSE93272 dataset) revealed significant upregulation of all five genes in RA samples (Figure 2B).

Further analyses of the expression of these five genes in other autoimmune diseases SLE, PSS, and AS were conducted. Using the GSE154851 dataset, *RSAD2, IFIT3, OAS1, IFIT2*, and *SAMD9L* were found to be significantly upregulated in SLE samples compared to normal samples (Figure 2C). Similarly, using the GSE84844 dataset, these genes were significantly upregulated in PSS samples (Figure 2D). *SAMD9L* was significantly upregulated in AS samples based on the GSE73754 dataset (Figure 2E).

OA is a degenerative joint disease characterized by the gradual breakdown of the cartilage in joints, leading to pain, stiffness, and reduced mobility [23], while RA is an autoimmune disorder that involves inflammation of the synovium, leading to joint damage and systemic symptoms. While there are clear distinctions between RA and OA, there can be challenges to distinguish them in certain situations, such as in the early stages of the diseases or when there are overlapping symptoms. The GSE48556 dataset indicated no significant difference in the expression of these five genes in OA compared to normal samples (Figure 2F). Additionally, the GSE39340 dataset revealed a significant difference in the expression of *IFIT3* between RA and OA samples (Figure 2G), showing *IFIT3*'s potential for early diagnostic between RA and OA.

Based on the PPI network results and the significance of differential expression, we have focused on *IFIT3* for subsequent study. To further evaluate the potential diagnostic potential of *IFIT3* in distinguishing immune disease samples from control samples, the ROC analysis was then performed. Our results indicated that the AUC values of *IFIT3* in distinguishing RA, SLE, PSS, AS, and OA samples were 0.718, 0.801, 0.877, 0.567, and 0.491, respectively (Figure 2H). Furthermore, the expression of the *IFIT3* gene in blood and synovial tissue (synovium) of RA patients was validated using the GSE17755 and GSE55235 datasets. The results showed a significant upregulation of *IFIT3* in RA samples compared to normal samples (Figure 2I,J).

3.4 | Promising TFs Regulation of IFIT3

Motivated by the need to understand the regulatory mechanisms influencing *IFIT3* expression in RA, we identified a set of 19 differentially expressed TFs in RA using the GSE93272 dataset (Figure 3A). Subsequently, utilizing mRNA expression data from the GSE93272 dataset, the correlation between the expression levels of these 19 TFs and *IFIT3* gene was analyzed. Correlation results indicated that among them, eight TFs, *ZNF230, TFEC, BCL11B, BATF2, ZNF117, KIN, ZNF267*, and *BAZ2B*, showed significant correlations with *IFIT3* expression based on the standard of $p < 0.05$ (Figure 3B). *BCL11B* was significantly negatively correlated with *IFIT3* expression, while other TFs were positively correlated with *IFIT3* expression levels. Using Cytoscape, we created a network diagram depicting the interactions between the eight TFs and *IFIT3* (Figure 3C).

For the upstream 3000 bp region of the *IFIT3* promoter, we searched for TF binding sequences employing the public database. Based on a p value threshold of $< 10^{-4}$, we identified a potential binding sequence for the TF *BCL11B* around 2132 bp upstream of the *IFIT3* promoter (UN0114.1.meme) (Table S5), implying the potential regulation of TF *BCL11B* on *IFIT3* gene expression by binding to its upstream region. Further validation was performed by searching the Cistrome chip-seq public database (http://cistrome.org/db/#/). In the Thymus dataset GSM2247496 [24] with a score of 0.008, the *BCL11B* chip-seq results revealed significant binding peaks on the *IFIT3* gene (Figure 3D), supporting *BCL11B*'s role in regulating *IFIT3* gene expression through direct binding.

3.5 | Functions and Pathways Potentially Influenced by IFIT3 in RA

To further elucidate the role of *IFIT3* in the pathogenesis of RA, GSEA analysis was performed using the GSE93272 cohort of RA samples, and the samples were ordered based on the expression levels of *IFIT3*. Samples with *IFIT3* expression above the median were defined as $IFIT3^{high}$ group, and those below the median as the $IFIT3^{low}$ group. GSEA enrichment analysis between these groups revealed that 87 pathways were significantly enriched ($p < 0.05$) in the high compared to the low-expression group. The pathways enriched by GSEA included Cell cycle, NOD-like receptor signaling pathway, SLE, RA, TNF signaling pathway, and Toll-like receptor signaling pathway, as shown in Figure 4A.

DEGs between $IFIT3^{high}$ and $IFIT3^{low}$ groups were also subjected to DO enrichment analysis, which identified 41 significantly enriched diseases ($p < 0.05$) such as hepatitis and immunodeficiency diseases. The top 30 significantly enriched pathways are depicted in Figure 4B. Detailed enrichment results are provided in Table S6.

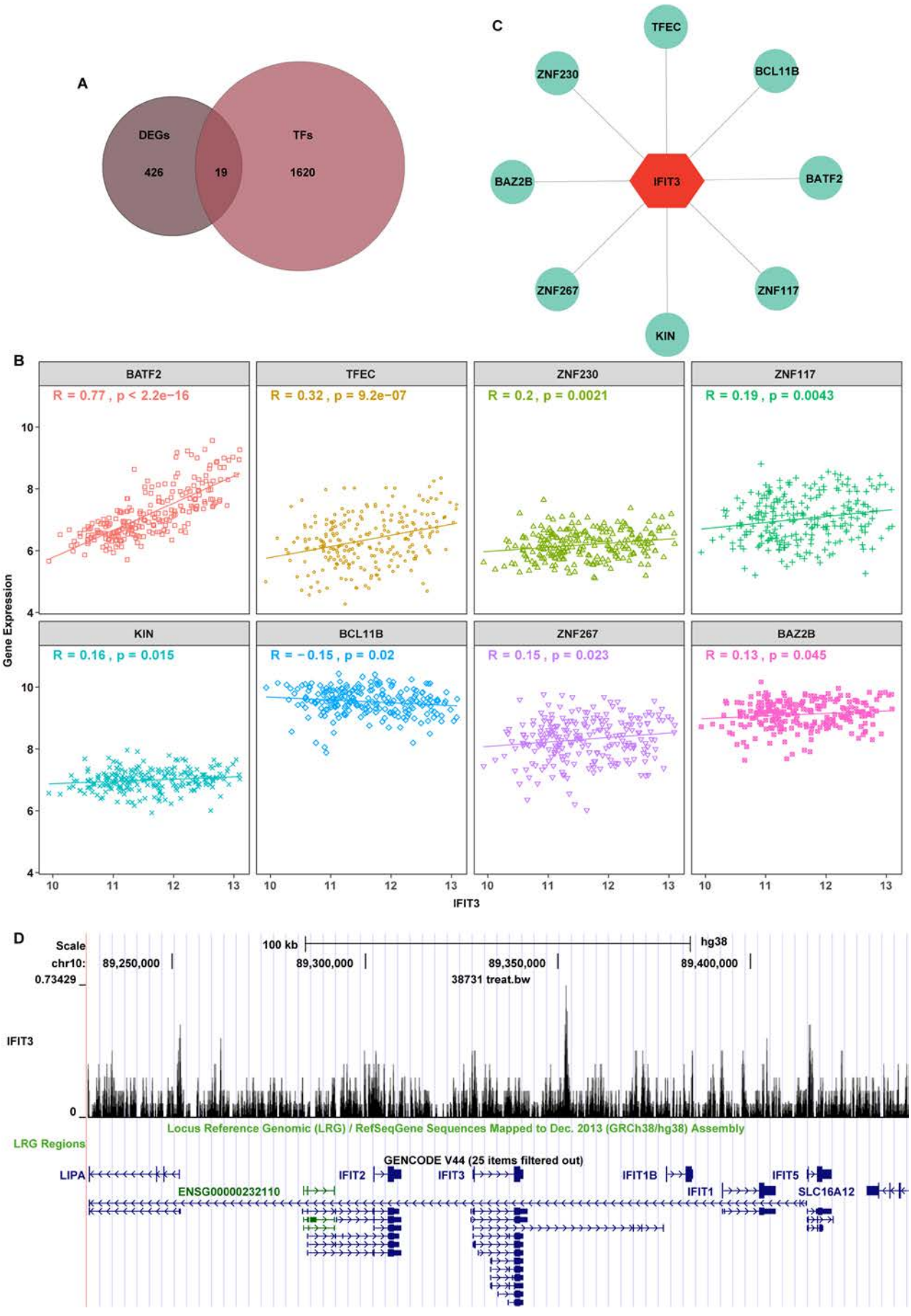

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FIGURE 3 | Prediction of TFs regulation of *IFIT3*. (A) Intersection of DEGs and TFs list. (B) Correlation between *IFIT3* expression levels and eight TFs BATF2, TFEC, ZNF230, ZNF117, KIN, BCL11B, ZNF267, and BAZ2B. (C) Network of TFs and *IFIT3*. (D) Results from Chip-seq database.

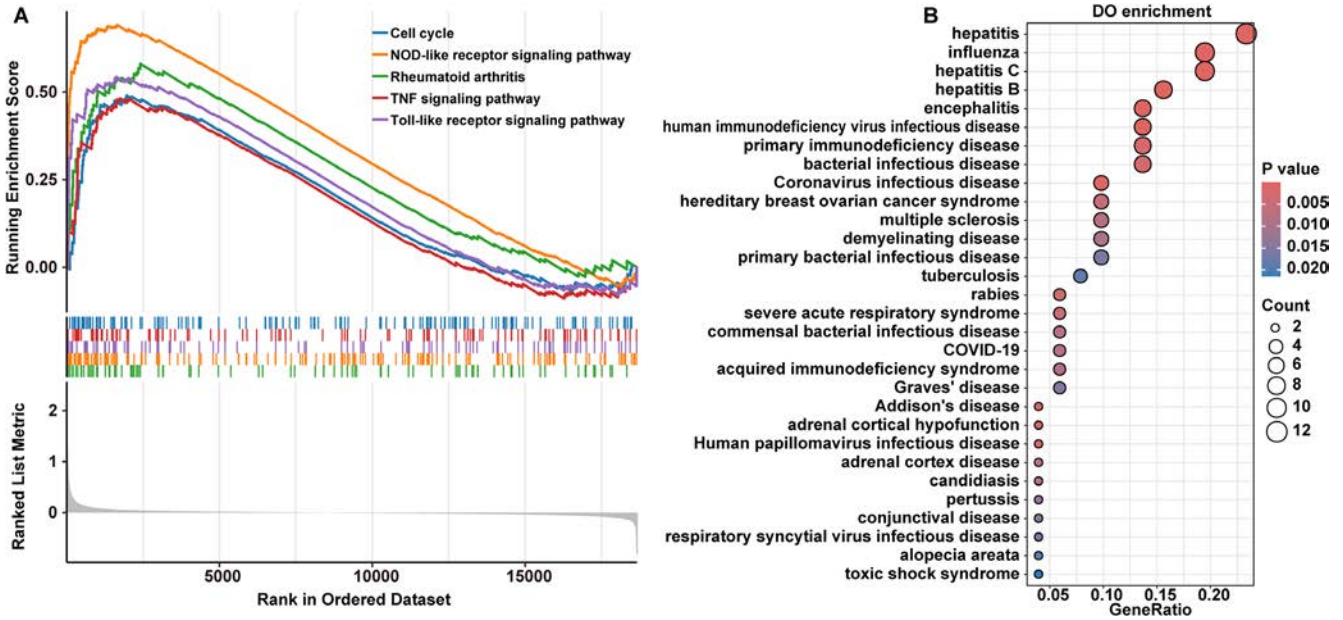

FIGURE 4 | Pathways and functions potentially influenced by *IFIT3* in RA. (A) GSEA analysis. (B) DO analysis.

3.6 | Immune Landscape Influenced by IFIT3

RA is an autoimmune disease; hence, exploration of *IFIT3* influence on the immune landscape will aid in understanding the mechanism lying behind the disease. Using the CIBERSORT algorithm, we calculated the relative abundance of 22 types of immune infiltrating cells in the RA samples from the GSE93272 (Figure 5A). Based on the median expression value of the gene *IFIT3*, the RA samples were divided into $IFIT3^{high}$ and $IFIT3^{low}$ groups. We analyzed the differences in immune cell infiltration between these groups and found significant differences in memory B cells (high), naive B cells (low), plasma cells (high), Macrophages M0 (low), Macrophages M1 (high), and activated dendritic cells (high) between $IFIT3^{high}$ and $IFIT3^{low}$ groups (Figure 5B). Further analysis of the Pearson correlation between expression levels of *IFIT3* and the significantly different immune infiltrating cells revealed that *IFIT3* was significantly negatively correlated with naive B cells and Macrophages M0 and positively correlated with memory B cells, plasma cells, Macrophages M1, and activated dendritic cells (Figure 5C).

Furthermore, we analyzed the expression levels of *IFIT3* across different immune cell types using the GSE93776 dataset. The results showed that the expression levels of *IFIT3* were significantly higher in monocytes and natural killer T (NKT) cells in RA compared to the control group (Figure 5D,E). Conversely, in the CD8 T CD45RO memory cells, the expression levels of *IFIT3* were significantly reduced in RA compared to the control group (Figure 5F). However, for other immune cell types, including naive CD8 T cells, effector memory CD8 T cells, central memory CD8 T cells, CD4 T cells, neutrophils, and natural killer (NK) cells, no significant differences in *IFIT3* expression were observed between RA patients and the control group (Figure S3A–F). Additionally, we found no significant differences between $IFIT3^{high}$ and $IFIT3^{low}$ groups in adaptive immunity and innate immunity (Figure S3G). Similarly, there were no significant differences in T-cell responses between the two groups (Figure S3H).

3.7 | Correlation Between Drugs and IFIT3

In the GSE224330 dataset, which contains transcriptomic data from RA patients after various drug treatments and healthy individuals, we analyzed the changes in *IFIT3* gene expression between RA patients and healthy controls following different treatments. The results showed that there were no significant differences in *IFIT3* expression between the RA group and the healthy group after treatments with Abatacept, TNFalpha, MTX, and Tocilizumab (Figure 6A–D).

Additionally, we analyzed the expression levels of the *IFIT3* gene in response to Rituximab and TNFi treatments, comparing responders and nonresponders using the GSE54629 and GSE129705 datasets. The results indicated that there were no significant differences in *IFIT3* expression between Rituximab responders and nonresponders (Figure 6E). However, the *IFIT3* expression was significantly higher in TNFi responders compared to nonresponders (Figure 6F).

Subsequently, basing on drug prediction using CMAP, we found that a total of 295 drugs showed good binding potential with *IFIT3*, with binding scores > 90 (Table S7). Among the top five drugs with the highest binding scores, we noticed that naringin was of great potential in treating RA; thereby, the

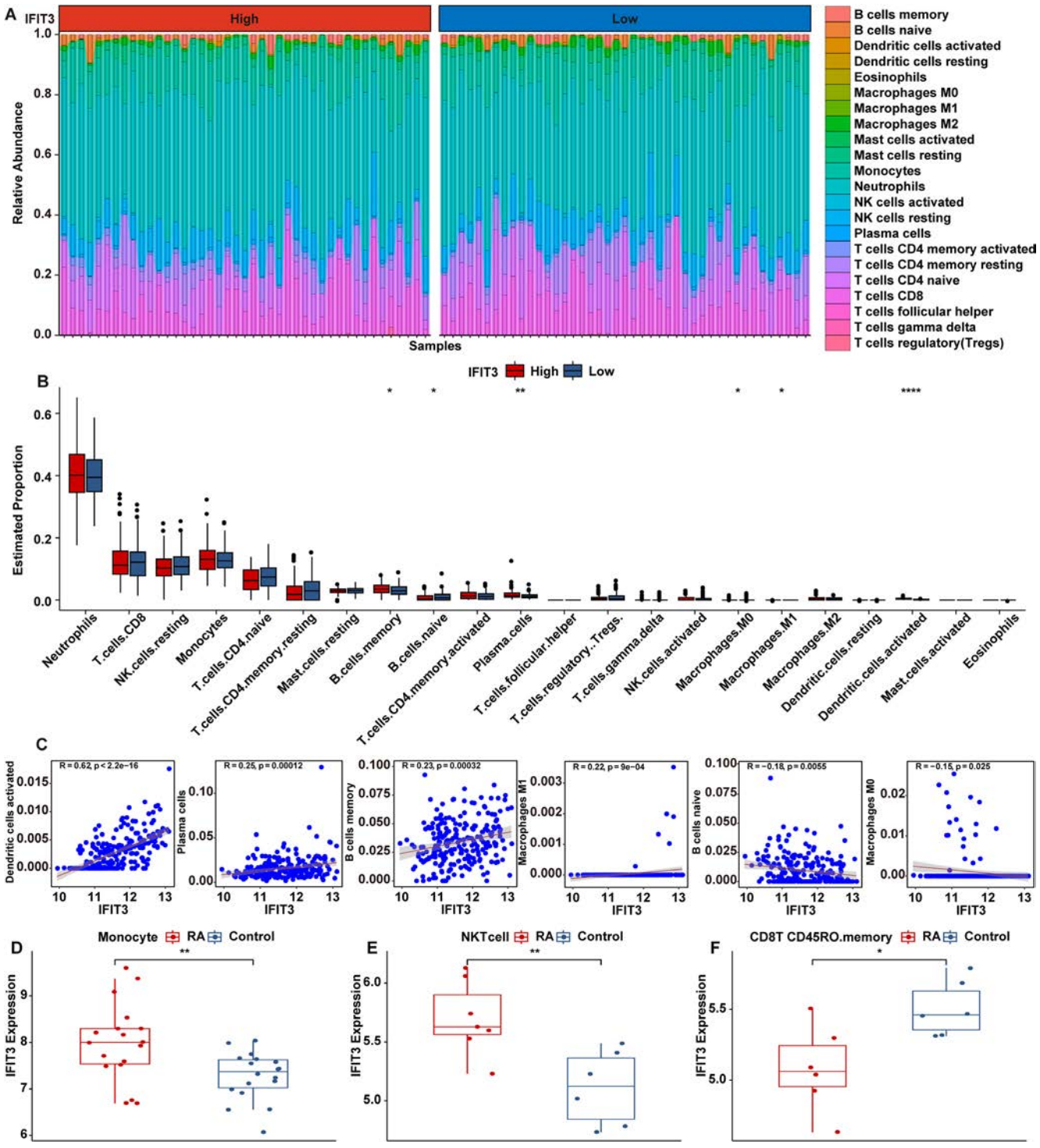

FIGURE 5 | Immune landscape influenced by *IFIT3*. (A) Infiltration proportion of 22 types of immune cells. (B) Infiltration level of 22 types of immune cells between *IFIT3*^{high} and *IFIT3*^{low} groups. (C) Correlation of *IFIT3* expression and 10 immune infiltrating cells significantly different between *IFIT3*^{high} and *IFIT3*^{low} groups. (D–F) Expression levels of *IFIT3* in monocytes, NKT cells, and CD8 T CD45RO memory cells. (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, ns indicates nonsignificance).

binding status between *IFIT3* and naringin was focused on in our following analysis.

The molecular docking results indicated that naringin could bind to the active pocket of IFIT3 composed of ASN-206, GLN-240, TYR-208, GLN-41, LYS-148, HIS-182, ARG-245, LYS-249, TYR-181, SER-246, ARG-37, and LEU-147 (Vina score = −8.18 cal/mol), forming 10 hydrogen bonds (Figure 7A–C). Meanwhile, molecular dynamics simulations were also conducted to confirm the stability of the IFIT3-naringin complex. The results suggested that the IFIT3-naringin complex system exhibited good stability during the simulation process. The protein backbone

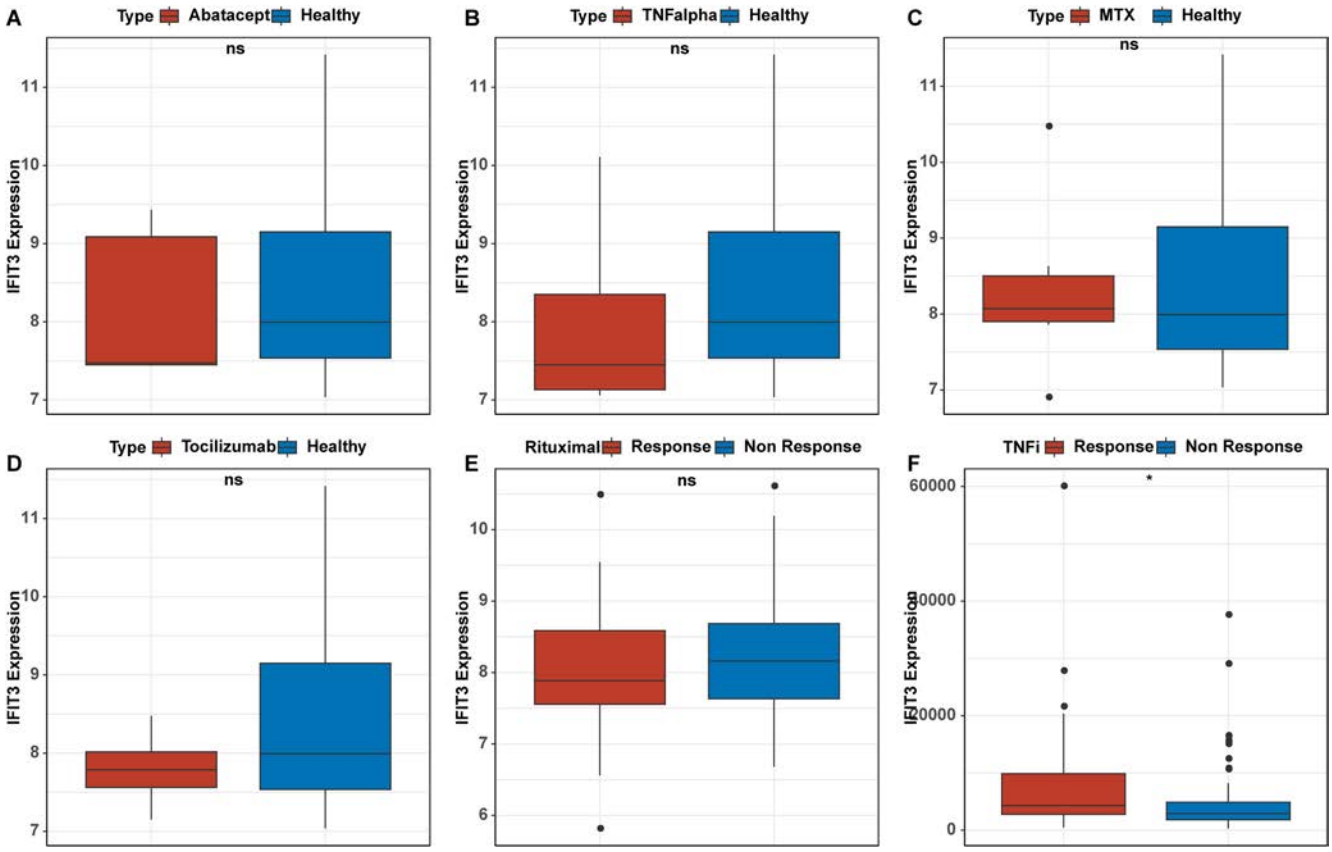

FIGURE 6 | Correlation between drugs and *IFIT3*. (A–D) *IFIT3* expression in RA patients after Abatacept, TNFalpha, MTX, Tocilizumab treatment and healthy individuals. (E–F) *IFIT3* expression in Rituximab, TNFi drug response and nonresponse groups.

structure fluctuated <0.2 nm between 6 ns and 10 ns, tending towards stability (Figure 7D). The RMSF curve showed that the residue structures in IFIT3 were relatively stable, with small RMSF values (Figure 7E). The total energy curve indicated that the total energy was lower after 6 ns, exhibiting a more stable receptor-ligand binding (Figure 7F) and compact folding structure (Figure 7G). After 6 ns, the system still maintained a stable two to three hydrogen bonds (Figure 7H).

4 | Discussion

The findings of this study provide valuable insights into the role of $CD8^+$ T cells related genes in RA and their potential as therapeutic targets. The identification of 58 candidate genes, particularly the top five genes *RSAD2, IFIT3, OAS1, IFIT2*, and *SAMD9L*, which are upregulated in RA and other autoimmune diseases, suggests a significant association with the disease pathology. The functional enrichment analysis further supports the involvement of these genes in critical pathways. *IFIT3*'s potential as a diagnostic marker in RA, as well as its potential indication for various treatments' effects, highlights its potential as a therapeutic target.

The *IFIT3* gene, a member of the IFN-Induced Proteins with Tetratricopeptide Repeats (IFIT) family, plays a crucial role in the host's antiviral defense mechanisms [25]. It is often induced by IFNs in response to viral infections, contributing to the inhibition of viral replication [26]. Additionally, *IFIT3* has been implicated in immune modulation beyond antiviral responses, with associations to autoimmune diseases [27]. *IFIT3* has been identified as a regulatory factor and treatment target in an autoimmune skin disease, psoriasis, being able to stimulate the growth and immune response in keratinocytes, contributing to the onset of psoriasis [28]. In another autoimmune disease, multiple sclerosis (MS), *IFIT3* is upregulated in macrophages M1, and when microglia are polarized towards M1, the expression of *IFIT3* is enhanced. Knockdown of *IFIT3* can ameliorate MS. *IFIT3* preferentially enhances the polarization of microglia towards an M1 state, potentially playing a role in the progression of MS [29]. In our study, macrophages M1 were significantly positively correlated with *IFIT3* expression, possibly suggesting a similar mechanism behind RA's onset. Consistent with our study, *IFIT3* has also been found to be upregulated in SLE [30], AS [31], and RA [32]. In SLE, *IFIT3* may promote the differentiation of monocytes into dendritic cell-like cells [33, 34]. $CD8^+$ T cells induce IFN-responsive microglia where *IFIT3* is upregulated [33, 34].

Eight TFs potentially regulating *IFIT3* were identified; among them, the binding sequence of *BCL11B* was detected in the upstream region of *IFIT3*. *BCL11B*, also known as B-cell lymphoma/leukemia 11B, is a crucial TF that plays a significant role in the regulation of gene expression and cellular development, particularly in the context of hematopoiesis and neural development. It expresses in various types of cells and suppresses T-cell maturation [35, 36]. According to our study, the expression of *BCL11B* was significantly negatively correlated

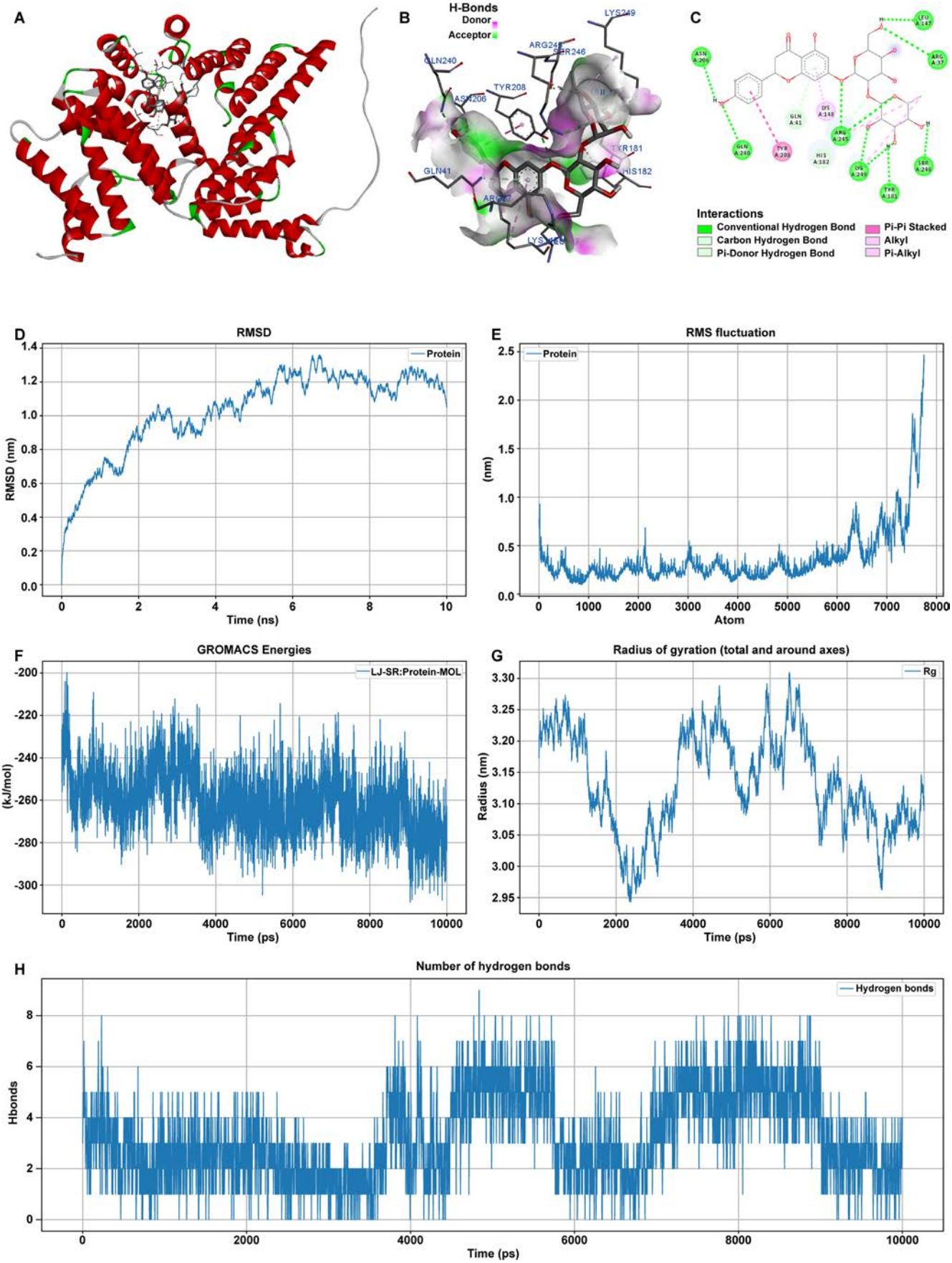

FIGURE 7 | IFIT3-related drug prediction and molecular docking. (A) The 3D structural diagram between IFIT3 and naringin. (B) The active pocket of IFIT3. (C) The binding mode between naringin and protein residues. (D) RMSD curve. (E) RMSF curve. (F) Total energy curve. (G) Rg curve. (H) The number curve of hydrogen bonds.

with *IFIT3*, suggesting its inhibition of *IFIT3*. *BCL11B* has been found to be hypomethylated in RA compared to OA, and it may be related to the pathogenesis of RA [37, 38]. In our research, *IFIT3* expressed significantly higher in RA compared to the control group. The expression level differences were not significant after treatment with Abatacept, TNFalpha, MTX, and Tocilizumab. Although the samples were not the same before and after treatment, it might suggest *IFIT3* expression levels changed to normal levels after treatment. T-lymphocyte-related gene *BCL11B* is positively associated with the response to antirheumatic drug treatment, indicating that downmodulation of T-cell activation pathway may be one of the mechanisms of the drug treatment [39].

As a $CD8^+$ T-cell-related gene, *IFIT3* is expressed significantly lower in RA compared to the control group in the CD8 T CD45RO memory cells. However, in the analysis of immune cell infiltration, the infiltration of $CD8^+$ T cells was not detected to be significantly different between $IFIT3^{high}$ and $IFIT3^{low}$ groups. The lack of significant differences in $CD8^+$ T-cell expression could be attributed to several factors. Firstly, the immune system operates through a complex regulatory network, where different genes may exert their influence at distinct stages. Secondly, even though *IFIT3* is associated with $CD8^+$ T cells, its specific regulatory role might not be prominently manifested during immune infiltration. Other genes or factors may also play a role in regulating $CD8^+$ T-cell activity. Additionally, the data may not have captured variations in subtypes of $CD8^+$ T cells. Lastly, the dynamic changes in immune infiltration may not be fully reflected in the available data.

Although the role of *IFIT3* was first revealed in the present work, there were still some limitations in our study. Despite including multiple public cohorts, the candidate hub genes were mainly validated based on transcriptomic data. The validations in clinical samples would further improve the robustness of our study. In future research, a more comprehensive analysis of the dynamic changes in $CD8^+$ T-cell infiltration and activation in response to various treatments with clinical samples would be of great significance, as well as the exploration of the interplay between *BCL11B* and *IFIT3*.

5 | Conclusions

In conclusion, this study has identified a set of candidate genes associated with $CD8^+$ T cells that are potentially involved in the pathogenesis of RA. The top five genes, *RSAD2, IFIT3, OAS1, IFIT2*, and *SAMD9L*, have been found to be upregulated in RA and other autoimmune diseases, suggesting their significant role in disease pathology. Notably, *IFIT3* has shown potential as a diagnostic marker for RA and as an indicator of treatment response, highlighting its potential as a therapeutic target. The study also revealed the regulatory role of the TF *BCL11B* in *IFIT3* expression, which could be a key mechanism in the modulation of T-cell activation in RA. The findings provide valuable insights into the complex interplay between genetic factors, immune cell dynamics, and the inflammatory response in RA, contributing a step to future research into targeted therapies and personalized treatment strategies.

Author Contributions

Kangsong Tian: conceptualization. **Kangsong Tian:** methodology. **Kangsong Tian:** software. **Jie Guo and Qian Yan:** validation. **Jie Guo, Qian Yan:** formal analysis. **Jie Guo, Qian Yan:** data Curation. **Kangsong Tian:** writing – Original Draft. **Ning Wang:** writing – Review and Editing. **Jie Guo, Qian Yan:** visualization. **Ning Wang:** project administration. All authors read and approved the final version to be published.

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The authors have nothing to report.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data analyzed in this study are openly available in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The results of PCA basing on various cohorts. **Figure S2:** Functional enrichment analysis of candidate genes. (A). Significantly enriched KEGG pathways. (B). The top 10 significantly enriched GO terms of three aspects, BP, CC, and MF. **Figure S3:** (A–F). Expression levels of *IFIT3* in naive $CD8^+$ T cells, effector memory CD8 T cells, central memory CD8 T cells, CD4 T cells, neutrophils, and NK cells. (G). Differences between $IFIT3^{high}$ and $IFIT3^{low}$ groups in adaptive immunity and innate immunity. (H). Differences between $IFIT3^{high}$ and $IFIT3^{low}$ groups in T-cell response. **Table S1:** Genes screened by WGCNA. **Table S2:** 58 candidate genes. **Table S3:** Results of GO, KEGG enrichment analysis. **Table S4:** Top five genes calculated by MNC algorithm. **Table S5:** Prediction of binding sequence for the TF BCL11B. **Table S6:** Results of GSEA and DO analysis. **Table S7:** List of 295 drugs exhibiting binding potential with IFIT3.

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ORIGINAL ARTICLE

Toward Identifying a Multivariate Correlation of Septic Arthritis With a Machine Learning Approach: Time to Reset the Current Australasian Guidelines?

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ABSTRACT

Objectives: To understand the complexity of disease pathology through the prism of septic arthritis, especially the reliability of popular and, yet, arbitrary thresholds like synovial leucocyte counts of ≥100,000/μL suggestive of it, with the help of statistical analysis and logistic regression.

Methods: An anonymized patient dataset comprising 360 swollen joint episodes was collated along with a range of patient attributes, including age, gender, comorbidity (e.g., diabetes, gout, pseudogout, immunosuppression), prior administration of antibiotics and washout of the affected joint, isolation of crystals from synovial aspirate, blood/synovial fluid culture growth, and synovial aspirate cell count. The dataset was subjected to statistical analysis (e.g., sensitivity, specificity, predictive and likelihood ratios) and logistic regression modeling, with results compared to the synovial leucocyte count thresholds of ≥100,000/μL and ≥50,000/μL.

Results: The logistic regression model (sensitivity 50%, specificity 97.04%) outperformed the models based on arbitrary thresholds like a synovial leucocyte count of ≥100,000/μL (sensitivity 48.21%, specificity 88.16%) or ≥ 50,000/μL (sensitivity 64.29%, specificity 69.74%) in predicting septic arthritis. Independent variables like age, presence of gout, and autoimmune arthritis as comorbidities, hip joint involvement, synovial aspirate leucocyte count, and crystals in aspirated fluid demonstrated a significant ($p < 0.05$) correlation to septic arthritis.

Conclusion: Septic arthritis presents a multivariate correlation that deserves a holistic oversight rather than singling out individual factors. Data mining platforms like logistic regression can investigate the complex interplay among these individual variables while making a diagnosis not only in septic arthritis but also in other diseases with multisystem involvement, infective or non-infective alike.

1 | Introduction

The association of septic arthritis (SA) with rheumatoid arthritis (RA), especially in RA patients on anti-tumor necrosis factor-alpha therapy, is well established and is often considered a hallmark of worse prognosis [1]. Joint sepsis is frequently caused by bacterial infection (80%) [2], although viruses [3], mycobacteria [4], and fungi [5] may also be responsible. Other than *Neisseria gonorrhoeae*, which causes gonococcal arthritis in young sexually active adults [6], *Staphylococcus aureus* (including Methicillin-Resistant *Staphylococcus Aureus* (MRSA) strains, 40%), *Streptococcus pneumoniae* (28%), *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Escherichia coli* are frequently isolated from the affected joints in non-gonococcal arthritis [3]. The association between SA and RA is attributed to a variety of factors, including immunosuppression (often due to prolonged steroid therapy) [7], damaged articular surfaces with synovial neovascularization [8], and impaired phagocytosis by the polymorphonuclear leucocytes in the synovial fluid [9].

Other than RA, underlying risk factors of SA include diabetes [10], osteoarthritis [11], immunosuppressed states due to senility [12], prior medication or HIV infection [13], recent joint replacement [14], gout [15], and a history of intravenous drug abuse [16]. The incidence of SA varies between 4 and 29 per 100,000 person-years, while 85% of patients reported the contributing factors mentioned earlier [17]. In Australia, the incidence of SA at the Top End of the Northern Territory between 1978 and 1994 was 9.2 per 100,000 person-years and 29 per 100,000 person-years among Aboriginal Australians [18]. Diabetes, corticosteroid administration, pre-existing joint diseases, and alcohol abuse were identified among the commonest risk factors. Non-gonococcal SA accounted for 88% of cases, while 80%–90% were monoarticular episodes.

An early diagnosis remains crucial for the effective management of SA with limited disability and morbidity. Due to a lack of clinical correlation and inadequate symptomatic findings, arthrocentesis remains the mainstay, while synovial aspirate bacterial culture is the gold standard of SA diagnosis [19]. Unfortunately, a global consensus on the synovial leucocyte count threshold to segregate the SA cases from aseptic arthritis (AA) is missing, although the current Royal College of Pathologists Australasia (2019) guidelines, followed extensively in Australia and New Zealand, use synovial leucocyte counts of $\geq$ 100000/μL and $\geq$ 50,000/μL as diagnostic markers of SA and bacterial arthritis, respectively [20].

However, using such synovial leucocyte count thresholds to separate the SA cohort from AA remains controversial. Drawing such arbitrary watershed lines between SA and AA also ignores the role of contributing factors. The way a *disease* is defined in modern medicine is undergoing a major overhaul, and a paradigm shift is emerging where, instead of a univariate model, a multivariate analysis of disease etiology is gaining traction [21]. The core concept here encompasses an idea that the disease is a collective expression, clinically ingrained as the *signs* and *symptoms*, of a broader, interrelated web of factors that, in cohesion, cause the pathology as an outcome. Thus, other factors must be weighed to make a clinical diagnosis methodologically robust, clinically justified, and rationally defensible.

Finally, synovial leucocyte count and culture vary based on other factors, such as prior antibiotic therapy, a common scenario in SA that commences before joint aspiration and invariably influences the synovial leucocyte count [22]. Similarly, peripheral blood (sensitivity 26%–36%) or synovial fluid (sensitivity 29%–65%) culture, including Gram staining, has produced weaker correlation patterns that, apart from being statistically insignificant, also suffered from other inadequacies. Despite being a helpful research tool, the Newman classification [23–25], which linked isolated microorganisms from the affected joint or elsewhere in the patient's body to corroborate SA, also suffers from erratic correlation.

The aims and objectives of the study were: (i) to collect and curate a retrospective (anonymized) dataset of patients with arthritis; (ii) to explain the dataset and understand the extent of SA or AA with descriptive statistical parameters; (iii) to analyze the dataset and develop a binary (SA or AA) modeling paradigm with logistic regression; (iv) to comprehend how the independent variables, including synovial leucocyte count and allied comorbidities, influenced the final diagnosis (dependent variable) of SA or AA; (v) to compare the obtained results with the ones derived from synovial leucocyte count-based thresholds of 100000/μL and 50000/μL on the merit of performance and discrimination capability.

2 | Patients and Methods

2.1 | Ethical Approval

Ethics statement was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research to conduct an observational retrospective cross-sectional study for patients managed in public hospitals within the Top End Health Service (Royal Darwin Hospital, Katherine District Hospital, and Gove District Hospital), with an approval code: EFILE2020/25864—HREC 20–3766.

2.2 | Patient Selection and Data Collection

Data were extracted from the local Labtrak pathology database for synovial fluid leucocyte counts of affected joints from 01/01/2017–31/12/2019 (three years). Episodes were excluded if it occurred outside the study period, had inaccessible medical records, involved extra-articular samples, duplicate samples from the same joint, or unavailable crystal microscopy or culture results. Two researchers reviewed the Electronic Medical Record and supplemented it with paper charts, as required for the inpatient stay and correspondence from relevant clinics within three months of the index hospital discharge.

Diagnoses were defined based on what was documented in the hospital discharge summaries or outpatient clinic correspondence. Modified Newman criteria were used to classify SA cases into three tiers: definite (Newman A), probable (Newman B),

and possible (Newman C). Definite SA indicated that a clinically significant microorganism was isolated from the joint and identified by Gram staining, culture, or polymerase chain reaction. Probable SA was defined as a significant microorganism isolated in a clinical specimen collected from elsewhere other than the affected joint. Finally, a possible SA had no microorganism isolated, but physicians made the diagnosis based on available information, including (but not limited to) clinical examination, synovial fluid cell counts, radiology, histology, and response to antibiotics. The length of stay included hospital-in-the-home and inpatient rehabilitation. Immunosuppression was defined as per the hospital guidelines. The duration of antibiotic administration included treating coexisting infections, such as endocarditis or osteomyelitis.

The collated data included information on (anonymous) patient ID, gender, age, presence of immunosuppression, diabetes, gout, and pseudogout, autoimmune arthritis (RA and seronegative arthritis), Charlson comorbidity index (CCI) [26], any history of prior administration of antibiotics (and if yes then duration in days), Newman score (A, B, and C), length of hospital stay in days, synovial aspirate cell count, history of joint washout(s), any microbial growth noticed in blood or synovial fluid culture, presence of crystals in synovial fluid, and diagnosis (as mentioned in the discharge summary). The attributes were further classified as metric (age, antimicrobial duration, aspirate cell count/μL, and hospital stay), ordinal (CCI), and nominal (patient ID, gender, immunosuppression, diabetes, gout, pseudogout, autoimmune arthritis, antibiotic, joint aspirated, single joint, washout, synovial culture, blood culture, crystals, limb (upper/lower), (if) inflammatory, and diagnosis). The assimilated data were tabulated in an MS Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA). A collinearity check between the independent variables was not feasible, as many of them were ordinal or nominal data; therefore, a correlation matrix could not be generated. However, such collinearity analysis is advisable in future studies when feasible.

2.3 | Data Analysis

The descriptive statistical analysis and logistic regression modeling were conducted online at DATAtab (https://datatab.net/), a web-based statistical application with a live calculation tracker. The MS Excel spreadsheet was uploaded into its interface using the *Export/Import* tab, followed by designating the columns as nominal, ordinal, or metric variables. The *Descriptive Statistics* wizard was then used to calculate the location (mean and median) and dispersion (standard deviation, variance, and range) parameters.

2.4 | Logistic Regression Modeling

A logistic regression analysis was conducted as an *Inferential Statistics* exercise where the logistic function $f(z)$ was defined as:

$$f(z) = \frac{1}{1 + e^{-z}}$$

$$\Rightarrow z = b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + \ \ldots \ + b_{k-1} \cdot x_{k-1} + b_k \cdot x_k + b$$

Here, x_1, x_2, x_3, ..., x_{k-1}, x_k were independent variables, whereas, b_1, b_2, b_3, ..., b_{k-1}, b_k and b stood for the regression coefficients and regression constant, respectively.

For binary logistic regression analysis (SA or AA), the same online DATAtab online platform was used, while the reference categories for the attributes were as follows: gender–female, immunosuppression–absent, diabetes–absent, gout–absent, pseudogout–absent, autoimmune arthritis–absent, joint aspirated–elbow, single joint–yes, CCI–0, and crystals–no. Data on prior joint washout and inflammation were excluded as confounding factors. The duration of antibiotic therapy was excluded from the predictive model due to the retrospective nature of this study, while its selection would have skewed the predictive ability of the model (by pre-selecting patients who were treated for SA already).

The Chi-square (χ^2) value was assessed, with a statistical significance level set at $p < 0.05$. Furthermore, the sensitivity and specificity were determined. The null hypothesis was that there was no relationship between the observed and expected frequencies of the outcomes predicted by the model. The goodness of fit was measured by −2 Log-Likelihood and Cox & Snell R^2 [27], Nagelkerke R^2 [28], and McFadden's R^2 [29] values. A receiver operating characteristic (ROC) curve [30] was developed based on logistic regression and synovial leucocyte-based thresholds of ≥ 100,000/μL and ≥ 50,000/μL, with estimations of the area under the curve (AUC) for all models.

2.5 | Statistical Analysis

Using a confusion matrix for logistical regression modeling, the following statistical parameters were measured: sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, prevalence, and accuracy. For comparison purposes, similar confusion matrices were created for the commonly used synovial leucocyte count thresholds of ≥ 100,000/μL and ≥ 50,000/μL, while identical statistical parameters were calculated. Data plotting was done with OriginPro 2024b software (OriginLab Corporation, Northampton, MA, USA).

3 | Results

3.1 | Patient Demographics and Descriptive Statistics

A cohort of 477 aspirated joints was identified from the Labtrak database, and 117 were excluded based on the exclusion criteria, resulting in a final dataset comprising 360 episodes (Figure 1). Within the dataset, 15.56% (56/360) were diagnosed as SA and 84.44% (304/360) as AA. The mean age of the entire cohort was 50.57 years (range 0–90 years, standard deviation/SD 18.98 years). The cohort consisted of 71.11% males (range 90 years, mean age 51.52 years, SD 17.09 years) and 28.89% females (range 84 years, mean age 48.23 years, SD 19.91 years). Patients with SA were younger (mean age 44.13 years) than the AA (mean age 51.76 years) cohort. Among the 56 SA episodes,

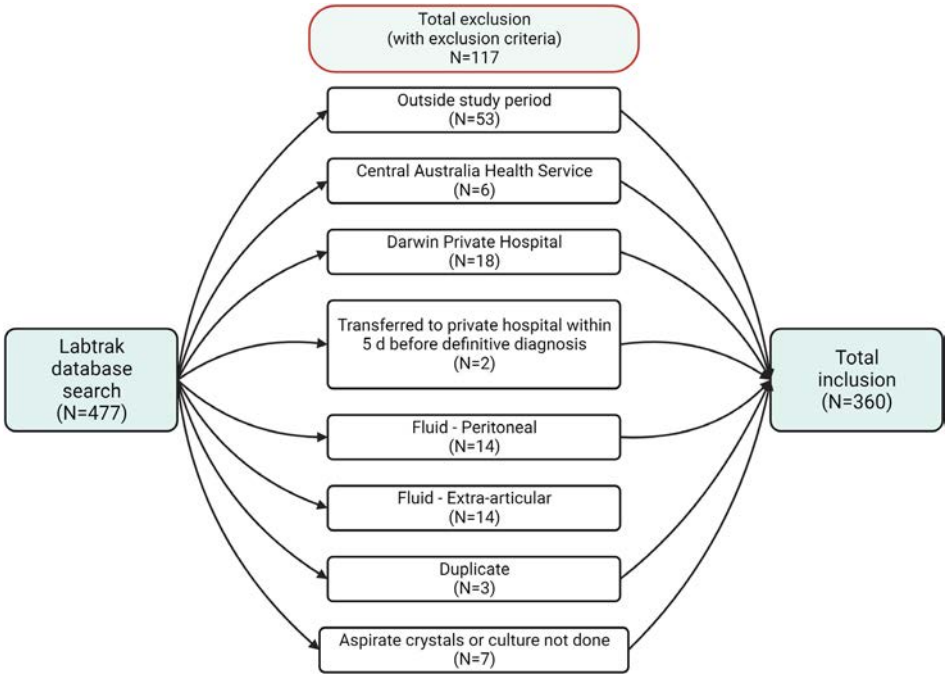

FIGURE 1 | Scheme showing the methodology followed, including the exclusion criteria, to build the dataset. The number of included or excluded episodes is shown as counts (*N*).

15 were categorized as Newman A, 15 as Newman B, and 26 as Newman C. The commonest microorganism identified in SA episodes was methicillin-sensitive *Staphylococcus aureus* (9/56), followed by MRSA strain (2/56), *Neisseria gonorrhoeae* (3/56), and (Group A) *Streptococcus pyogenes* (3/56). Other microorganisms detected included *Neisseria gonorrhoeae*, *Burkholderia pseudomallei*, *Escherichia coli*, *Streptococcus* sp. (Groups C and G), and *Kingella kingae*. Key patient demographics, comorbidity, and clinical data are summarized in Table 1, while the entire dataset is provided as Supporting Information S1.

The mean synovial leucocyte count of the cohort was 60,578/μL (range 827 - 987/μL, SD 100785). Crystals were detected in 46% of cases, and synovial cultures were positive in 3% of patients. When collected, blood cultures were positive in 11% of patients (18/159). The SA cases (Newman A, B, and C) had a similar distribution of leucocyte counts, while the mean leucocyte count in SA (range 827 - 950/μL, mean leucocyte count 141 642/μL, SD 177523/μL) was higher than in AA (range 678 - 987/μL, mean leucocyte count 45 645/μL, SD 69831/μL). The mean CCI of the entire cohort was 2.04 (range 11, SD 2.26). Relevant comorbidities included crystal-induced arthritis (32%), diabetes (24%), autoimmune arthritis (12%), or immunosuppression (8%). Knees were the predominant joint aspirated (80.56%), while 81% were monoarticular episodes.

3.2 | Effect of Pre-Existing Comorbid Conditions on Synovial Leucocyte Count

The mean synovial leucocyte counts for comorbid conditions (gout, pseudogout, diabetes, and immunosuppression) are shown in Figure 2. The results were filtered for each comorbid condition based on the final diagnosis: SA and AA. In the case of gout (Figure 2A), the mean synovial leucocyte counts for SA and AA were 198,600/μL and 56,129/μL, respectively. Interestingly, these values were diminished for the SA (138,418/μL) and AA (40,272/μL) without gout. On the contrary, the mean synovial leucocyte counts were higher in the absence of pseudogout (Figure 2B) for both SA (143,628/μL) and AA (45,649/μL) than in its presence: SA (32,400/μL) and AA (45,421/μL).

In diabetes as a pre-existing condition (Figure 2C), the overall trend was similar to gout, where the mean synovial leucocyte counts in SA were lower in the absence (134 625/μL) than in the presence (159 186/μL), whereas for AA, the readings were comparable (presence: 41056/μL; absence: 47043/μL). The trend for immunosuppressed patients (Figure 2D) was similar to that of pseudogout, with a higher mean synovial leucocyte count for both SA and AA in the absence (SA: 145991/μL; AA: 46623/μL) in comparison to the presence (SA: 64800/μL; AA: 35603/μL) of immunosuppression.

3.3 | Logistic Regression Analysis

The regression analysis correctly predicted (accuracy) the SA or AA in 323/360 (89.72%) patients in the entire cohort ($N = 360$). The sensitivity (correct predictions for SA) was 50% (28/56), while the specificity (correct predictions for AA) was 97.04% (295/304). In comparison, when a synovial leucocyte count threshold of ≥ 100,000/μL was used, the sensitivity was 48.21% (27/56) and specificity 88.49% (269/304). Similarly, a threshold of ≥ 50,000/μL yielded a sensitivity and specificity of 64.25% (36/56) and 69.74% (292/304), respectively. The confusion matrices with different measured statistical parameters are provided in Table 2.

TABLE 1 | Summary of patient demographics of the studied dataset ($N = 360$). Where applicable, the % contributions to the representative cohort are also marked.

	Entire cohort		Only SA		Only AA	
	# Episodes	%	# Episodes	%	# Episodes	%
Total	360	100	56	100	304	100
Male	256	71.11	34	60.71	222	73.03
Female	104	28.89	22	39.29	82	26.97
Age (years)						
Mean ± SD	50.57 ± 17.98		44.13 ± 23.31		51.76 ± 16.6	
Comorbidities						
Immunosuppression	30	8.33	3	5.36	27	8.88
Diabetes	87	24.17	16	28.57	71	23.36
Gout	106	29.44	3	5.36	103	33.88
Pseudogout	7	1.94	1	1.79	6	1.97
Autoimmune arthritis	43	11.94	2	3.57	41	13.49
Joint aspirated						
Knee	290	80.56	39	69.64	251	82.57
Ankle	29	8.06	2	3.57	27	8.88
Elbow	17	4.72	2	3.57	15	4.93
Hip	10	2.78	5	8.93	5	1.64
Shoulder	9	2.5	7	12.5	2	0.66
Wrist	5	1.39	1	1.79	4	1.32
Relevant findings						
Crystals	166	46.11	9	16.07	157	51.64
Charlson Comorbidity Index						
Mean ± SD	2.04 ± 2.26		2.18 ± 2.5		2.01 ± 2.22	

Abbreviations: AA, aseptic arthritis; SA, septic arthritis; SD, standard deviation.

The χ^2 value of the logistic regression model was estimated to be 123.82 with 26° of freedom and a p value of < 0.001, suggesting a zero probability of observing a χ^2 value of 123.82 if the null hypothesis was upheld. Furthermore, a p value of < 0.001 indicated a statistically significant relationship at the 5% level. The −2 Log-Likelihood value was 187.38, which indicated the model's fit relative to a perfect fit. The Cox & Snell and Nagelkerke R^2 values of 0.29 and 0.5 meant that these models could explain 29% and 50% of the observed variances, respectively. McFadden's R^2 was estimated to be 0.4. These data collectively established that the model was significant.

The data from the logistic regression analysis, including the regression coefficients (b_x) and p values, are shown in Table 3 and were used to develop an interactive model to determine the probability % of SA when the independent variables were adjusted. With a measured AUC of 0.917, the ROC curve (Figure 3) also demonstrated the logistic regression model's improved performance compared to the synovial leucocyte count-based thresholds of ≥ 100,000/μL (AUC 0.653) and ≥ 50,000/μL (0.573).

4 | Discussion

The logistic regression analysis highlighted the complex interplay between correlative factors that contributed to a diagnosis of SA and its distinction from AA. The male gender showed a positive correlation, although it was insignificant. Interestingly, age showed a significant correlation ($p < 0.05$) to SA characterized by a negative regression coefficient. Immunosuppression, on the other hand, despite an insignificant correlation, demonstrated a positive regression coefficient and an odds ratio of 3.47. The correlation to SA also varied among upper and lower limb joints. In the upper limb, the shoulder joint elicited a positive correlation, whereas the wrist joint demonstrated a negative correlation (both statistically insignificant). Conversely, all the lower limb joints (knee, hip, and ankle) exhibited positive regression coefficients. However, only the hip joint demonstrated a significant correlation ($p < 0.05$), a worthwhile finding from a clinical perspective.

The presence of comorbidities (e.g., diabetes, pseudogout) positively correlated with SA, indicating their contributory roles.

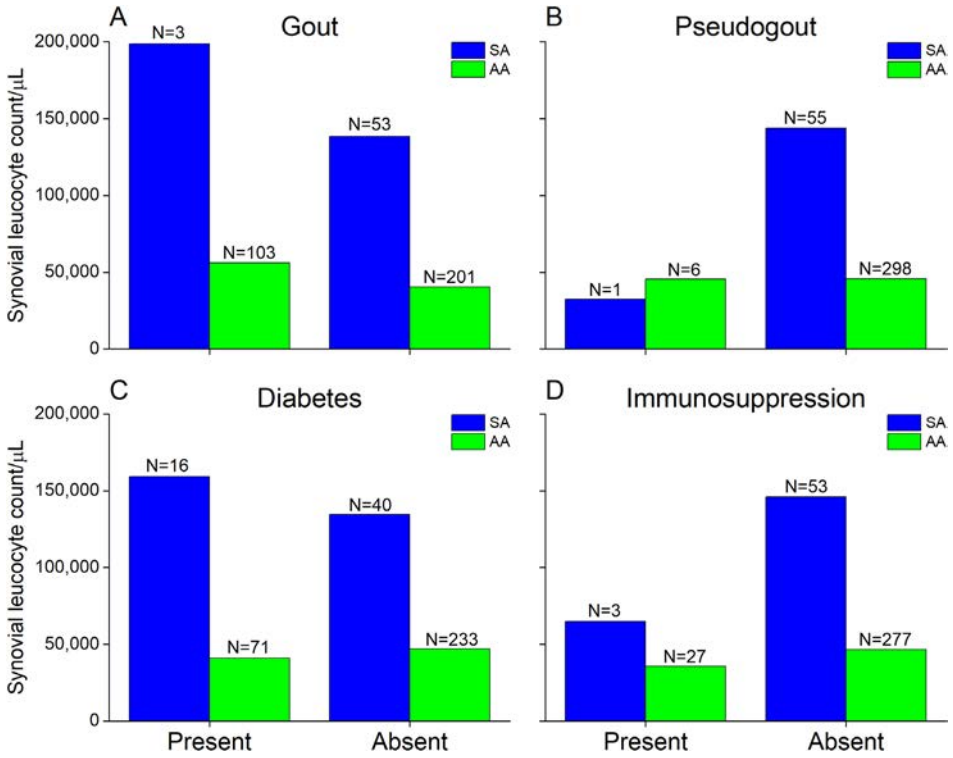

FIGURE 2 | The mean synovial leucocyte counts in patients with comorbidities (A) gout; (B) pseudogout; (C) diabetes; and (D) immunosuppression. The results were further filtered along the lines of discharge diagnosis (SA and AA) while the respective counts (*N*) are mentioned.

TABLE 2 | Summary of the parameters derived from confusion matrices from the logistic regression model and the two synovial leucocyte count thresholds of 100,000/μL and 50,000/μL.

Method	TP #	FP #	TN #	FN #	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+ (%)	LR− (%)	Accuracy (%)
Logistic regression	28	28	295	9	50	97.04	75.68	91.33	16.89	0.52	89.72
≥ 100 000/μL	27	36	268	29	48.21	88.16	42.86	91.38	4.34	0.59	81.94
≥ 50 000/μL	36	92	212	20	64.29	69.74	28.13	91.38	2.12	0.56	68.89

Abbreviations: FN, false negative; FP, false positive; LR−, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

Conversely, gout and autoimmune arthritis showed significant ($p < 0.05$) yet negative correlations. The presence of crystals in joint aspirates also exerted a significantly negative correlation, and it would be apt to view the results through the lens of coexisting gout or pseudogout. It is known that, especially in monoarthritic cases, crystal-induced arthritis and SA often coexist [31], and the presence of crystals does not exclude SA or *vice versa* [32, 33]. In the presence of gout, the mean leucocyte count in SA was threefold higher than in AA. However, a similar trend emerged even without gout, making it difficult to comment on whether gout had an additional impact on synovial leucocyte count.

Further interesting patterns emerged when the mean synovial leucocyte counts were filtered by the presence or absence of diabetes and immunosuppression. In the presence of diabetes, the mean leucocyte count was higher in SA than in AA, and this trend was also observed in its absence. However, although a similar trend was noticed for immunosuppression, the mean synovial leucocyte count in SA without immunosuppression was higher than in those with it. However, the gross disparity between the number of SA ($N = 56$) and AA ($N = 304$) cases in the entire cohort makes it difficult to draw firm conclusions, and further verification from analysis on larger datasets with a comparable number of SA and AA cases is necessary. Unfortunately, avoiding such disparity of instances in a retrospective study like this one is difficult. Moreover, attempting to enforce a numerical equivalence, for example, by randomly selecting 56 AA cases to match the 56 SA ones, risks leaving useful variables unexplored and excluded from the model, thereby rendering it untrustworthy with poor performance.

The logistic regression modeling of SA, or its absence, outperformed diagnoses made on isolated and arbitrary thresholds,

TABLE 3 | The obtained model after logistic regression analysis of the dataset. The numerical values were approximated to the second decimal place, while p values < 0.05 (statistical significance) were marked in bold fonts.

	Coefficient b_x	Standard error	Z	p	Odds ratio	95% CI
Constant	−1.41	1.4	1	0.315	0.24	0.02–3.83
Gender—Male	0.35	0.43	0.82	0.412	1.42	0.61–3.29
Age	−0.05	0.02	3.1	**0.002**	0.95	0.92–0.98
Immunosuppression—Present	1.25	1.42	0.88	0.38	3.47	0.22–56.12
Diabetes—Present	0.92	0.61	1.5	0.134	2.5	0.75–8.28
Gout—Present	−1.71	0.77	2.23	**0.026**	0.18	0.04–0.81
Pseudogout—Present	1.93	1.43	1.35	0.177	6.91	0.42–114.22
Crystals—Yes	−2.07	0.54	3.84	**< 0.001**	0.13	0.04–0.36
Autoimmune arthritis—Present	−3.16	1.49	2.13	**0.033**	0.04	0.0–0.78
Single joint—No	−0.66	0.57	1.15	0.249	0.52	0.17–1.59
Aspirate cell count/μL	0	0	4.49	**< 0.001**	1	1–1
CCI 1	−0.64	0.88	0.73	0.466	0.53	0.09–2.94
CCI 2	1.25	0.83	1.52	0.129	3.51	0.69–17.73
CCI 3	2.33	0.97	2.41	**0.016**	10.32	1.54–68.97
CCI 4	1.23	1.28	0.96	0.335	3.42	0.28–41.75
CCI 5	1.36	1.09	1.25	0.21	3.91	0.46–32.88
CCI 6	1.8	1.27	1.41	0.157	6.02	0.5–72.58
CCI 7	1.1	1.5	0.73	0.465	3	0.16–56.85
CCI 8	−16.12	8107.4	0	0.998	0	0–∞
CCI 9	−19.08	16289.72	0	0.999	0	0–∞
CCI 10	−18.23	16289.72	0	0.999	0	0–∞
CCI 11	2.22	1.81	1.23	0.219	9.18	0.27–316.38
Joint aspirated—Shoulder	3.04	1.63	1.87	0.062	20.96	0.86–512.76
Joint aspirated—Wrist	−0.91	1.9	0.48	0.632	0.4	0.01–16.73
Joint aspirated—Hip	3.44	1.58	2.18	**0.029**	31.34	1.41–694.72
Joint aspirated—Knee	1.28	1.3	0.99	0.323	3.6	0.28–45.56
Joint aspirated—Ankle	0.4	1.5	0.27	0.79	1.49	0.08–28.46

Abbreviations: CCI, Charlson comorbidity index; CI, confidence interval.

such as the synovial leucocyte counts of ≥100,000/μL or ≥ 50,000/μL. The improvement elicited by logistic regression, often remarkably, was grossly reflected in many of the considered parameters, including specificity and accuracy, and AUC in ROC curves, demonstrating its edge over synovial leucocyte count-driven models. Regrettably, regarding sensitivity, the logistic regression model failed to demonstrate an edge over the synovial leucocyte count thresholds. Its sensitivity was less than the 50,000/μL threshold, although slightly better than 100,000/μL.

However, when estimated over diverse parameters, including accuracy and AUC ROC, the logistic regression model outperformed the 50,000/μL and 100,000/μL thresholds. Similarly, in terms of specificity, it outperformed the other two threshold-driven models. Taken together, despite a disappointing show on sensitivity, which may stem from a mismatch between the numbers of SA and AA cases in the dataset, the overall performance of logistic regression made a valid case over the other two threshold-based models. However, acquiring a trustworthy, robust dataset with sufficient case numbers and homogeneously distributed independent variables across genders is necessary to enhance the performance of such a machine learning platform.

As an online tool, DATAtab was a helpful and intuitive resource that provided step-by-step guidance and explanations that clinicians would find useful. The emergence of online platforms like DATAtab or other comparable software provides fresh opportunities for clinicians to explore their datasets with interactive

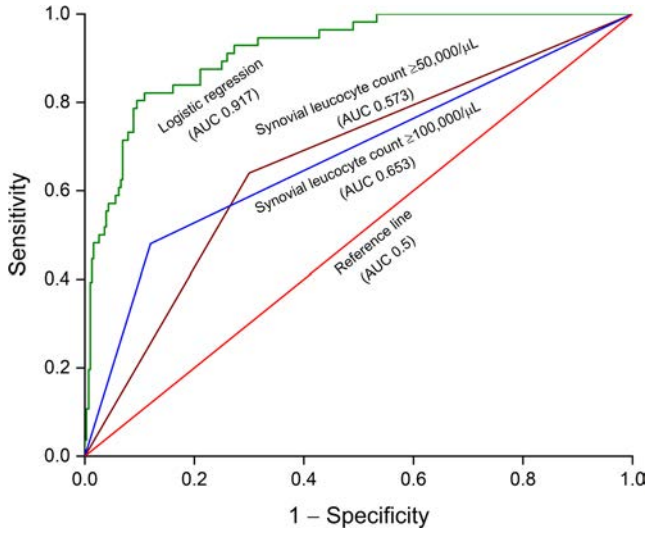

FIGURE 3 | The ROC curve (green trace) using the binary logistic regression model used in this study (AUC = 0.917) showed improved modeling of the data compared to the synovial leucocyte-based thresholds of ≥ 100,000/μL (blue trace) with an AUC of 0.653 and ≥ 50,000/μL (brown trace) with an AUC of 0.573. The reference line (AUC = 0.5) is marked in red.

visualization. Additionally, their integrated cloud computing systems obviate the need to download or run heavy applications and install dedicated workspaces with expensive data servers for saving data. This is particularly useful for improving the cost-effectiveness of analysis, while it also aids data or skill transfer.

It is challenging to determine the optimal case number when building such a dataset. On realistic grounds, it may be implausible to collect data from a sufficient number of patients, especially in rare diseases. Additionally, even in an adequate dataset, further specifics, such as the optimal ratios of male-to-female patients or SA-to-AA episodes, need to be resolved. Perhaps no standard guideline or *fit-for-all* model can explain the variance in all the datasets. With the increasing digitization of patient records, such a model might require further optimization and refinements using available local, regional, or national epidemiological data on the disease under consideration.

Only a CCI index of 3 exhibited a significant ($p < 0.05$) correlation with SA, while the remaining indices (1, 2, 4–11) did not. However, an interesting aspect was that the lower CCIs (1–7) demonstrated positive regression coefficients of lower magnitude (except for CCI 1, which had a low-magnitude negative coefficient), whereas the regression coefficients increased in magnitude (roughly around −20) for higher indices (8–11). Notably, higher comorbidities did not always increase the probability of SA in a patient.

Standardization of the independent variables is necessary for developing models that are applicable across the cross-section of various healthcare systems. However, from the perspective of this study, such standardization was avoided as the original scale of the independent variables was deemed necessary for regression analysis. Moreover, for a complex disease like SA that may involve multiple organs and systems, it is difficult to pinpoint the specific details of such variables. Medical organizations, such as the Royal College of Pathologists of Australasia or its equivalents, need to come forward and, when necessary, collaborate to suggest protocols and harmonize the current clinical and diagnostic paradigms.

Finally, the synovial aspirate leucocyte count, the pivotal topic of discussion here, showed a significant correlation with SA, with a p value of < 0.001. Thus, as an independent variable, the joint aspirate leucocyte count demonstrated an undeniable correlation to SA [34, 35]. Perhaps that also provided some rationality behind using arbitrary synovial count thresholds for diagnosing SA, or at least where the roots of such a pervasive concept rest. However, when taken in its entirety, the logistic regression analysis unveiled the precarious nature of any univariate diagnostic criterion of synovial leucocyte count and cautioned against singling out individual threads to judge the tapestry of a complex and multilayered pathology like SA.

A major strength of this study was its relatively adequate dataset, which allowed logistic regression to have ample ground for analysis, enabling predictive modeling to identify and correlate factors other than synovial cell count in the diagnosis of SA. However, the dataset suffered from its retrospective nature and associated bias, the identification of variables after confirmation of diagnosis (rather than prospectively), a lack of information limiting data collection, and, at times, an absence of data emerging from a standardized collection of cultures (including peripheral blood cultures). In a way, it highlighted the need for having a robust, well-thought-out, and well-formulated study design to be in place before data collection ensues. Such a structured study design is only possible through collaboration, dialog, and cross-pollination of ideas between the clinicians and data scientists. Future studies should include this vital range of information in their datasets. Conducting multi-center trials on larger datasets, and, most importantly, datasets collected based on standardized independent variables (if applicable), would be necessary to maximize the benefits of such a modeling-based approach, including diagnostics, for complex diseases like SA.

5 | Conclusion

A retrospective study of a patient-derived dataset comprising 360 aspirated inflamed joint episodes (15.56% SA and 84.44% AA) was conducted with statistical analysis and logistic regression to identify the correlation matrix of SA. The cohort featured 71.11% male and 28.89% female patients. Additionally, data on a set of independent variables—such as age, comorbidity (immunosuppression, gout, pseudogout, diabetes, autoimmune arthritis), CCI indices, aspirated joint (knee, shoulder, hip, ankle, wrist, elbow), and synovial aspirate cell count—were collected. A logistic regression analysis revealed a complex syntax of mechanical principles that drove the pathogenesis of SA (or its absence thereof) where patient age, presence of gout, involvement of the hip joint, synovial aspirate leucocyte count, and the presence of crystals in aspirated fluid significantly ($p < 0.05$) contributed toward a diagnosis of SA, often with high odds ratios.

The regression model overall outperformed the popular and yet inadequate (due to a lack of strong scientific rationale) guiding Australasian protocol, where synovial leucocyte counts of

≥ 100,000/μL or ≥ 50,000/μL are prioritized for a diagnosis of SA or bacterial arthritis, including the determination of sensitivity and specificity. The results firmly established the grounds for a multivariate correlation of disease etiology which, in conjunction with a robust and scientifically justifiable diagnosis, is also likely to guide the management protocols, with its scope need not be confined to SA only, but also inclusive of other multisystemic disorders. Finally, the availability of user-friendly machine learning platforms is providing a range of tools for application in clinical practice and building models that may facilitate and upgrade—if not rectify—the medical wisdom that, despite serving humanity over millennia, needs a rehash and, fortunately, remains receptive to such emerging techniques.

Author Contributions

S.B.: conceptualization, data curation, formal analysis, visualization, and writing – original draft preparation. I.C.H.: conceptualization, data collection, data curation, visualization, and writing – review and editing. S.V.: conceptualization, data collection, methodology, software, visualization, and writing – review and editing. R.W.B: conceptualization, data collection, visualization, and writing – review and editing. S.K.: conceptualization, data collection, visualization, and writing – review and editing. All authors have read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are incorporated into the article and its online Supporting Information. Further information and clarification(s) may be obtained 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. **Supporting Information S1:** Original dataset collated as an MS Excel spreadsheet.

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LETTER TO THE EDITOR

Case Report: Anifrolumab for Successful Treatment of Refractory Ulcers due to Cutaneous Lupus Vasculitis

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Dear Editor,

The type I interferon (IFN) pathway and its activation play a critical role in the pathogenesis of systemic lupus erythematosus (SLE) [1]. Anifrolumab, a monoclonal antibody against the type I IFN receptor, has exhibited high efficacy against active SLE in clinical trials [1]. The European Alliance of Associations for Rheumatology (EULAR) recommendations for the management of SLE state that anifrolumab should be considered in patients not responding to hydroxychloroquine or who are unable to reduce glucocorticoids below doses suitable for chronic use [2]. Regarding skin manifestations, post hoc analysis of pooled data from randomized clinical trials of anifrolumab showed that SLE patients receiving anifrolumab were more likely to achieve reductions in Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) score compared with patients receiving placebo [3], and anifrolumab can be considered as second-line therapy for cutaneous manifestations in SLE [2]. However, most real-world studies of anifrolumab for skin manifestations in SLE have investigated the efficacy in patients with discoid lupus erythematosus (DLE), and reports of its efficacy in non-DLE patients remain insufficient [4]. The types of skin manifestation for which anifrolumab is suitable remain unclear. We have previously reported the efficacy of anifrolumab for acute digital ischemia due to cutaneous vasculitis in a patient with SLE, so anifrolumab may represent a promising treatment for cutaneous vasculitis of SLE [5]. Here, we present the case of a Japanese man with refractory cutaneous ulcers due to lupus vasculitis who was successfully treated using anifrolumab.

A 39-year-old man was diagnosed with SLE based on leukopenia, thrombocytopenia, erythema of the lower extremities, Raynaud's phenomenon, arthritis, interstitial pneumonia with the usual interstitial pneumonia pattern, and positive results for both anti-nuclear antibodies (1:2560, speckled pattern) and anti-ribonucleoprotein antibodies. The patient reported no history of smoking cigarettes. The patient showed no findings of swollen "sausage-like" fingers or sclerodactyly and displayed negative results for systemic sclerosis-specific autoantibodies, including anti-RNA polymerase III, anti-Scl-70, and anti-centromere antibodies. Negative results were obtained for lupus anticoagulants, anticardiolipin immunoglobulin G and M antibodies, and anti-cardiolipin/β2 glycoprotein I antibodies. No hypocomplementemia or elevated creatinine kinase was observed. Spirometry showed pulmonary restriction, with forced vital capacity (FVC), 3.58 L (77% predicted); forced expiratory volume in 1 s ($FEV_{1.0}$), 2.98 L; and $FEV_{1.0}$% (($FEV_{1.0}$/FVC)×100), 83%. Skin biopsy of erythematous leg skin showed leukocytoclastic vasculitis. Administration of oral prednisolone at 20 mg/day and tacrolimus at 3 mg/day resulted in gradual improvement of leg erythema and cytopenia. Prednisolone was reduced to 5 mg/day 2 years later and is being maintained at this dose. When prednisolone was tapered to 4 mg/day at 45 years old, the patient developed cutaneous ulcers of the right extremity and increased ground-glass opacities in both lungs on computed tomography, indicating slight exacerbation of interstitial pneumonia. Mycophenolate mofetil was added at 1500 mg/day but resulted in exacerbation of cutaneous ulcers. At 47 years old, skin biopsy of the leg ulcer revealed cutaneous vasculitis below the ulcer (Figure 1A,B). Pathological findings showed infiltration of inflammatory cells in and around the vessel wall below the ulcer (Figure 1A) and disrupted internal elastic lamina (Figure 1B). No findings of panniculitis were observed. The results of pulmonary function testing at this point were as follows: FVC, 2.92 L (63% predicted); $FEV_{1.0}$, 2.62 L; $FEV_{1.0}$%, 89%; and diffusion capacity of the lung

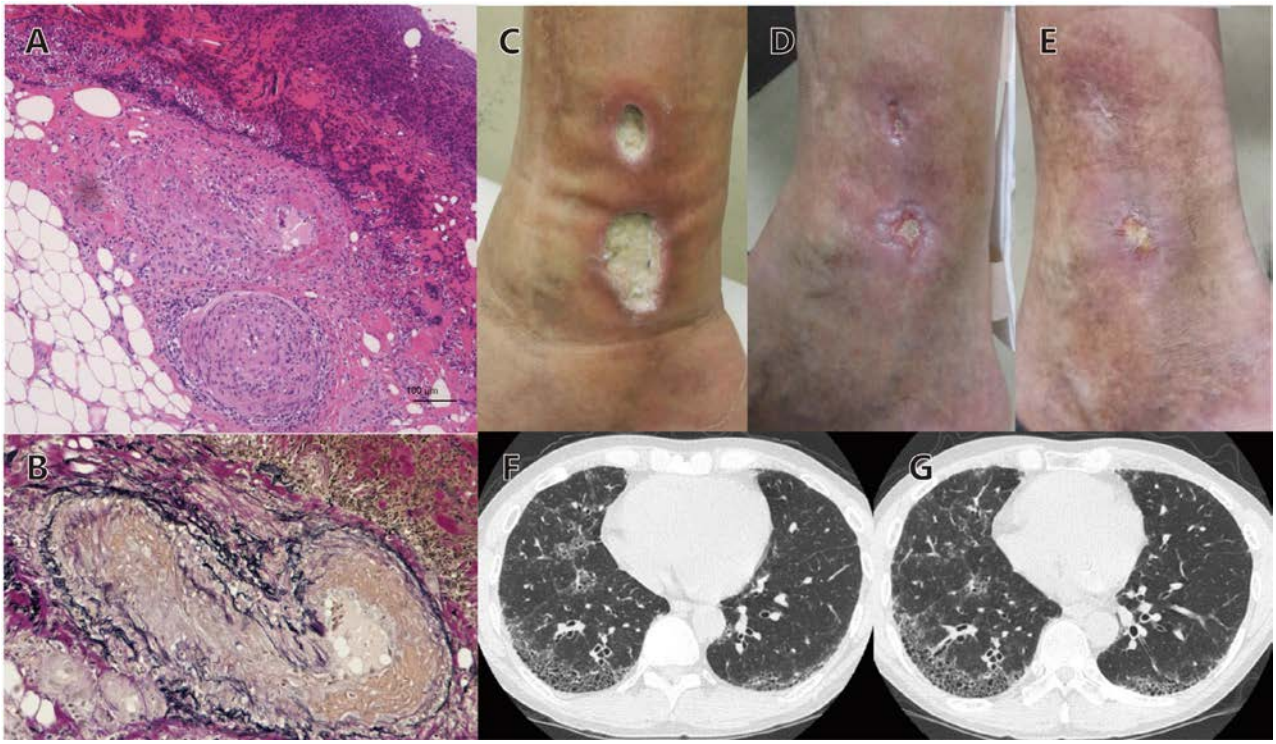

FIGURE 1 | Skin ulcers and cutaneous vasculitis in the right lower extremity and computed tomography of the lung. Pathological findings from skin biopsy of ulcers on the right lower extremity. (A, B) Infiltration of inflammatory cells is seen in and around the vessel wall below the ulcer (A) and at the disrupted internal elastic lamina (B). (A) Hematoxylin and eosin staining; (B) Elastica van Gieson staining. (C–E) Skin ulcers of the right lower extremity in our patient before anifrolumab treatment (C), and 6 months (D) and 1 year (E) after starting anifrolumab. (F, G) Computed tomography of the lung before (F) and 1 year after starting anifrolumab (G).

for carbon monoxide, 10.32 mL/min/mmHg (44% predicted). These findings indicated progression of interstitial lung disease. Prednisolone was increased to 40 mg/day, with intravenous infusion of cyclophosphamide at 500 mg every 4 weeks and discontinuation of tacrolimus. After six infusions of cyclophosphamide, interstitial pneumonia improved and was maintained with prednisolone at 10 mg/day, but no improvement of cutaneous ulcers was observed (Figure 1C). No significant bacteria were detected from the ulcers by culturing. Cyclophosphamide was switched to anifrolumab (300 mg infusions every 4 weeks) and cutaneous ulcers gradually improved over 1 year (Figure 1D,E). Although prednisolone was tapered to 5 mg/day, no deterioration of interstitial pneumonia was observed under treatment with a combination of anifrolumab and nintedanib, which is an inhibitor of tyrosine kinases (Figure 1F,G).

To the best of our knowledge, this represents the first report to suggest the efficacy of anifrolumab for refractory cutaneous ulcers due to lupus vasculitis. Although few clinical studies have examined immunosuppressive agents for SLE patients with skin manifestations not responding to first-line therapy [2], anifrolumab has shown efficacy against mucocutaneous involvements in SLE patients using CLASI [3]. However, the types of skin involvements for which anifrolumab shows efficacy remain unclear. The efficacy of anifrolumab for various types of lupus-associated cutaneous manifestations has recently been described in various case reports and case series. One case series demonstrated the efficacy of anifrolumab for not only discoid lupus but also subacute cutaneous lupus erythematosus, lupus panniculitis, chilblain lupus, alopecia, and tumid lupus [4]. The efficacy of anifrolumab for refractory cutaneous manifestations (including severe lupus panniculitis and alopecia due to discoid lupus erythematosus) has also been reported [6, 7]. In those patients, cutaneous manifestations proved refractory to glucocorticoids, hydroxychloroquine, several immunosuppressants, and belimumab, but were successfully treated using anifrolumab. Another case report showed re-pigmentation of depigmented lesions due to discoid lupus erythematosus after anifrolumab administration, which may be a potential effect of anifrolumab [8]. As in those reports, the efficacy of anifrolumab against diverse cutaneous manifestations of SLE has been reported in recent years, but the efficacy of anifrolumab against lupus vasculitis has not been reported. A previous study demonstrated that the serum type I IFN signature was associated with cutaneous vasculitis, oral ulcers, and alopecia, but was not significantly associated with malar rash [9]. Another study reported that serum IFN activity was higher in SLE patients with oral ulcers or acute cutaneous lupus, but not in those with alopecia, subacute cutaneous, or discoid lupus [10]. In patients with cutaneous lupus erythematosus, expressions of type I IFN and type I IFN response genes were significantly increased in the skin lesions of patients compared to healthy skin [11]. Given these results, type I IFN is expected to be deeply involved in the pathogenesis of skin manifestations in SLE, but whether type I IFN expressions differ between different types of skin manifestations has yet to be investigated. Patients with type I interferonopathies may show monogenic diseases in which constitutive production and dysregulation of type I IFN can be observed, including skin manifestations such as digital

vasculitis and/or necrosis, chilblains, livedo, and panniculitis [12]. These involvements can be observed in SLE patients, and specific skin manifestations may be induced by type I IFN. Indeed, we have reported the efficacy of anifrolumab for cutaneous vasculitis in a patient with SLE [5], so cutaneous vasculitis may be a type I IFN-driven manifestation. Anifrolumab can thus prove effective for cutaneous vasculitis in SLE patients. Cutaneous ulcers in our patient proved refractory to high-dose glucocorticoid and intravenous cyclophosphamide. Although upregulation of type I IFN pathways has been reported in patients with SLE, glucocorticoids showed lower efficacy for suppressing type I IFN-stimulated genes in an in vitro study [13]. That study suggested that high activity of type I IFN can induce a reduction of glucocorticoid effect and resistance to glucocorticoid therapy [13]. Moreover, another study reported a patient with SLE who exhibited a high-IFN signature status receiving intravenous cyclophosphamide. In that patient, cyclophosphamide temporarily and partially reduced the type I IFN signature, but type I IFN status returned to baseline immediately after intravenous cyclophosphamide [14]. Some SLE patients with high-type I IFN status may be resistant to glucocorticoids and cyclophosphamide, and early use of anifrolumab can be considered in such cases. To date, no treatment strategy for cutaneous ulcers by lupus vasculitis has been available. In the reports on cutaneous lupus vasculitis and digital ischemia, high-dose glucocorticoid combined with methotrexate, azathioprine, cyclosporine, cyclophosphamide, or rituximab suppressed the progression of ischemia [15, 16]. Although anifrolumab has not shown superiority over other immunosuppressive drugs against cutaneous manifestations of SLE, EULAR stated that anifrolumab can be considered as a second-line therapy for cutaneous manifestations of SLE [3]. Given the possible contribution of type I IFN to lupus vasculitis, anifrolumab may also be positioned as second-line therapy in lupus vasculitis and resulting skin ulcers, as in this recommendation. In addition, how inhibition of the type I IFN signal affects interstitial pneumonia remains unclear. Our patient showed no change in interstitial pneumonia on computed tomography after 1 year of anifrolumab therapy. Interstitial lung disease has been reported in patients with type I interferonopathies [17]. Type I IFN may thus be involved in the pathogenesis of interstitial lung disease. How the inhibition of type I IFN modifies the course of interstitial pneumonia should be carefully monitored in our patient.

In conclusion, anifrolumab may provide a promising therapeutic option for cutaneous lupus vasculitis. SLE is a heterogeneous disease, so case-based studies are required to further clarify the relationship between type I IFN and cutaneous lupus vasculitis.

Author Contributions

All authors had access to the data and played a role in writing the manuscript. S.H.: conceptualization, visualization, writing – original draft, writing – review and editing. Y.K., M.W., K.I., S.K., and D.N.: writing – review and editing.

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Consent

Written informed consent was obtained from the patient by the corresponding author. The signed consent forms are retained by the corresponding author. Patient details were anonymized as much as possible.

Conflicts of Interest

S.H. has received speaking fees from AstraZeneca. All other authors declare no conflicts of interest.

Data Availability Statement

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

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LETTER TO THE EDITOR

Bleed and Blister: A Case Report of a 40-Year-Old Woman With Hemorrhagic Bullous Henoch-Schonlein Purpura

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ABSTRACT

Henoch-Schonlein Purpura (HSP) is a rare immune vasculitis affecting small blood vessels. Hemorrhagic-bullous conversion of HSP is even rarer, especially in adults, and remains poorly characterized. We present a unique case of a 40-year-old female with hemorrhagic-bullous HSP. The patient presented with maculopapular and vesicular rashes, progressing to bullae predominantly on the bilateral legs. Past history included a similar episode at 17 years old. Laboratory findings showed elevated inflammatory markers, microscopic hematuria, and fecal occult blood. Skin biopsy confirmed leukocytoclastic vasculitis. Immunosuppression with hydrocortisone, colchicine, and azathioprine was initiated. Methylprednisolone pulse therapy was given; transitioning to prednisone. Concurrent urinary tract infection and nephrolithiasis were addressed. Lesions regressed, and the patient was discharged on a tapering prednisone regimen. HSP, particularly the hemorrhagic-bullous variant, is a diagnostic challenge; management remains varied. This case contributes to understanding this rare manifestation and emphasizes the importance of a multidisciplinary approach for optimal patient outcomes.

1 | Introduction

HSP is a type of immune vasculitis that involves the small blood vessels affecting the joints, kidneys, skin, and gastrointestinal tract [1]. It is seen more commonly in children; rarely, it may also be seen in adults [2]. In the adult population, the incidence is reported to be at 3–14/million cases [3]. Hemorrhagic-bullous transformation, reported in only 16%–60% of adult HSP cases, remains poorly characterized [4]. In this case report, we present a case of a 40-year-old female with Hemorrhagic-Bullous HSP in a tertiary hospital in Northern Luzon, Philippines.

2 | Case

We report a case of a 40-year-old woman with no known comorbidities who presented with macular, palpable papular, and vesicular rashes of the lower extremities. She had no fever or abdominal pain. Lesions were multiple, discrete, raised, reddish to violaceous, round to ovoid coalescing purpuric macules, papules, and plaques with areas of crusting, vesicles, and bullae formation, predominantly on the dorsum of the bilateral feet and legs, with purpuric papules along the thighs, hypogastric area, forearms, and back (Figure 1). The patient had a history of a similar occurrence of pruritic, palpable, purpuric rashes along the lower extremities, without any note of inciting events, and with associated colicky abdominal pain, loose bowel movement, joint pains, and bipedal edema when she was 17 years old, which regressed spontaneously without scars or hyperpigmentation after 2 weeks.

Complete blood count (RBC 4.97×10^{12}/L, WBC 9.67×0^{9}/L with 56% neutrophils and 30% lymphocytes, platelet count 357×10^{9}/L), procalcitonin (0.133 ng/mL, reference range

< 0.5 ng/mL), C3 level (129.8 mg/dL, reference range 90–180 mg/dL) were within normal levels. Additionally, anti-nuclear antibody test (16 U/mL) was negative.

Inflammatory markers including C-reactive protein (29.86 mg/L, reference range < 10 mg/L), erythrocyte sedimentation rate (25 mm/h, reference range < 20 mm/h) were slightly elevated.

Microscopic hematuria (25–50 red cells/HPF on urinalysis) and fecal occult blood (945 ng/mL, reference range < 99 ng/mL) were also detected. Colonoscopy was done, revealing an ileal ulcer, with red blood cells seen along the intestinal glands at biopsy (Figure 2).

Urinary tract infection complicated by nephrolithiasis was seen on urinalysis and CT stonogram, respectively, which could be the possible inciting event of the recurrence of the patient's skin lesions.

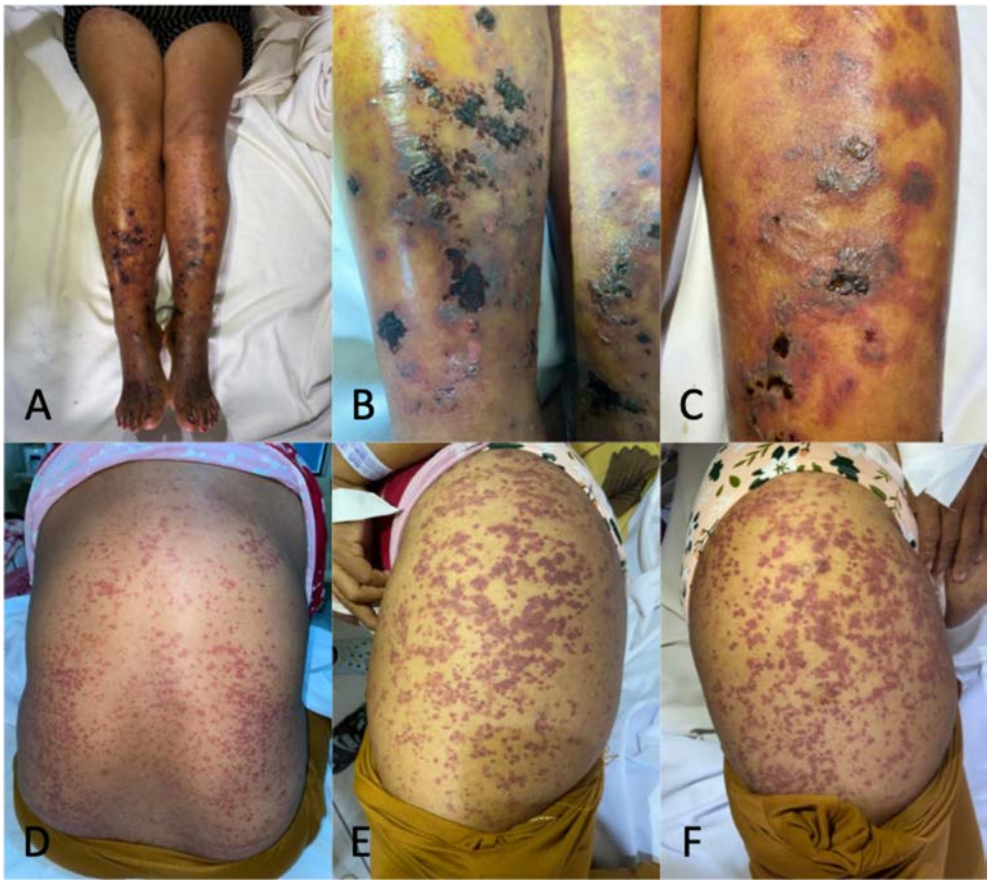

FIGURE 1 | Patient's skin lesions. (A) Lower extremities; (B, C) closer view of the legs, right and left; (D) back; (E, F) left and right lateral thighs.

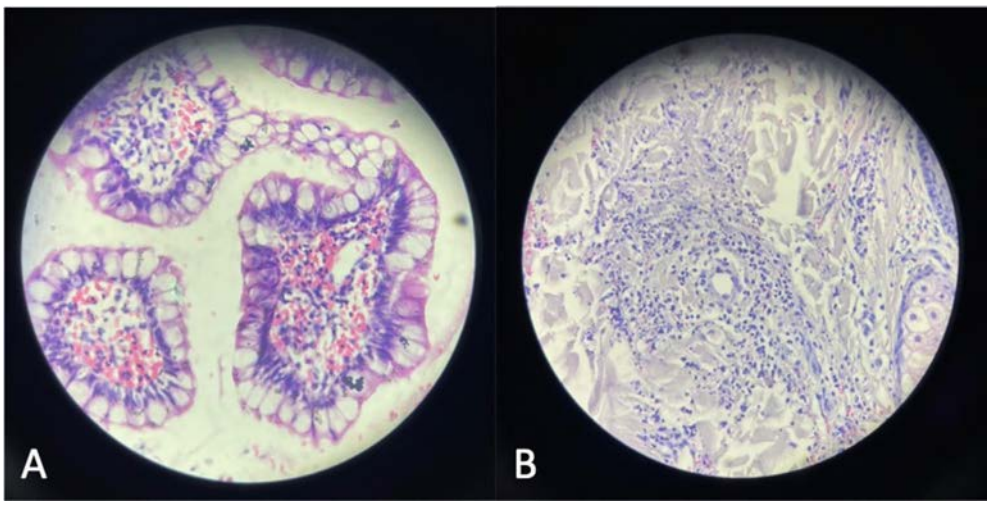

FIGURE 2 | Photomicrographs. (A) Biopsy of ileal ulcer taken from colonoscopy showing microhemorrhages; (B) skin from punch biopsy showing leukocytoclastic vasculitis.

The patient's skin lesions started as macular and papular rashes along the bilateral legs, ankles, and dorsum of the feet around 2 weeks prior to consult. The lesions rapidly progressed to the thighs, began to coalesce, and became more erythematous. Five days prior to consult, the lesions became red-purplish in color, more elevated, and vesicles erupted. Lesions continued to evolve in terms of size, height, and color, with note of bullae formation.

Incision biopsy of the skin showed leukocytoclastic vasculitis (Figure 2). Direct immunofluorescence (DIF) study for IgA was not done due to the unavailability of the test in the institution, and serum IgA level could not be measured for the same reason.

Hydrocortisone 1.5 mg/kg IV every 8 h, colchicine 500 mcg tablet thrice a day, and azathioprine 50 mg tablet once a day were started on admission (day 0). Methylprednisolone 1 g/day was pulsed on days 3–5, after which oral prednisone 1 mg/kg/day was introduced and tapered over 8 weeks (Figure 3).

The urinary tract infection was treated with Ciprofloxacin; the patient was referred to the Urology service for comanagement regarding the obstructing nephrolithiasis seen on CT stonogram.

The patient's lesions regressed, flattened, and became skin-colored, almost unnoticeable along the back, forearms, and thighs. Scabs continued to fall off along the legs and feet. The patient was sent home with colchicine 500 μg/tab TID, azathioprine 50 mg/table OD, tapering dose of prednisone, calcium and vitamin D 600/400 IU/table OD, and omeprazole 40 mg/cap OD (Figure 3).

At 1-week outpatient follow-up, the patient's cutaneous lesions continued to regress, and the scabs had dissolved. At 3 months, follow-up laboratory evaluation demonstrated a normal complete blood count (RBC 5.27×10^{12}/L, WBC 11.02×10^{9}/L with 52% neutrophils and 36% lymphocytes, platelet count 414×10^{9}/L); urinalysis showed persistent microscopic hematuria attributed to nephrolithiasis, which was removed via open nephrolithotomy by the Urology service; renal function studies revealed a BUN 2.96 mmol/L and creatinine 71.82 umol/L; and the ESR was 32 mm/h, by which time all skin lesions had completely resolved.

3 | Discussion

HSP is an IgA-mediated immune vasculitis involving the small vessels of the joints, kidneys, gastrointestinal (GI) tract, skin, and less commonly, the central nervous system and lungs. Patients with this disorder typically present with gastrointestinal complaints, palpable purpura, arthralgias, and renal involvement [1].

HSP is a rare disorder that typically affects children; however, the disorder can also be seen in adults and adolescents [1]. It is the most common childhood vasculitis, affecting 10–20/100000 children per year, [2] and the incidence in adults varies from 3 to 14/1,000,000 cases [3]. Another article reports that HSP is the most common form of childhood vasculitis in the West with an incidence of 20/100,000 aged < 17 years, but it is rarer in adults (13/million) [5].

Hemorrhagic bullous conversion is a rare occurrence compared to the typical HSP, and it has been observed more frequently in adults with HSP estimated to be ranging from 16% to 60%, compared to children with an incidence of less than 2% [4].

To our knowledge, there are only two reported or published cases of HSP in the Philippines who are above 18 years old. Most reported cases are from the pediatric population. The first is a 25-year-old male who presented with purpuric rashes on the thighs and buttocks, with gastrointestinal manifestations several days after onset of the disease, and accompanying arthralgia and polyarthritis more on the lower extremities. Acute kidney failure was noted in the course of the disease. He was given corticosteroid, and management was geared toward treatment of symptoms. The symptoms improved and no subsequent renal sequelae were observed [6]. The second is a 61-year-old female who presented with upper gastrointestinal bleeding with vasculitic type of skin lesions (black tarry stools and rashes). Upper GI endoscopy revealed findings of multiple ulcers and erosions. She was treated using a short course of corticosteroid therapy and proton pump inhibitor with significant clinical improvement [7].

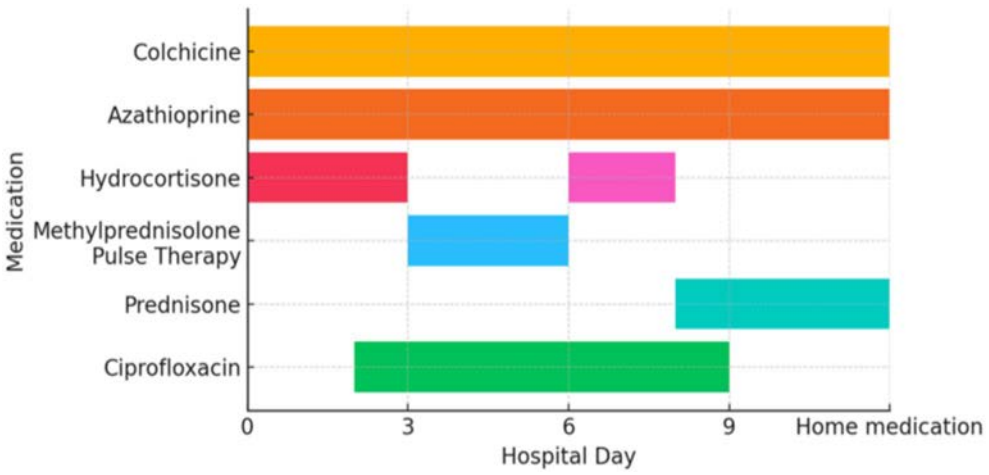

FIGURE 3 | Treatment timeline. This illustrates the initiation, discontinuation, and completion of in-hospital therapies over hospital days 0–9, and the continuation of medications at home beyond hospital day 9.

To the extent of our knowledge and search, there are no reported cases of hemorrhagic bullous type of HSP in the Philippines so far.

HSP is diagnosed using the diagnostic criteria developed by the European League Against Rheumatism, the Paediatric Rheumatology International Trials Organization and the Paediatric Rheumatology European Society (EULAR/PRINTO/PRES), wherein a mandatory criterion must be fulfilled: purpura or petechiae with lower limb predominance; and a minimum of one out of four criteria must be met: (1) diffuse abdominal pain with acute onset, (2) histopathology showing leukocytoclastic vasculitis or proliferative glomerulonephritis, with predominant immunoglobulin A (IgA) deposits, (3) arthritis or arthralgia of acute onset, or (4) renal involvement in the form of proteinuria or hematuria [2]. In our case, the patient met the mandatory criterion. Furthermore, skin biopsy revealed leukocytoclastic vasculitis, with accompanying renal involvement being observed.

Although the mechanism is still not completely known, available data have shown that most cases of HSP have been associated with an upper respiratory tract infection as its trigger, regardless of the identified pathogen. In this case, the patient's potential triggers include urinary tract infection or nephrolithiasis. The vascular endothelial cells and the leukocytes interaction plays a central role in the pathogenesis of HSP. The clinical manifestations arise from the endothelial damage, perivascular leukocytic infiltrates, chemokines, and cytokines that contribute to the underlying process. Vascular deposition of IgA1-containing immune complexes plays a pathogenic role. HSP has been associated with an IgA-mediated dysregulated immune response to an antigen. Through binding and activation of complement factors, IgA cross-reacts with endothelial cells and damages the cells. The dysregulated immune response may result in inflammation and vasculitis without a granulomatous reaction [2]. The progression or conversion of the lesions in HSP to vesicular eruption and bullae formation has not yet been explored; hence, the mechanism still remains unclear.

In a third world country, such as in the Philippines, often we encounter diagnostic challenges due to unavailable imaging studies or tests and/or financial constraints. With the development of the diagnostic criteria for HSP, which was introduced by EULAR/PRINTO/PRES, diagnosis has become more standard. In our case, immunofluorescence to demonstrate IgA or C3 deposits was not available; hence, we relied on the clinical manifestations and H&E-stained incision biopsy of the skin for diagnosis. Although skin biopsies are the gold standard in diagnosing cutaneous vasculitis, to date, many authors argue that histopathology is not indicated unless the diagnostic criteria based on clinical manifestations are not met, or the case has an atypical presentation. According to the American College of Rheumatology/Association of Rheumatology Health Professionals (ACR/ARHP), leukocytoclastic vasculitis is present in 92% of adult patients with HSP, and 81% have IgA deposition in dermal vessels through direct immunofluorescence [2].

It was unfortunate that DIF for IgA and serum IgA were not done due to the unavailability of the tests in the institution. However, although DIF for IgA is highly specific, its sensitivity is also variable and falls sharply when samples are taken beyond 7 days from lesion onset [8, 9]. If available, it is still recommended to test since there is a strong association between IgA deposits on DIF and HSP with perivascular deposits of IgA in lesional skin being seen with a frequency of 75%–100%. The presence of these, however, may not correlate with disease activity [10] with some studies showing positive findings mainly early in the disease course [11–14]. Serum IgA, which is elevated in only about 60% of adult HSP patients, is considered supportive but not essential for diagnosis [15, 16].

As of to date, there is no consensus in the management of bullous lesions in HSP. Corticosteroids remain first line for severe cutaneous or gastrointestinal involvement. Steroids act through inhibition of AP-1 binding activity in the nucleus in association with a reduction of nuclear factor-kappa B (NF-κB) and decreased MMP-2 and MMP-9 concentrations [17]. Colchicine and dapsone have been used as adjuncts for refractory disease. Colchicine is beneficial for its neutrophil-modulating, steroid-sparing properties [18]. Azathioprine, Mycophenolate mofetil, or Calcineurin inhibitors serve as steroid-sparing agents in relapsing courses. In our case, Azathioprine was initiated early because adult-onset HSP carries a higher risk of chronicity and it also showed efficacy in steroid-resistant HSP without renal involvement in a case series of pediatric patients [19]. Our patient received both Colchicine and Azathioprine early, allowing successful prednisone taper without flare. Generally, however, bullous HSP responds well to early corticosteroid therapy and has a good outcome and prognosis [20].

4 | Conclusion

Hemorrhagic-Bullous Henoch-Schonlein Purpura is a rare and complicated form of HSP. Early multidrug immunosuppression, vigilant laboratory surveillance, and urologic management of nephrolithiasis facilitated complete cutaneous remission and renal preservation in this rare adult hemorrhagic-bullous HSP case.

A high index of suspicion needs to be present when diagnosing hemorrhagic-bullous HSP, as there are several similar entities with the same presentation of bullous or vesicular lesions. Standard diagnostic criteria have been developed and have become very helpful in establishing the diagnosis. Management still needs to be standardized, although most cases benefit from early use of corticosteroids and steroid-sparing therapy. Early recognition and management are essential to avoid complications and progression to renal involvement.

Author Contributions

Brylle Domerson Turalba: conceptualization, data curation, formal analysis, writing – original draft, writing – review and editing. **Hannah Urbanozo-Corpuz:** conceptualization, writing – review and editing. **Allan Corpuz:** conceptualization, formal analysis, writing – original draft, writing – review and editing.

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Ethics Statement

The authors have nothing to report.

Consent

Written informed consent was taken from the patient for this case 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.

Brylle Domerson Turalba
Hannah Urbanozo-Corpuz
Allan Corpuz

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LETTER TO THE EDITOR

Investigation of Genetic Causality Between Epstein–Barr Virus Infection and Risk of Autoimmune Diseases

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Dear Editor,

Although a large number of studies have established the crucial roles of genetic susceptibility, environmental risk factors, and immune dysregulation in the pathogenesis of autoimmune diseases (ADs) [1], some evidence increasingly suggests a complex interplay between exogenous virus infections and the onset and development of ADs. Epstein–Barr virus (EBV) has been widely investigated due to its association with various health conditions, including infectious mononucleosis. Observational studies have suggested that ADs patients often exhibited a history of EBV infection [2]. It is hypothesized that EBV may contribute to ADs by triggering abnormal immune responses or molecular mimicry [3, 4]. However, establishing definitive causality using observational designs is challenging due to inherent limitations, including residual confounding, reverse causation, and potential measurement error. Mendelian randomization (MR) studies offer a powerful approach to infer causality by minimizing confounding. While previous studies have reported associations between EBV seropositivity and specific ADs, to our knowledge, no study has comprehensively evaluated causal relationships between multiple specific EBV antibody responses (reflecting distinct infection/immunity phases) and a broad spectrum of ADs. In this context, we employed two-sample MR (TSMR) and multivariable MR (MVMR) analyses to systematically evaluate the causal links of five distinct EBV infection markers (anti-EBV IgG seropositivity, EBV EA-D antibodies, EBV EBNA-1 antibodies, EBV VCA-p18 antibodies, and EBV ZEBRA antibodies) with the risk of seven major ADs: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes (T1D), Crohn's disease (CD), ulcerative colitis (UC), ankylosing spondylitis (AS), and psoriasis.

This study adhered to the Specifications for Reporting of Observational Epidemiological Studies in MR (STROBE-MR) guidelines and three fundamental assumptions of MR, including correlation, independence, and exclusivity (Table S1 and Figure 1) [5]. It used publicly available summary data from genome-wide association studies (GWAS) (Table S2). We utilized serological GWAS data on infectious agents from the UK Biobank (UKB) as markers for EBV infection [5]. A total of 5

Xiao Hu, Mei Fang and Ping-Ting Zhou contributed equally to this work and should be considered co-first authors.

serum immune markers related to EBV infection was selected, which consisted of anti-EBV IgG seropositivity, EBV EA-D antibodies, EBV EBNA-1 antibodies, EBV VCA-p18 antibodies, and EBV ZEBRA antibodies, as shown in Text S1. To ensure the stability and reliability of the results, we included multiple sources of outcome data when inferring causal estimates between EBV infection and ADs. The discovery outcome datasets, consisting of various types of ADs, were sourced from UKB, and the validation datasets were derived from the FinnGen consortium (Table S2).

We extracted exposure-associated variants as *instrumental variables* (*IVs*). Initially, the standard for extracting independent IVs is typically set at $p < 5 \times 10^{-8}$. However, due to the limited genetic variants at this standard, we have to relax the statistically significant threshold of $p < 1 \times 10^{-5}$. Additionally, we utilized a linkage disequilibrium (LD) threshold (R^2) for aggregation, specifically setting independence at $R^2 = 0.001$ and a physical distance of 10000 kb. We excluded certain genetic variants that were not present in the outcomes. The strength of IVs was evaluated using the *F*-statistic, and the R^2 was calculated to determine the proportion of variance explained by the exposure IVs [6].

Four methods were applied in two-sample MR analyses, including inverse variance weighted (IVW), weighted median (WM), weighted mode, and MR-Egger. The positive results of both IVW and WM methods were considered statistically significant ($p < 0.05$). The IVW method was the most reliable tool for causal estimates if there was no horizontal pleiotropy [7]. When the presence of invalid IVs exceeded 50%, the WM method obtained the most accurate results [8]. Considering the possibility of false-positive results in multiple testing, we implemented the False Discovery Rate (FDR) method for result correction [9]. It has been shown that EBV infection is commonly associated with infectious mononucleosis [10]. Thus, further MVMR analyses were conducted to investigate the direct effects of EBV infection and ADs after adjusting for mononucleosis. Sensitivity analysis was performed to assess the stability of causal estimates. First, the MR-PRESSO method was employed to test for possible horizontal pleiotropy of selected IVs [11]. Second, the Cochran's *Q* test was used to assess heterogeneity [12]. In addition, leave-one-out analysis was performed to examine the potential impacts of individual SNPs on the causal estimates. All analyses were performed using R software (version 4.2.2), with the use of "TwoSample MR" and "MVMR" packages, and the results visualization was performed using "ggplot2" package.

Our study identified 50 SNPs associated with anti-EBV IgG seropositivity, 44 SNPs associated with EBV VCA-p18 antibodies, 37 SNPs associated with EBV EBNA-1 antibodies, 51 SNPs associated with EBV ZEBRA antibodies, and 23 SNPs associated with EBV EA-D antibodies (Table S3). Genetically predicted higher EBNA-1 antibody levels were associated with decreased risk of

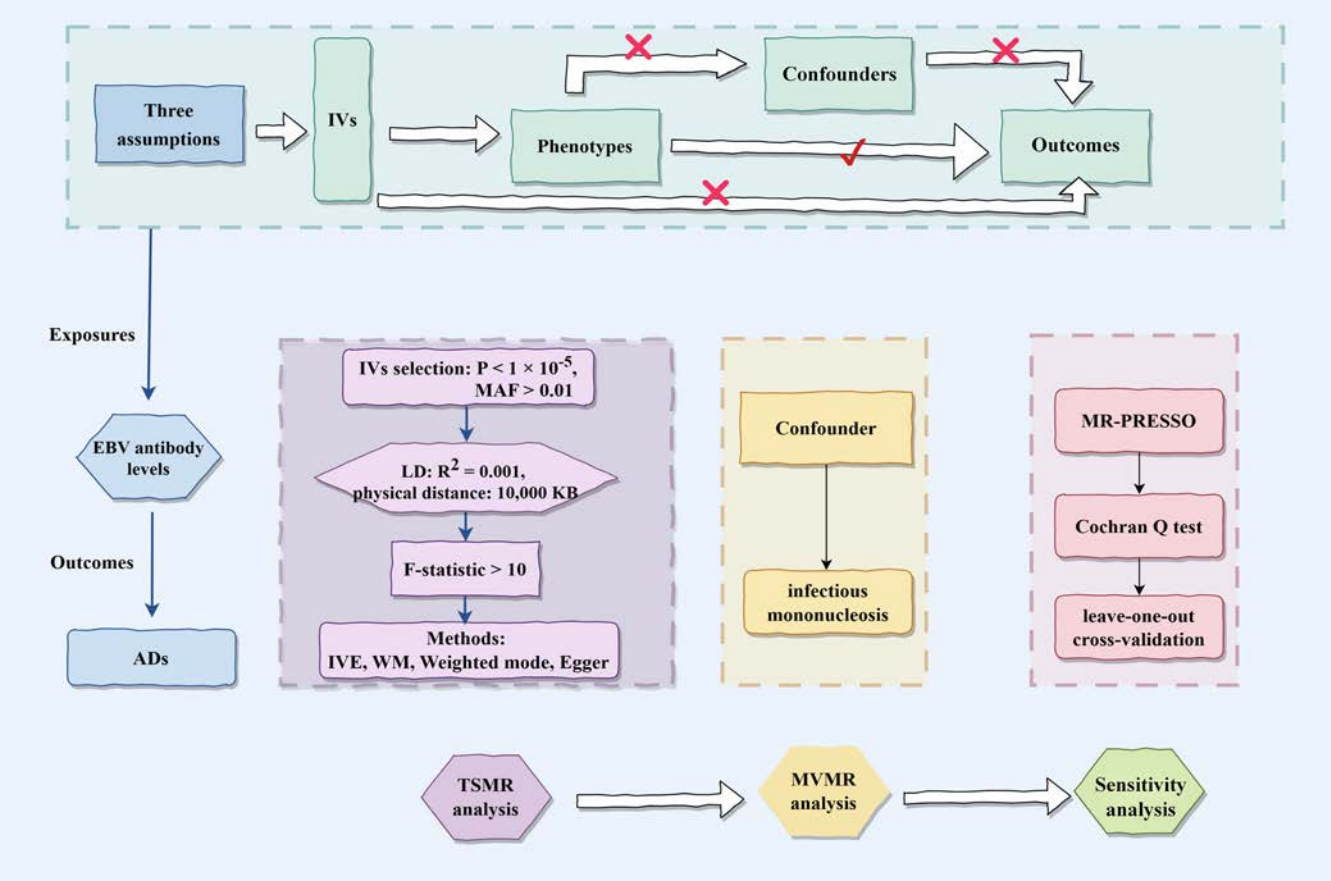

FIGURE 1 | The study design of causal inference between EBV infection and ADs. ADs, Autoimmune diseases; AS, Ankylosing spondylitis; CD, Crohn's disease; EBV, Epstein–Barr virus; MR, Mendelian randomization; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus; T1D, Type 1 diabetes; UC, Ulcerative colitis.

psoriasis in both discovery and replication analyses (Discovery: OR = 0.99, 95% CI: 0.98 to 0.99, $p_{FDR} < 0.05$; Replication: OR = 0.63, 95% CI: 0.46 to 0.85, $p_{FDR} < 0.05$) (Figure 2A). The results of MR analyses were displayed in Figure 2A,B and Tables S4–S10. After FDR correction, discovery and replication analyses confirmed causal association (Figure 2C,D).

Our study identified a total of 150 SNPs associated with SLE, 44 SNPs associated with RA, 88 SNPs associated with psoriasis, 285 SNPs associated with T1D, 21 SNPs associated with AS, 56 SNPs associated with CD, and 34 SNPs associated with UC (Table S3). Genetic susceptibility to AS was causally linked to elevated EA-D antibodies (Discovery: $\beta = 14.11$, 95% CI: 2.21 to 26.01, $p_{FDR} < 0.05$; Replication: $\beta = 0.02$, 95% CI: 0.01 to 0.03, $p_{FDR} < 0.05$). Furthermore, genetic susceptibility to T1D was associated with lower VCA-p18 antibodies (Discovery: $\beta = -0.03$, 95% CI: −0.04 to −0.02, $p_{FDR} < 0.05$; Replication: $\beta = -0.05$, 95% CI: −0.08 to −0.01, $p_{FDR} < 0.05$). Sensitivity analyses supported the stability of these three pairs of causal associations.

Furthermore, we implemented MVMR to assess the direct effects of EBV infections on ADs after adjusting for the impact of infectious mononucleosis. The inverse association observed between genetically predicted EBV EBNA-1 antibodies and psoriasis risk persisted after MVMR adjustment for infectious mononucleosis (OR = 0.99, 95% CI: 0.98 to 0.99, $p_{IVW} = 0.042$). Conversely, the direct causal associations between genetic susceptibility to ADs and the risk of EBV infection were also evaluated, and the results demonstrated a direct causal association between genetically predicted T1D and lower levels of VCA-p18 antibodies ($\beta = -0.03$, 95% CI: −0.06 to −0.01, $p_{IVW} = 0.040$).

In the current study, exposures of EBV infection were defined as infectious agents with seropositivity rates greater than 15% [13].

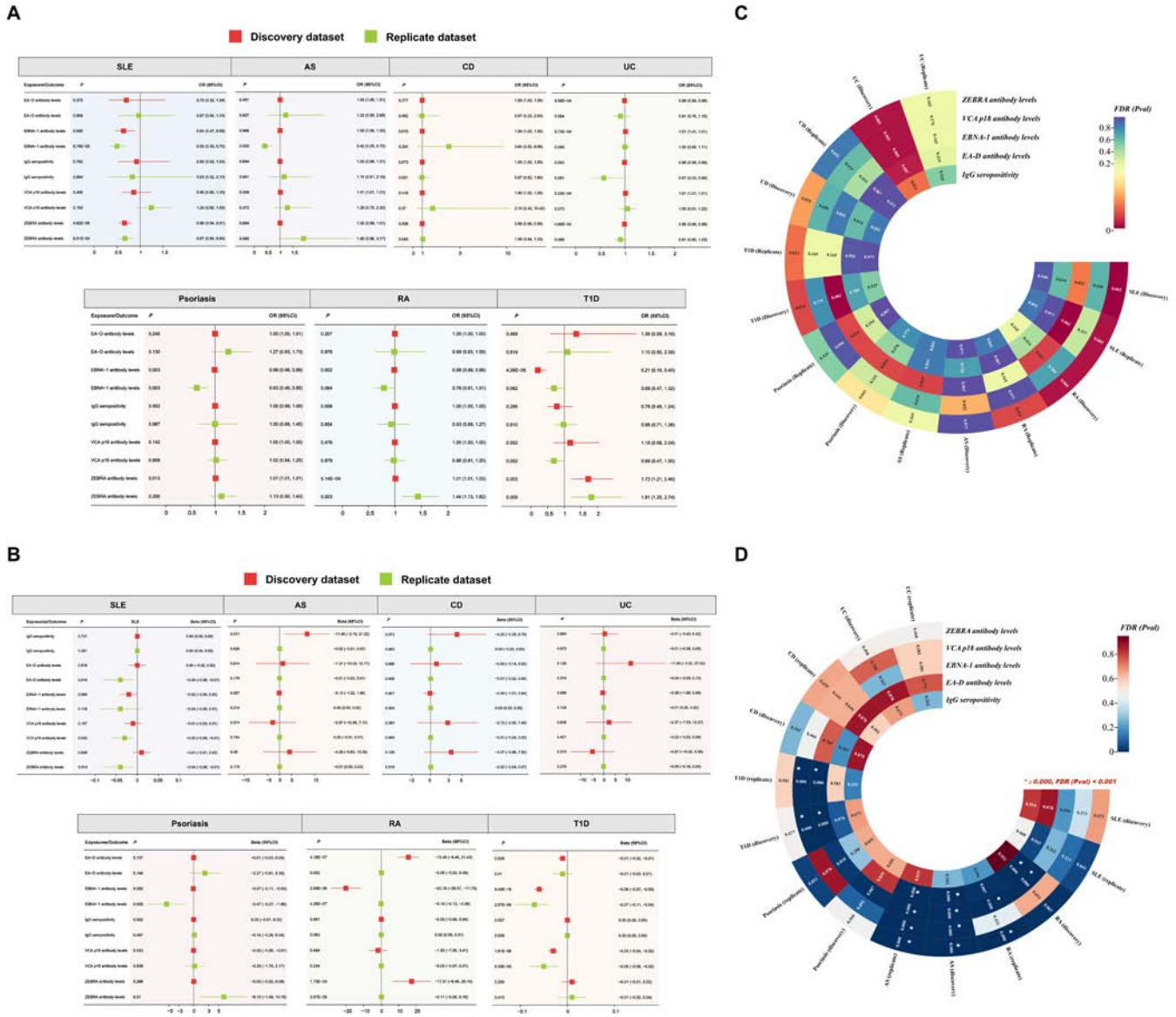

FIGURE 2 | Causal associations between EBV infection and ADs. (A) Forest plot of causal associations of EBV antibodies with ADs. (B) Forest plot of causal associations of ADs with EBV antibodies. (C) The results of FDR correction of EBV antibodies with ADs. (D) The results of FDR correction of ADs with EBV antibodies. ADs, Autoimmune diseases; AS, Ankylosing spondylitis; CD, Crohn's disease; EBV, Epstein–Barr virus; FDR, False Discovery Rate; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus; T1D, Type 1 diabetes; UC, Ulcerative colitis.

Lower seropositivity rates (< 15%) are known to substantially reduce power to identify genetic associations, which is critical for the robustness of MR analyses requiring reliable IVs [14]. Serological testing is a well-established proxy for EBV infection status, as antibody titers reflect both past exposure and current virological activity. Specifically, IgG seropositivity indicates prior infection and viral latency, while elevated EA-D (early antigen) and VCA-p18 (viral capsid) antibodies correlate with lytic viral replication and reactivation. Although serology efficiently identifies asymptomatic infections and immune resolution, cross-reactivity with non-specific antibodies remains a limitation. Thus, serological testing can identify asymptomatic individuals who have been infected and have produced the appropriate antibodies. It can also identify those who have achieved self-healing through the production of antibodies by their immune system after infection. Serological testing is relatively rapid, cost-effective, and reliable [15]. However, it is important to note that quantitative analyses are limited to the seropositive threshold, as there is a risk of cross-binding with non-specific antibodies not involved in the infection [16]. Therefore, serological antibody testing is based on the host immune response and may provide new evidence for exploring the mechanisms of viral etiology in ADs.

Our MR analyses identified an inverse association between genetically predicted EBV EBNA-1 antibody levels and psoriasis risk, which was consistently observed in both discovery and replicate datasets. While this finding suggests a potential protective role of EBNA-1 antibodies in psoriasis, it is critical to interpret these results within the framework of MR. The genetic variants used as instruments influence antibody levels, but this association does not definitively establish a causal relationship between the antibodies themselves and reduced psoriasis risk. Instead, EBNA-1 antibody levels likely serve as proxies for complex immune responses to latent EBV infection or viral control mechanisms. Several alternative explanations should be considered, such as: (1) confounding by other immune factors that influence both EBNA-1 levels and psoriasis susceptibility; (2) reverse causation; (3) horizontal pleiotropy, where genetic variants affect psoriasis risk through pathways unrelated to EBV antibodies. Sensitivity analyses did not detect significant pleiotropy or heterogeneity, which strengthens the robustness of the observed association. However, we acknowledge the inherent limitations of MR in proving direct biological mechanisms. While our findings provide evidence for a potential relationship, we do not claim that EBNA-1 antibodies are direct, causal mediators of reduced psoriasis risk. Previous studies have suggested that immune competition between cytomegalovirus (CMV) and EBV may weaken the immune response to EBV, potentially reducing the risk of developing ADs [17, 18]. However, most cases of psoriasis associated with EBV infection are linked to immunosuppression, making it difficult to draw direct conclusions about the role of EBNA-1 antibodies. The immunological significance of elevated EBNA-1 antibodies in relation to psoriasis pathogenesis requires further investigation. Specifically, it is important to explore whether these antibodies reflect enhanced viral control, altered T-cell responses, or modulation of inflammatory pathways. Additionally, genetic susceptibility to AS was correlated with elevated EBV EA-D antibodies. EBV infection may increase AS risk by dysregulating B-lymphocyte activity [19], potentially due to impaired immune control of EBV [19]. Our findings suggest that AD-related immune states may directly influence EBV serological responses. Specifically, genetic susceptibility to T1D was associated with reduced VCA-p18 antibodies, consistent with clinical observations of impaired EBV-specific immunity in T1D patients [20]. This may reflect generalized B-cell dysfunction or altered immunoregulation in pre-diabetic states that attenuate humoral responses to viral antigens.

The relationship between viral etiology (particularly EBV infection) and ADs is not well understood. However, previous studies have proposed several potential mechanisms. Harley et al. found that EBNA-2 influences the progression of ADs such as RA, SLE, T1D, and MS by promoting the transcription of high-risk genetic alleles [21]. Eric et al. suggested that the similarity between EBV proteins and self-antigens could explain how EBV interferes with ADs and induces disease [22]. An interesting concept is that EBV initially infects lymphocytes and modulates the expression of viral antigens, which in turn affects the host's immune system. This ultimately leads to varied immune responses in individuals, depending on factors like the type of infected cells, reactivation sites, and immune status [2]. These factors may contribute to the onset and development of various ADs. EBNA-1, a nuclear antigen critical for maintaining viral latency, has been implicated in molecular mimicry due to structural homology with host proteins, potentially triggering cross-reactive autoantibodies in SLE [23]. Furthermore, EBNA-1 can disrupt immune regulation by inhibiting apoptosis and interfering with antigen presentation pathways [24]. VCA-p18, a component of the viral capsid, may contribute to pathogenesis through similar mimicry mechanisms or by promoting chronic inflammation during lytic replication [25]. Elevated VCA-p18 antibodies often signify viral reactivation, which could exacerbate immune dysregulation and tissue damage in susceptible hosts [26]. Nevertheless, further study is still needed to elucidate the molecular mechanism of how EBV infection contributes to immune abnormalities and disease progression of ADs.

Author Contributions

Xiao Hu: conceptualization, methodology, software, writing – original draft. **Mei Fang:** conceptualization, validation, writing – review and editing. **Ping-Ting Zhou:** methodology, software. **Yu-Chen Liu:** investigation, methodology. **Yue-Can Gao:** software, visualization. **Li Gu:** validation, visualization. **Wen-Wen Ding:** visualization, software. **Ye-Hai Liu:** software, visualization. **Hai-Feng Pan:** conceptualization, methodology. **Peng Wang:** conceptualization, supervision.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and material that support the findings of this study are available from public datasets that could be found in NHGRI EBI GWAS Catalog and Yang Lab.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** STROBE-MR. **TABLE S2:** Detailed information regarding studies and datasets used in the present study. **TABLE S3:** Single nucleotide polymorphisms (SNPs) associated with exposures and outcomes (used as IVs of Mendelian randomization). **TABLE S4:** Causal association between Epstein–Barr virus and SLE. **TABLE S5:** Causal association between Epstein–Barr virus and RA. **TABLE S6:** Causal association between Epstein–Barr virus and AS. **TABLE S7:** Causal association between Epstein–Barr virus and Psoriasis. **TABLE S8:** Causal association between Epstein–Barr virus and T1D. **TABLE S9:** Causal association between Epstein–Barr virus and CD. **TABLE S10:** Causal association between Epstein–Barr virus and UC. **Text S1:** Details of Infectious Agents Studied.

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EDITORIAL

Leveraging Bibliometric Methods to Uncover the Research Landscape Within the Field of Rheumatology

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Bibliometrics originated in 1955 with Garfield's "citation analysis" theory [1]. As a quantitative tool, bibliometrics reveals research trends and structures through systematic literature analysis. It includes citation analysis, co-occurrence analysis, and collaboration network mapping (Table S1). Bibliometrics has become a powerful tool for mapping research landscapes. Its application in rheumatology has grown notably in recent years. This editorial reviews current applications in rheumatology, evaluates their role in identifying research frontiers, and discusses both methodological strengths and limitations.

1 | Application of Bibliometric Approaches in Rheumatology Research

Rheumatic diseases (RD) encompass a broad spectrum of disorders affecting joints, bones, muscles, and soft tissues (e.g., ligaments, synovium). RD are etiologically classified as inflammatory (e.g., rheumatoid arthritis), metabolic (e.g., gout), degenerative (e.g., osteoarthritis), soft tissue (e.g., fibromyalgia), or infectious (e.g., purulent arthritis). They cause substantial global health and economic burdens, often leading to chronic pain, disability, and reduced quality of life. RD research spans immunology, genetics, and clinical medicine, while facing persistent challenges such as early diagnosis and personalized treatment. Bibliometric analysis can help track academic trends, foster interdisciplinary collaboration, and drive innovation in RD research.

2 | Research Hotspots and Cutting-Edge Topics

Based on a summary of bibliometric studies from PubMed, Web of Science, Embase, Cochrane, and Scopus (Figure 1 and Table S2), most publications appeared between 2021 and 2024, with China contributing the largest share. Among 87 studies, 10 analyzed the entire field of RD, while 77 focused on specific subtypes (Table S3). Of these, 37 addressed broad RD topics, and others explored molecular mechanisms, therapies, comorbidities, symptoms, and technical aspects (Figure S1). Details of literature screening and analysis methods are provided in Supporting Information 1 and Table S4.

These 37 studies highlight key research areas in diagnosis (e.g., artificial intelligence), pathogenesis (e.g., gut microbiota, NETs, microRNAs), and treatment (e.g., infliximab, acupuncture, tocilizumab) (Table S5). These trends reflect urgent needs and rapid progress in RD diagnosis, mechanisms, and therapies. The reviewed literature demonstrates the broad and mature application of bibliometrics across disciplines in RD research. In diagnosis, AI and big data have enhanced imaging interpretation and improved diagnostic accuracy. For instance, AI models now detect early features of rheumatoid arthritis and ankylosing

Jian-Peng Wang and Yi-Pin Yang have contributed equally to this work and share the first authorship.

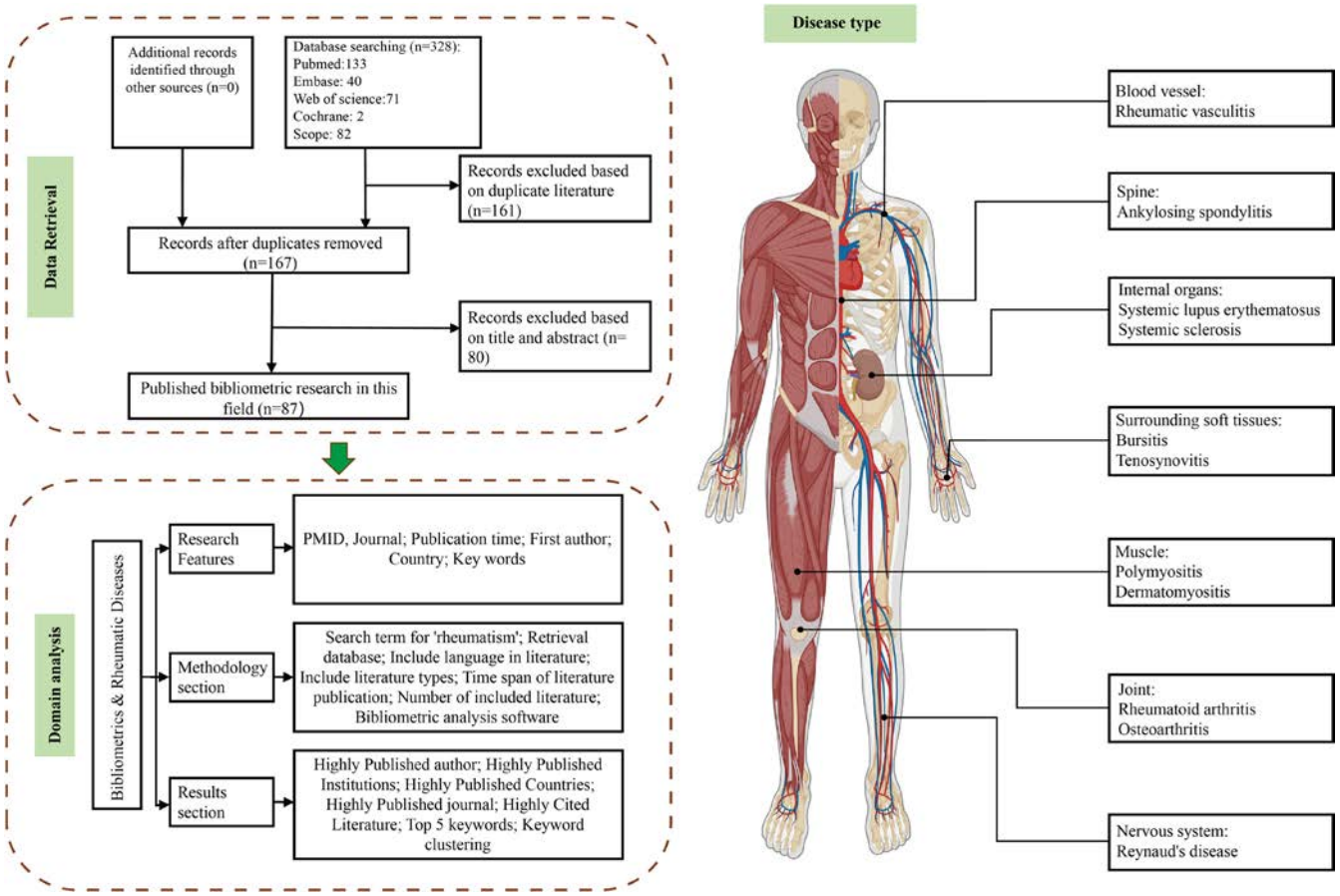

FIGURE 1 | Research inclusion flow chart.

spondylitis, increasing diagnostic sensitivity and specificity. AI is also expected to help uncover disease mechanisms and therapeutic targets [2]. Gut microbiota imbalance has emerged as a key factor in RD pathogenesis [3]. Regulating microbiota composition has shown therapeutic effects, offering insights into disease mechanisms. Huang et al. [4] conducted a bibliometric analysis of gut flora in RD, summarizing its immunomodulatory functions. At the molecular level, macrophages, neutrophil extracellular traps (NETs) [5], and microRNAs [6] play crucial roles in inflammation, autoimmunity, apoptosis, and may serve as therapeutic targets. The bibliometric analysis revealed that research on these molecular mechanisms primarily focused on their regulatory roles in autoimmune reactions, inflammatory responses, apoptosis, and other processes, as well as their therapeutic potential as targets in disease progression. Validating the efficacy, safety, and mechanisms of drugs using modern pharmacological approaches remains a key research focus [7]. Techniques such as acupuncture and arthroscopy provide additional clinical options [8]. Current research emphasizes their clinical feasibility and optimization. Notably, their roles in underlying disease mechanisms have also gained attention as emerging hotspots in RD research [9].

3 | Key Development Directions

Comprehensive bibliometric analyses in RD research highlight biological agents (e.g., infliximab) and emerging molecular mechanisms (e.g., DNA methylation). Biological agents, a major advancement in RD treatment, effectively target tumor necrosis factor α (TNF) and other key molecules, reducing inflammation, alleviating symptoms, and potentially altering disease progression in immune-mediated conditions like rheumatoid arthritis and ankylosing spondylitis. The expanding repertoire of biologics, especially multi-target drugs addressing diverse immune pathways, has established biological therapy as a cornerstone of RD management [10]. Concurrently, advances in molecular mechanism research offer new insights for early diagnosis, pathogenesis, and personalized therapies. For example, epigenetic modifications such as DNA methylation link aberrant immune activation to RD, revealing promising molecular targets for novel therapies. As these studies evolve, targeted treatments within precision medicine frameworks are expected to provide more effective, individualized options. Despite progress, systematic bibliometric analyses focusing on clinical applications, molecular mechanisms of biologics, and targeted drug development remain scarce. Therefore, such analyses are essential to comprehensively assess these strategies, guide future research, and foster innovation in RD.

4 | Limitations of Bibliometric Studies in Rheumatology Research

Despite its value in RD research, bibliometrics has notable limitations (Table S6). Search strategies are often subjective, though refined methodologies can mitigate bias. The standard approach involves combining subject headings and free-text terms, but

inconsistent use across RD studies may yield incomplete or biased results. Database selection also affects outcomes. WOSCC is widely used for its stringent inclusion criteria and reliable data quality. However, sole reliance on it may restrict research scope and introduce bias. Additionally, the focus on English-language literature, while reflecting mainstream trends, may overlook emerging research in other languages. Moreover, excluding non-journal sources such as conference papers and technical reports may omit innovative insights, especially in interdisciplinary fields. While these sources can be valuable, their lack of peer review and inconsistent quality pose challenges. Including them indiscriminately may compromise analytical robustness.

5 | Summary

Bibliometric analysis is a powerful tool in rheumatology, systematically mapping research trends and breakthroughs across prevention, diagnosis, treatment, and molecular mechanisms. In prevention, it identifies modifiable risk factors (e.g., obesity, smoking) to inform public health strategies. Diagnostic studies highlight the growing role of AI in automated imaging and improving accuracy, particularly for rheumatoid arthritis. Treatment-focused bibliometrics evaluate biological agents like infliximab and emerging therapies such as acupuncture, while underscoring the potential of multi-target biologics for personalized medicine. Regarding molecular mechanisms, it accelerates understanding of factors such as gut microbiota imbalance, neutrophil extracellular traps, and epigenetic modifications, revealing novel therapeutic targets. Furthermore, bibliometric approaches promote interdisciplinary collaboration among immunology, genetics, and bioinformatics, driving innovation in RD diagnosis and therapy. Overall, this methodology not only identifies emerging frontiers but also strategically guides future rheumatology research.

Author Contributions

H.-F.P.: conceptualization; J.-P.W.: formal analysis, methodology, writing – original draft; Y.-P.Y.: data curation, software, validation; C.-Y.P.: writing – original draft, writing – review and editing; Y.-C.L. and H.-F.P.: supervision, validation, funding. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Classification of research directions in the field of rheumatology bibliometric analysis. **Table S1:** Research methods of bibliometrics. **Table S2:** Characteristics of the included studies. **Table S3:** Types of diseases included in the bibliometric studies. **Table S4:** Retrieval methods for bibliometric research in the field of rheumatology. **Table S5:** Results of included studies. **Table S6:** Methodology of included studies.

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LETTER TO THE EDITOR

Case Report: Overlapping Rheumatoid Arthritis and Systemic Sclerosis in an Elderly Male With Positive Fibrillarin Antibody

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ABSTRACT

This case report describes a 67-year-old male with confirmed overlap syndrome of rheumatoid arthritis (RA) and systemic sclerosis (SSc), exhibiting fibrillarin autoantibody positivity. Therapeutic intervention with methylprednisolone, methotrexate, and adjuvant medications resulted in substantial amelioration of articular symptoms. This case underscores the potential diagnostic and prognostic utility of fibrillarin autoantibodies in SSc, while highlighting the rarity of RA-SSc overlap syndrome with concurrent fibrillarin autoantibody expression.

1 | Introduction

A 67-year-old male presented with a 6-month history of facial and bilateral lower extremity edema accompanied by cutaneous hyperpigmentation. One month prior to admission, he developed symmetric swelling and pain involving the proximal interphalangeal joints of both hands. On admission, physical examination revealed sclerotic and indurated facial and hand skin with reduced turgor and plication. Laboratory investigations demonstrated elevated anticyclic citrullinated peptide (anti-CCP)

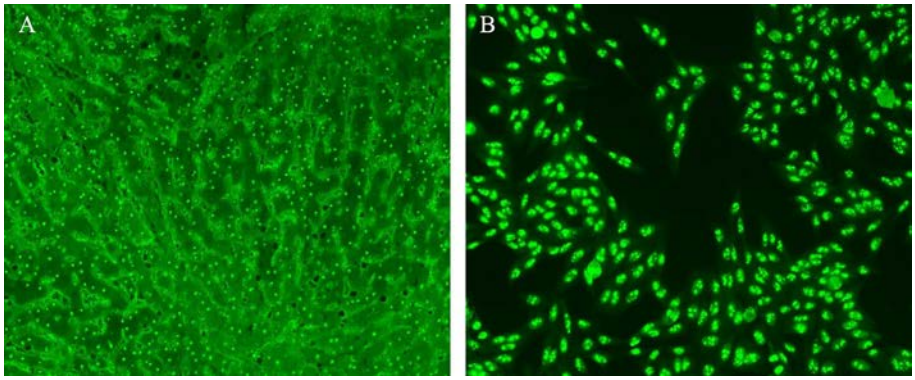

FIGURE 1 | ANA by use of indirect immunofluorescence and two commercially available substrates. (A) Mouse liver; (B) Human epithelial cells.

TABLE 1 | Summary of reported cases of cooccurring RA and SSc.

Case report (year)	Patient demographics	Initial symptoms	Organ involvement	RF	ANA	Other antibodies	Treatment and outcome	Other complications
Kono et al. (2017)	Female, 25 years	Polyarthralgia	Lung	ND	+	Anti-Scl-70	Prednisolone, tocilizumab; significant improvement	ND
Kono et al. (2017)	Male, 32 years	Small joint swelling	Lung	ND	+	Anti-U1-RNP	Prednisolone, tocilizumab; significant improvement	ND
Horiki et al. (1996)	Female, 67 years	Raynaud's phenomenon	Lung, esophagus	+	+	ND	Died of subarachnoid hemorrhage	ND
Horiki et al. (1996)	Female, 59 years	Raynaud's phenomenon	Lung, heart failure	+	+	ND	Prednisolone; aggravation	Chronic thyroiditis
Horiki et al. (1996)	Male, 57 years	Raynaud's phenomenon	Lung, esophagus	+	+	ND	Sulfasalazine; symptom improvement	ND
Horiki et al. (1996)	Female, 70 years	Raynaud's phenomenon	Lung, esophagus	+	2+	ND	ND	Subcutaneous calcification
Horiki et al. (1996)	Female, 64 years	Raynaud's phenomenon	Lung, esophagus	+	1:160	ND	Died of pneumonia	ND
Rozenbaum et al. (1993)	Female, 41 years	Symmetric arthritis	Lung	+	ND	ND	D-penicillamine, methotrexate; partial remission	ND
Zhu et al. (2018)	Female, 34 years	Raynaud's phenomenon	Lung	+	1:640	Anti-Scl-70	Prednisone, cyclophosphamide; significant improvement	Sweet's syndrome
Baron et al. (1982)	Female, 77 years	Raynaud's phenomenon	Lung, esophagus	+	1:160	ND	NSAIDs; aggravation	Subcutaneous calcification
Alkassab et al. (2007)	Female, 51 years	Skin thickening	ND	+	ND	Anti-Scl-70	Methotrexate, etanercept; symptom improvement	ND
Motegi et al. (2014)	Female, 68 years	Raynaud's phenomenon	ND	+	ND	Anti centromere	Methotrexate, etanercept; symptom improvement	Nodulosis
Sherman et al. (1968)	Female, 52 years	Hydroderma	ND	ND	ND	ND	ND	Interstitial calcinosis
Iwata et al. (1999)	Female, 49 years	Raynaud's phenomenon	Lung, esophagus	+	+	Anti-U1-RNP, anti-Sm, anti-dsDNA	Triple antituberculous therapy; symptom improvement	Mycobacterium kansassi pulmonary infection

(Continues)

TABLE 1 | (Continued)

Case report (year)	Patient demographics	Initial symptoms	Organ involvement	RF	ANA	Other antibodies	Treatment and outcome	Other complications
Lee et al. (2018)	Female, 53 years	Skin thickening	ND	+	1:640	Anti-Scl-70, anti centromere	Leflunomide, methotrexate; symptom improvement	Dystrophic calcinosis
Cohen et al. (1982)	Male, 44 years	Polyarthralgia	Lung, esophagus	+	+	ND	Penicillamine; without improvement	Sicca, parotid swelling
Cohen et al. (1982)	Male, 57 years	Knee pain and swelling	Lung, esophagus	+	+	ND	Penicillamine; without improvement	Subcutaneous calcification
Cohen et al. (1982)	Female, 47 years	Multiple arthritis	Lung	+	1:640	ND	Penicillamine; without improvement	Pleural and pericardial effusion
Cohen et al. (1982)	Female, 46 years	Morning stiffness	Lung	+	+	ND	Penicillamine; without improvement	Sicca, parotid swelling
Wada et al. (2011)	Female, 67 years	Raynaud's phenomenon	Lung, kidney, heart failure	+	1:640	Anti-Scl-70	Etanercept; symptom improvement	Reactive AA amyloidosis
Ingegnoli et al. (2007)	Female, 71 years	Morning stiffness	Lung, esophagus	+	1:160	ND	Methylprednisolone, azathioprine; symptom improvement	Bilateral vocal fold immobility
Hamaguchi et al. (2007)	Female, 43 years	Morning stiffness	ND	+	+	ND	Prednisolone, methotrexate; symptom improvement	ND
Maekawa et al. (1992)	Female, 49 years	Raynaud's phenomenon	Lung	+	+	ND	ND	Primary biliary cirrhosis
Shimomura et al. (1989)	Male, 37 years	Raynaud's phenomenon	ND	+	+	ND	ND	ND
Nishioka et al. (2004)	Female, 48 years	Small joint swelling	Kidney	−	1:160	ND	Prednisolone, enalapril; symptom improvement	ND
Yamakawa et al. (2017)	Female, 61 years	Raynaud's phenomenon	Lung	+	1:640	Anti-Scl-70	Prednisolone; partial remission	ND
Kono et al. (2017)	Male, 32 years	Small joint swelling	Lung	ND	+	Anti-U1-RNP	Prednisolone, tocilizumab; significant improvement	ND
Horiki et al. (1996)	Female, 67 years	Raynaud's phenomenon	Lung, esophagus	+	+	ND	Died of subarachnoid hemorrhage	ND

(Continues)

TABLE 1 | (Continued)

Case report (year)	Patient demographics	Initial symptoms	Organ involvement	RF	ANA	Other antibodies	Treatment and outcome	Other complications
Horiki et al. (1996)	Female, 59 years	Raynaud's phenomenon	Lung, heart failure	+	+	ND	Prednisolone; aggravation	Chronic thyroiditis
Horiki et al. (1996)	Male, 57 years	Raynaud's phenomenon	Lung, esophagus	+	+	ND	Sulfasalazine; symptom improvement	ND
Horiki et al. (1996)	Female, 70 years	Raynaud's phenomenon	Lung, esophagus	+	2+	ND	ND	Subcutaneous calcification
Horiki et al. (1996)	Female, 64 years	Raynaud's phenomenon	Lung, esophagus	+	1:160	ND	Died of pneumonia	ND
Rozenbaum et al. (1993)	Female, 41 years	Symmetric arthritis	Lung	+	ND	ND	D-penicillamine, methotrexate; partial remission	ND
Zhu et al. (2018)	Female, 34 years	Raynaud's phenomenon	Lung	+	1:640	Anti-Scl-70	Prednisone, cyclophosphamide; significant improvement	Sweet's syndrome
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Alkassab et al. (2007)	Female, 51 years	Skin thickening	ND	+	ND	Anti-Scl-70	Methotrexate, etanercept; symptom improvement	ND
Motegi et al. (2014)	Female, 68 years	Raynaud's phenomenon	ND	+	ND	Anticentromere	Methotrexate, etanercept; symptom improvement	Nodulosis
Sherman et al. (1968)	Female, 52 years	Hydroderma	ND	ND	ND	ND	ND	Interstitial calcinosis
Iwata et al. (1999)	Female, 49 years	Raynaud's phenomenon	Lung, esophagus	+	+	Anti-U1-RNP, anti-Sm, anti-dsDNA	Triple antituberculous therapy; symptom improvement	Mycobacterium kansassi pulmonary infection
Lee et al. (2018)	Female, 53 years	Skin thickening	ND	+	1:640	Anti-Scl-70, anticentromere	Leflunomide, methotrexate; symptom improvement	Dystrophic calcinosis

Abbreviations: ANA, antinuclear antibodies; ND, not described; RF, rheumatoid factor.

antibodies (> 300 U/mL), rheumatoid factor (RF) (123 IU/mL), and C-reactive protein (CRP) at 13.2 mg/L, normal erythrocyte sedimentation rate. Antinuclear antibodies (ANA) with a nucleolar staining pattern at 1:3200 titer (Figure 1). Notable negative findings included anti-SCL-70 and anticentromere antibodies. Additional parameters revealed elevated fibrillarin autoantibody positivity (a myositis-associated autoantibody) and Epstein–Barr virus (EBV) IgM antibodies measuring 3.35 AU/mL. Chest computed tomography (CT) identified subpleural inflammatory infiltrates in both lungs. The Disease Activity Score in 28 joints (DAS28) was calculated at 5.18, indicative of high disease activity.

The patient presents with joint swelling and pain, predominantly affecting small joints such as the proximal interphalangeal joints of both hands and the second and third metacarpophalangeal joints of the right hand, with a disease duration of 1 month. Laboratory tests revealed elevated titers of anti-CCP antibodies and RF, along with elevated CRP, yielding a total score of nine points according to the 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis. Besides, the patient exhibited skin thickening of the fingers extending beyond the metacarpophalangeal joints, accompanied by Raynaud's phenomenon in both hands. At presentation, no pulmonary, renal, or other extra-articular organ involvement was observed, resulting in a total score of 12 points per the 2013 ACR/EULAR Classification Criteria for Systemic Sclerosis. Based on these criteria, the patient was diagnosed with an overlap syndrome of RA and SSc. Additional findings included fibrillarin autoantibody positivity and EBV infection. The therapeutic regimen included methylprednisolone (6 mg daily), hydroxychloroquine (0.2 g twice daily), methotrexate (10 mg weekly), and technetium-99 m methylenediphosphonate (10 mL for 6 days) for disease management. For antiviral therapy, intravenous ganciclovir (0.4 g for 6 days) was administered. After 8 days of treatment, the patient reported improvement in articular symptoms. At the latest follow-up, no hand joint swelling or pain was observed, with a DAS28 of 2.35 (≤ 2.6), indicative of disease remission per established thresholds.

2 | Discussion

Fibrillarin antibodies are detected in 4%–10% of SSc patients [1], contributing to improved diagnostic precision and facilitating early disease identification as these autoantibodies may precede overt clinical manifestations [2–4]. In instances where anti-Scl-70 and anticentromere antibodies yield negative results, fibrillarin antibodies may serve as a diagnostic marker for SSc. The patient tested negative for anti-Scl-70 and anticentromere antibodies but positive for fibrillarin antibodies, with cutaneous sclerosis characterized by bilateral hand skin thickening, diminished dermal pliability, and reduced skin turgor—findings collectively suggestive of an early or preclinical SSc phase. Fibrillarin antibodies have also been associated with an unfavorable prognosis, correlating with higher rates of visceral organ involvement including pulmonary fibrosis, pulmonary arterial hypertension (PAH), and scleroderma renal crisis [5]. A comprehensive organ assessment is advised, comprising high-resolution computed tomography (HRCT) and pulmonary function testing for lungs, echocardiography with right heart catheterization for suspected PAH, and barium swallow studies or endoscopic evaluation for the gastrointestinal tract. Fibrillarin antibodies represent a diagnostic marker for SSc overlap syndrome. A recent study validated the association between SSc and myositis overlap, demonstrating significantly higher skeletal muscle involvement in fibrillarin-positive patients compared to antibody-negative counterparts (31.4% vs. 12.2%), with a 50% incidence rate observed among those with concurrent diffuse cutaneous involvement [6].

We did not identify any reports of fibrillarin autoantibody-positive RA-SSc overlap syndrome in the databases we searched (Table 1). However, given the limitations of our search scope, such cases may still exist but appear to be rarely reported.

3 | Conclusion

Immune dysregulation represents a shared pathogenic pathway underlying both RA and SSc, potentially serving as an effective therapeutic target for these conditions [7]. During treatment planning, it is essential to conduct a comprehensive evaluation of disease activity, complications, and visceral organ involvement for both RA and SSc.

Consent

Patient consent was received for publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available upon reasonable request by any qualified researchers who engage in rigorous, independent scientific research; and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). All data relevant to the study are included in the article.

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Jian Lu
Chang Liu
Meiling Li
Zhichun Liu

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LETTER TO THE EDITOR

Case Report: Pigmented Villonodular Synovitis in a Patient With Sjögren's Syndrome

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Dear Editor,

Pigmented villonodular synovitis (PVNS) is a rare but locally aggressive proliferative lesion that arises in the synovium, tendon sheaths, and bursae. According to the 2020 WHO Classification of Tumours of Soft Tissue and Bone, PVNS is now classified as diffuse-type tenosynovial giant cell tumor (diffuse-TGCT) [1].

PVNS primarily affects large joints, most commonly the knee, followed by the hip, ankle, and shoulder. It is characterized by nonspecific symptoms, such as joint pain, swelling, stiffness, and limited range of motion, which often leads to misdiagnosis as other inflammatory or degenerative joint disorders [1].

While the exact etiology of PVNS remains unclear, it is believed to involve neoplastic and inflammatory components. Recent studies suggest that a neoplastic process driven by a translocation involving colony-stimulating factor 1 (CSF1) and its receptor (CSF1R) leads to abnormal macrophage recruitment and infiltration of other inflammatory cells, thereby contributing to disease progression. This process leads to chronic inflammation, synovial proliferation, and cartilage destruction. The deposition of hemosiderin resulting from recurrent intra-articular bleeding contributes to increased tissue damage and fibrosis. Risk factors for progression include delayed diagnosis, incomplete surgical resection, recurrent synovial inflammation, and disease recurrence [2, 3].

Hemarthrosis detected through joint aspiration can be a crucial diagnostic clue for PVNS. Magnetic resonance imaging (MRI) is essential, typically revealing a well-defined lesion with hemosiderin deposition, characterized by low signal intensity on T2-weighted sequences. Histopathologically, PVNS is characterized by mononuclear cells, multinucleated giant cells, and hemosiderin-laden macrophages [4, 5].

The treatment primarily involves surgical resection, often through arthroscopic synovectomy; however, the recurrence rate remains high. In some cases, adjuvant therapies—such as radiotherapy or targeted molecular treatments such as tyrosine kinase inhibitors (e.g., pexidartinib)—may be considered to reduce the risk of recurrence [2, 6].

Deng-Ho Yang and Chia-Wen Kuo contributed equally as corresponding authors.

This case report describes a patient with an underlying diagnosis of Sjögren's syndrome who was diagnosed with PVNS based on pathological findings from a total knee replacement (TKR). We aim to highlight the key considerations in its diagnosis and management.

A 62-year-old Taiwanese woman presented with a 5-year history of persistent right knee pain and limited range of motion. She is a chronic hepatitis B carrier under regular monitoring. She does not smoke or drink. She has no significant family history. Initially, she was diagnosed with a knee effusion in her right knee and underwent arthroscopy in 2017. However, her symptoms gradually worsened, significantly impairing daily life. In 2022, she sought medical care at our orthopedic outpatient department, where a varus deformity of the right knee was observed. The physical examination revealed a positive patellar grind test, limited range of motion, and flexion contractures in both knees. The radiograph revealed joint space narrowing and obliteration in the right knee, with marginal spur formation. Joint aspiration yielded bloody effusion (Red blood cells: 591 700/μL, reference range: <100/μL). The initial diagnosis was osteoarthritis of the right knee.

Following discussion, a TKR was performed, during which PVNS was suspected. The histopathological examination revealed hyperplastic synovium with papillary projections, composed of mononuclear cells, hemosiderin-laden macrophages, and occasional multinucleated giant cells, consistent with PVNS, with no morphological evidence of malignancy (Figure 1).
Despite the surgery, her symptoms persisted. As a result, she underwent two revision surgeries in July and September 2023. Dry eyes and mouth were also observed during follow-up. Owing to postoperative fatigue, a preference for conservative treatment, and new symptoms, she was referred to the rheumatology clinic in January 2024. Laboratory results showed normal erythrocyte sedimentation rate, C-reactive protein, and white blood cell (WBC) count. The autoantibody panel was performed and showed positive anti-double-stranded deoxyribonucleic acid antibodies (40 IU/mL/; positive: > 35 IU/mL), positive anti-Sjögren's-syndrome-related antigen A autoantibodies (11 IU/mL; positive: > 10 IU/mL), and negative results for human leukocyte antigen B27, anti-nuclear antibodies, and anti-Sjögren's-syndrome-related antigen B autoantibodies. The clinical impression was Sjögren's syndrome. Subsequently, prednisone (5 mg twice daily), hydroxychloroquine (200 mg twice daily), and leflunomide (10 mg once daily) were prescribed for immunotherapy.

Although she underwent additional surgeries because of persistent symptoms, the interval between procedures increased significantly after initiating medical treatment (Figure 2). The primary method for monitoring the stability of PVNS is imaging, particularly MRI. However, in this case, MRI could not be performed due to the patient's history of total knee replacement (TKR) surgery, and conventional X-rays have limited sensitivity in detecting soft tissue lesions. Regarding blood markers, the literature suggests that the neutrophil-lymphocyte ratio (NLR) and CRP can be used for prognostic assessment [7]. In this case, the patient received immunotherapy to control systemic inflammation with normal levels of ESR and CRP, and active recurrent PVNS may be suppressed. Additionally, the patient's range of motion was measured and remained between grade 0 and 1, and

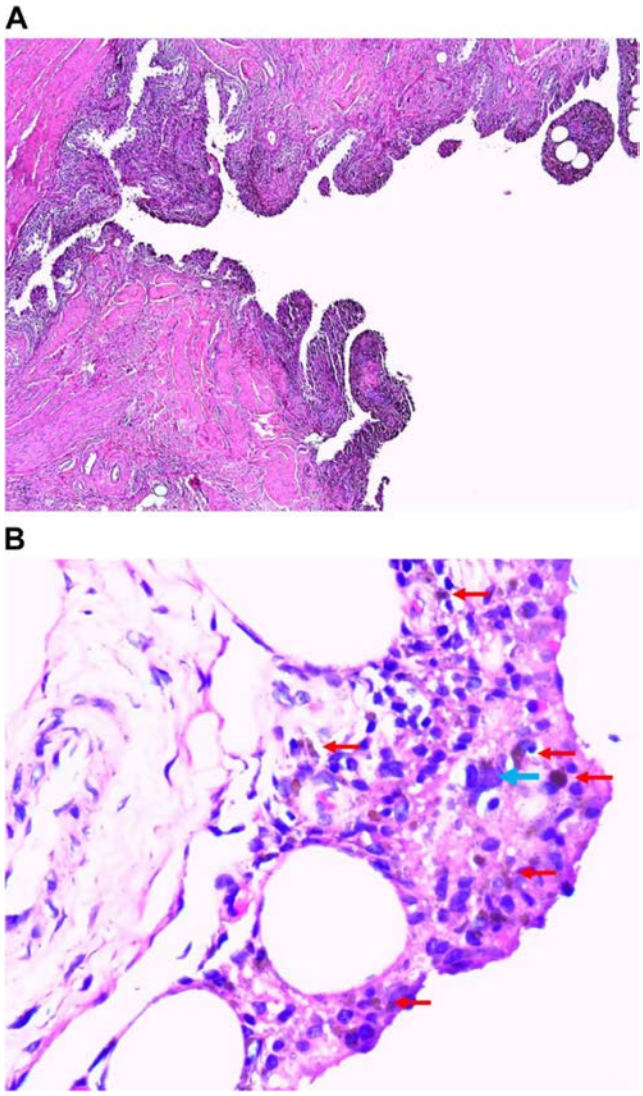

FIGURE 1 | Synovial biopsy microscopy image. (A) H&E stain, 40×: hyperplastic synovium with villous papillary projections. (B) H&E stain, 400×: admixture of mononuclear tumor cells, hemosiderin-laden macrophages (red arrows), and multinucleated giant cells (blue arrow) infiltrating the synovium.

there was also improvement in the Visual Analog Scale (VAS) pain score, which stabilized at 2–3 points. She remains under continuous monitoring and follow-up in the orthopedics and rheumatology clinics.
Initially, the patient was diagnosed with osteoarthritis based on chronic knee pain, joint stiffness, and limited range of motion—symptoms commonly seen in degenerative joint disease. The differential diagnosis for monoarthritis is broad, including septic arthritis, crystal-induced arthritis, hemarthrosis (from trauma or pigmented villonodular synovitis, PVNS), tumors, seronegative spondyloarthropathy, and osteoarthritis. Arthrocentesis is a key diagnostic step, distinguishing infection (WBC > 50 000/mm^3 and positive culture), crystal-induced inflammation, hemorrhagic conditions like PVNS, and degenerative causes (low WBC count) [8]. Although joint aspiration in this case revealed a bloody effusion (RBC: 591 700/μL), which should have raised suspicion for PVNS, the rarity of PVNS (about 1.8 per million people per year) meant it was not initially prioritized in the diagnostic workup [9].

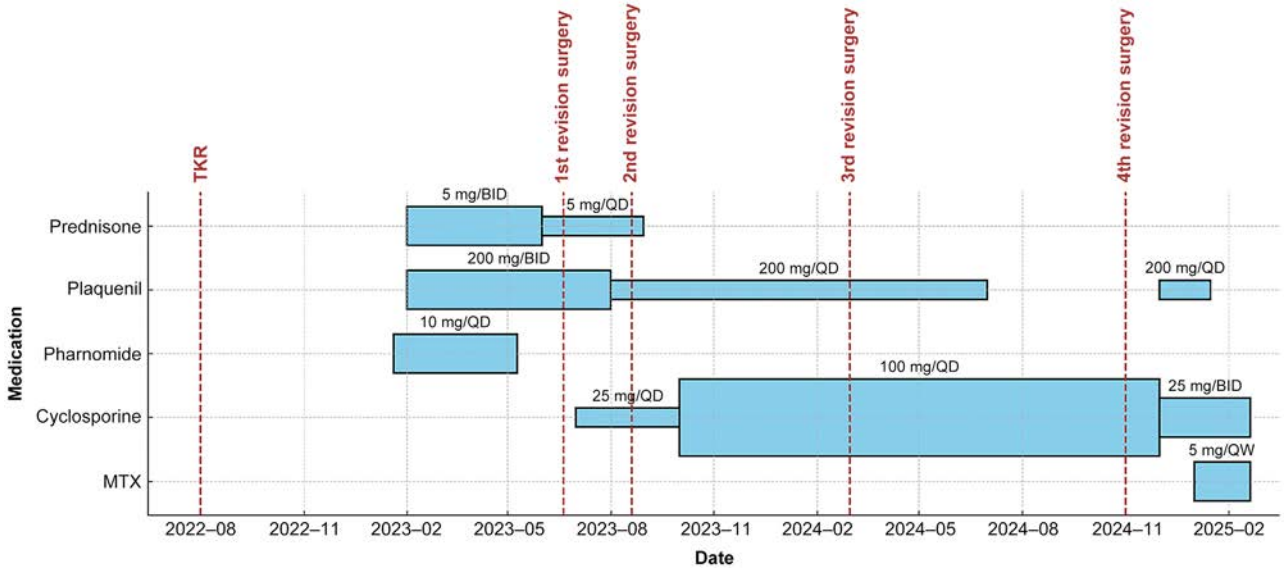

FIGURE 2 | Timeline of medication and surgery for this case.

Imaging studies are equally important. While plain radiographs showed joint space narrowing—findings that are often ascribed to osteoarthritis—closer examination later revealed more severe lateral compartment involvement, an atypical feature for degenerative disease. The lack of advanced imaging, particularly MRI, further limited early recognition of PVNS. Also, immunohistochemistry and molecular markers related to PVNS, such as CSF1 and CD68, are not routinely performed at our institution. Cost considerations and the perceived unlikelihood of rare conditions like PVNS meant MRI was not performed, and the diagnosis was made intraoperatively during TKR when synovial pathology was established.

This case emphasizes several important points for clinical practice: persistent monoarthritis with hemorrhagic effusion, rapid joint degeneration, or atypical radiographic features should raise suspicion for PVNS. In these situations, MRI—despite its cost—can significantly improve diagnostic accuracy. Enhanced awareness of PVNS and multidisciplinary discussions, along with careful review of imaging and aspiration findings, are essential to avoid missed or delayed diagnoses. Following these principles may help clinicians reduce missed PVNS cases and improve both perioperative planning and long-term patient outcomes.

The patient had a history of Sjögren's syndrome, a systemic autoimmune disease. The relationship between Sjögren's syndrome (SS) and pigmented villonodular synovitis (PVNS) remains speculative, but several immunopathological overlaps have been observed. Both diseases are characterized by marked immune cell infiltration—CD4+ and CD8+ T lymphocytes in SS and predominantly CD68+ macrophages (with some T cells) in PVNS. Chronic inflammatory environments in each condition include elevated cytokines such as IL-6, TNF-α, and IL-1β, suggesting that persistent immune activation in SS may create a milieu conducive to synovial proliferation or may influence the recurrence of PVNS [1, 10]. Therefore, active inflammation can be observed during the disease of SS, and this inflammation may be associated with recurrent PVNS.

The pathogenesis of PVNS is primarily driven by the overexpression of colony-stimulating factor 1 (CSF1) and activation of its receptor (CSF1R), resulting in abnormal recruitment and proliferation of monocyte/macrophage lineage cells. Recent research has identified IL-34 as an alternative ligand for CSF1R, independent of CSF1 [11, 12]. Notably, IL-34 has been found to be overexpressed in the inflamed salivary glands of patients with primary Sjögren's syndrome [13]. This overexpression is associated with the expansion of pro-inflammatory monocyte populations in affected tissue. These findings indicate that the CSF1/CSF1R/IL-34 axis may represent a shared pathological pathway between SS and PVNS.

Although immunohistochemistry for CSF1R was not performed in this case, current evidence underscores its central role not only in PVNS but also in various autoimmune diseases, such as rheumatoid arthritis, SLE, and psoriatic arthritis [14]. There are isolated reports of PVNS occurring in patients with systemic autoimmune diseases, including SS, SLE, RA, and psoriatic arthritis, which supports the possibility of a link; though causality has not been established [15–18].

Whether PVNS can result directly from SS-related chronic inflammation remains unproven. To establish a causal association, future studies are needed—incorporating epidemiological analysis, molecular profiling (including assessment of CSF1R and its ligands), and comparative studies of patients with overlapping syndromes. Ultimately, recognition of this potential overlap is important for timely diagnosis and may have implications for the use of systemic immunosuppressive therapies, as our case demonstrated a prolonged interval between surgical interventions after immunotherapy.

Author Contributions

Conceptualization, T.-Y.C., D.-H.Y., and C.-W.K.; methodology, D.-H.Y., C.-W.K., and C.-C.L.; investigation, T.-Y.C. and D.-H.Y.; resources, D.-H.Y. and C.-C.L.; writing – original draft preparation, T.-Y.C.; writing – review and editing, D.-H.Y. and T.-A.C.; supervision, C.-C.L. and

C.-W.K. All authors have read and agreed to the published version of the manuscript.

Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Tri-Service General Hospital (approval code: T63888).

Consent

Informed consent was obtained from all subjects involved in 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 from the corresponding author, D.-H.Y., upon reasonable request.

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ORIGINAL ARTICLE

The Value of Rheumatoid Arthritis Citrullinated Peptides (RACP) System in the Diagnosis of Rheumatoid Arthritis: A Retrospective Analysis of 2632 Patients

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ABSTRACT

Objectives and Aim: This study aimed to evaluate the diagnostic accuracy and prognostic value of the Rheumatoid Arthritis Citrullinated Peptides (RACP) assay in a real-world cohort, assessing its performance both independently and in combination with established biomarkers such as anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF), and anti-keratin antibodies (AKA).

Methods: A single-center retrospective analysis included 2632 patients who underwent serological testing between September 2022 and September 2023. Of these, 644 patients met ACR/EULAR classification criteria for rheumatoid arthritis (RA), while 1988 patients served as non-RA controls, including an osteoarthritis (OA) subgroup. RACP, anti-CCP, RF, and AKA levels were measured by enzyme-linked immunosorbent assay (ELISA). Diagnostic sensitivity, specificity, and combination strategies were assessed using chi-square and *t*-tests.

Results: RACP alone demonstrated a sensitivity of 76.86% and a specificity of 89.94%, closely comparable to anti-CCP (70.87% sensitivity and 89.92% specificity). Area under the curve (AUC) values were 0.805 for RACP, 0.783 for RF, 0.799 for anti-CCP, and 0.623 for AKA. Combining RACP with RF improved sensitivity (85.36%), while dual positivity (RACP + AKA) optimized specificity (96.61%). In the OA subgroup, biomarker positivity remained minimal, confirming strong discriminatory capacity. Patients positive for RACP or anti-CCP exhibited significantly higher tender joint counts, correlating biomarker positivity with clinical disease severity.

Conclusion: RACP is a robust biomarker for RA diagnosis, offering sensitivity and specificity comparable to or exceeding traditional assays. Combinations with RF or anti-CCP enhance diagnostic accuracy, supporting its utility in early RA detection, differential diagnosis from OA, and potentially reflecting disease activity. Prospective studies are warranted to confirm these findings.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by symmetrical, polyarticular inflammation that primarily involves the small joints of the hands and feet but can extend to other organ systems [1]. Globally, RA affects approximately 0.5%–1% of the adult population and is associated with progressive joint destruction, decreased quality of life, and increased morbidity and mortality [2].

The etiology of RA remains multifactorial, involving genetic predispositions, environmental triggers, and dysregulated immune responses. In 1979, Wada first demonstrated the presence of citrulline residues in proteins, laying the foundation

for our understanding of protein citrullination in RA [3]. This posttranslational modification is mediated by the peptidylarginine deiminase (PAD) enzyme family—particularly PAD2 and PAD4—whose expression is upregulated in inflamed synovial tissue [4]. Among the substrates for PADs, fibrinogen undergoes extensive citrullination to yield citrullinated fibrinogen (Cit-Fib) [5, 6].

Cit-Fib is a principal antigenic target for autoantibodies in RA, binding Anti-CCP and forming immune complexes that accumulate in the synovium [7]. These immune complexes activate the complement cascade and engage Fcγ receptors on innate immune cells, thereby amplifying local inflammation [8]. Moreover, in vitro and in vivo studies indicate that Cit-Fib contributes directly to bone destruction by promoting osteoclast activation and differentiation [9].

Under physiological conditions, native fibrinogen inhibits osteoclastogenesis by downregulating receptor activator of nuclear factor-κB ligand (RANKL) expression on osteoblast precursors, thus preserving bone homeostasis [10]. However, Cit-Fib loses this protective function and instead enhances osteoclast differentiation via upregulation of RANKL on synovial fibroblasts, leading to accelerated bone resorption [11].

Proteomic analyses have further identified specific structural motifs—termed multiple citrulline similar motifs (MCSM)—within citrullinated antigens present in RA patient sera. RACP is a detection process in which MCSM serves as the key substance for identifying citrullinated protein antigens in RA. The RACP assay utilizes anti-MCSM antibodies on an ELISA platform to detect these epitopes, demonstrating reactivity in 76% of RA cases with a sensitivity of 95% and specificity of 96% [7]. These performance metrics suggest that RACP may complement existing biomarkers, such as anti-CCP, RF, and AKA, in the early diagnosis of RA.

Additional serological markers under investigation include mutated citrullinated vimentin (MCV), scavenger receptor-A (SR-A), anti-RA 33 autoantibody, carp antibody, and antiperinuclear factor (APF), which have shown promise in reflecting disease activity and prognostic outcomes [12–15].

Despite these advances, the clinical utility of the RACP system has yet to be validated in large, diverse RA cohorts. Therefore, the present study aims to evaluate the diagnostic accuracy and prognostic value of the RACP assay in a well-characterized RA population, comparing its performance against conventional serological markers and correlating assay results with clinical measures of disease severity.

2 | Materials and Methods

2.1 | Study Design

This single-center, retrospective cohort study included 2632 consecutive patients who underwent RACP testing at The First Affiliated Hospital of Guangxi Medical University between September 2022 and September 2023. All data were extracted from the hospital's electronic medical record system (HIS). The Information Center generated a de-identified list of patients meeting the inclusion criteria, and clinical, demographic, and laboratory variables were then retrieved using an authorized HIS account.

2.2 | Study Subjects

After excluding patients with missing RACP results or incomplete clinical records, the final cohort comprised 2632 individuals. Of these, 644 were classified as RA (496 females and 148 males; mean age 52.7 ± 15.4 years) and 1988 served as non-RA controls (1310 females and 678 males; mean age 46.9 ± 16.9 years). Post hoc power calculation was performed for the current sample of 2632 and an effect size of 0.1; the achieved power was 0.9992 > 0.85.

RA was diagnosed according to the 1987 American College of Rheumatology (ACR) classification criteria and/or the 2010 ACR/EULAR (European Alliance of Associations for Rheumatology) criteria [16, 17]. The non-RA group included all patients tested by RACP who did not fulfill these RA classification criteria.

Among the 1988 non-RA patients, 81.7% were diagnosed with various rheumatic or musculoskeletal disorders—most commonly undifferentiated arthritis (42.3%), osteoarthritis (10.9%), low back pain (6.4%), gout (5.4%), unspecified musculoskeletal pain (5.2%), intervertebral disc disorders (3.7%), systemic lupus erythematosus (2.1%), undifferentiated connective tissue disease (1.9%), ankylosing spondylitis (1.5%), Sjögren's syndrome (1.4%), and other connective tissue diseases (10.1%)—while the remaining 18.3% presented with non-rheumatic conditions. From this cohort, 216 patients meeting established diagnostic criteria for osteoarthritis were designated as the OA subgroup [18, 19]. For all non-RA subjects, we extracted demographic information (age and sex), serological markers (RACP S/CO ratio, anti-CCP, RF, and AKA), and clinical assessments of disease activity (swollen joint count and tender joint count using the 28-joint method).

2.3 | Biomarker Analysis

Serological markers RACP, anti-CCP, RF, and AKA were measured in all participants. For each marker, sensitivity (the proportion of RA patients with a positive result) and specificity (the proportion of non-RA subjects with a negative result) were calculated.

2.4 | Combination Analysis

To determine whether adding RACP to conventional markers improved diagnostic accuracy, we evaluated four combination strategies—patients positive for either RACP or anti-CCP, either RACP or RF, both RACP and anti-CCP, and both RACP and AKA.

2.5 | Instruments and Reagents

All assays were performed using ELISA kits supplied by Guangzhou Lead Biological Technology Co. Ltd. Key laboratory

instruments included an Aikon AE65 fully automated enzyme immunoassay analyzer for incubation, washing, and optical reading; a Jingli LDZ5-2 centrifuge (Beijing) for serum separation; and a Ruicheng MS100 microplate shaker (Hangzhou) to ensure uniform reagent mixing. High- and low-titer quality control materials were run with each batch to maintain a coefficient of variation below 10%, thereby ensuring assay precision and repeatability.

2.6 | RACP Detection Method

Peripheral blood was collected into plain tubes, allowed to clot, and centrifuged within 2 h; hemolyzed, icteric, or lipemic samples were excluded. Serum aliquots were stored at room temperature for up to 12 h, at 2°C–8°C for no more than 48 h, or at −20°C ± 5°C for up to 6 months (≤ 5 freeze-thaw cycles). The ELISA procedure followed the manufacturer's protocol exactly: samples and controls were added to wells coated with MCSM peptides, incubated, washed, incubated with enzyme-conjugated detection antibody, and then reacted with substrate. The reaction was terminated at the prescribed time, and absorbance was measured at 450 nm with a 630 nm reference within 15 min. An S/CO ratio > 1.05 was interpreted as positive, while a ratio ≤ 1.05 was interpreted as negative.

2.7 | Statistical Analysis

All analyses were conducted using SPSS version 30.0 and GraphPad Prism version 9.0. G-Power (version 3.1.9.7) was used to analyze sample size calculation. Continuous variables were first assessed for normality by the Shapiro–Wilk test. Those conforming to a normal distribution are presented as mean ± SD and compared using two-tailed Student's *t*-tests; non-normal data are reported as median (interquartile range) and compared by the Mann–Whitney U test. Categorical variables are expressed as counts and percentages and compared using chi-square tests or Fisher's exact tests when expected cell counts were < 5.

Diagnostic performance metrics—including sensitivity, specificity—were calculated for each biomarker. To evaluate combined biomarker strategies, we compared sensitivities and specificities across combinations using chi-square testing. Differences between the RA and OA subgroups were also assessed by chi-square for categorical data and by *t*-test for continuous measures. A two-sided *p*-value < 0.05 was considered statistically significant.

3 | Results

Analysis of individual biomarkers revealed that RF achieved the highest sensitivity for RA diagnosis at 82.93% but had the lowest specificity (22.30%). Both RACP and anti-CCP demonstrated a more balanced diagnostic profile, with RACP yielding 76.86% sensitivity and 89.94% specificity, and anti-CCP yielding 70.87% sensitivity and 89.92% specificity. AKA, in contrast, provided excellent specificity (94.39%) but limited sensitivity (38.48%). All four markers significantly differentiated RA from non-RA subjects (χ^2 range 241.95–1117.03; $p < 0.001$) (Table 1). By constructing the ROC curves, we have obtained AUC values of 0.805 for RACP, 0.799 for ACCP, 0.783 for RF, and 0.623 for AKA.

Among the 200 non-RA patients who tested positive for RACP, undifferentiated arthritis predominated—comprising 66.0% of cases—while systemic lupus erythematosus accounted for 7.0% and undifferentiated connective tissue disease for 4.5% (Figure 1). A separate group labeled "Other Rheumatic and Immune Diseases," which included ANCA-associated vasculitis, palindromic rheumatism, gout, and Sjögren's syndrome, collectively represented another 4.5%, with each condition contributing up to 2.0%. Serological immunological abnormalities were identified in 3.5% of patients and osteoarthritis in 2.5%. Finally, 12.0% fell into an "Other Diseases" category,

TABLE 1 | Sensitivity, specificity, and *p*-values between the RA Group and the non-RA group.

Results	RA	non-RA	Sensitivity	Specificity	PPV	NPV	accuracy	t/χ^2	*p*
Age (year)	52.7 ± 15.3	46.9 ± 16.9	—	—				$t = 8.002$	< 0.001
Female	496 (77.0%)	1310 (65.9%)	—	—				$\chi^2 = 27.94$	< 0.001
RACP (+)*	495	200	0.7686	0.8994	0.7686	0.8993	0.8674	$\chi^2 = 1117.03$	< 0.001
RACP (−)	149	1788							
Anti-CCP (+)*	382	112	0.7087	0.8992	0.0.7087	0.8991	0.8369	$\chi^2 = 639.41$	< 0.001
Anti-CCP (−)	157	999							
RF (+)*	510	1157	0.8293	0.223	0.8292	0.2229	0.4001	$\chi^2 = 666.52$	< 0.001
RF (−)	105	332							
AKA (+)*	172	53	0.3848	0.9439	0.3847	0.9439	0.7643	$\chi^2 = 241.95$	< 0.001
AKA (−)	275	892							

Note: Sensitivity and specificity values are for positive results: RACP (+), Anti-CCP (+), RF (+), AKA (+). * indicates statistical significance $p < 0.05$.
Abbreviations: χ^2, chi-square statistic; AKA, anti-keratin antibodies; anti-CCP, anti-citrullinated protein antibodies; NPV, negative predictive value; PPV, positive predictive value; *p*-value, probability value; RA, rheumatoid arthritis; RACP, rheumatoid arthritis citrullinated peptides; RF, rheumatoid factor; *t*, *t*-value.

encompassing renal and endocrine disorders, lumbar or cervical disc herniation, and respiratory conditions.

To evaluate whether combining RACP with anti-CCP improved diagnostic discrimination, we classified patients as positive if they tested positive for either marker ("RACP + or anti-CCP +") and compared them to those negative for both. We also assessed the stricter criterion of dual positivity ("RACP + and anti-CCP +") against patients negative for at least one marker. Chi-square analysis demonstrated that both combination strategies significantly outperformed single-marker testing in differentiating RA from non-RA (see Table 2). Using the same approach, we similarly evaluated RACP in combination with RF and with AKA.

When RACP was combined with other biomarkers, overall sensitivity for RA diagnosis improved markedly: the "RACP+ or RF+" criterion detected 85.36% of RA cases, and "RACP+ or anti-CCP+" detected 77.92%, both exceeding the sensitivity of any individual assay. Conversely, specificity was maximized when requiring dual positivity: "RACP+ and AKA+" achieved 96.61% specificity, and "RACP+ and anti-CCP+" reached 91.80%, thereby reducing false-positive rates relative to single-marker use.

In our osteoarthritis (OA) subgroup of 216 patients—selected as a non-autoimmune comparator—the serological assays yielded uniformly low positivity rates: RACP 2.31%, anti-CCP 1.14%, RF 16.66%, and AKA 1.50% (Table 3). These findings confirm that, despite occasional background reactivity (particularly with RF),

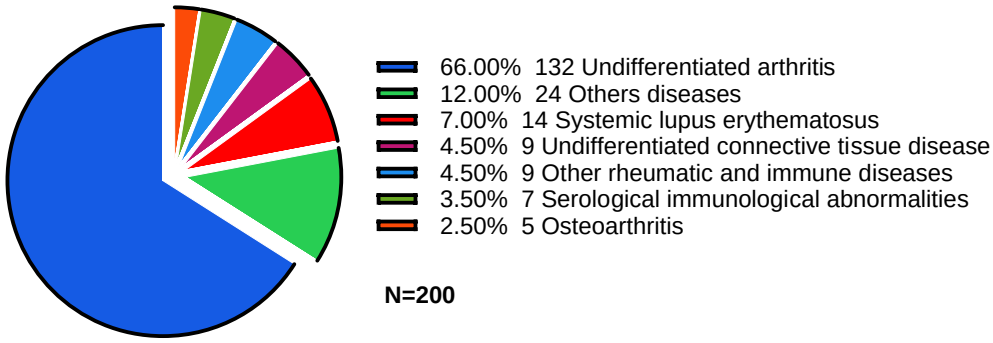

FIGURE 1 | Distribution of disease types among 200 non-rheumatoid arthritis patients with positive rheumatoid arthritis citrullinated peptide.

TABLE 2 | Specificity, sensitivity, chi-square (χ^2), and *p*-values of RA diagnosis using the RACP combined with other biomarkers.

Combination	Sensitivity	Specificity	χ^2	*p*
RACP+ or Anti-CCP+	0.7792	0.8415	606.19	<0.01
RACP+ or RF+	0.8536	0.7555	661.99	<0.01
RACP+ or AKA+	0.7695	0.8349	481.75	<0.01
RACP+ and Anti-CCP+	0.6864	0.9180	658.81	<0.01
RACP+ and RF+	0.7528	0.9019	907.33	<0.01
RACP+ and AKA+	0.3668	0.9661	278.19	<0.01

Abbreviations: AKA, anti-keratin antibodies; RA, rheumatoid arthritis; RACP, rheumatoid arthritis citrullinated peptide; RF, rheumatoid factor.

TABLE 3 | Sensitivity and specificity of RACP, anti-CCP, RF, and AKA in the RA and OA groups.

	RA	OA			Positive		
Results	(*n* = 644)	(*n* = 216)	χ^2	*p*	(OA)	Sensitivity	Specificity
RACP+	495	5	369.355	<0.01	0.0231	0.7686	0.9768
RACP−	149	211					
Anti-CCP+	383	2	257.871	<0.01	0.0114	0.7092	0.9885
Anti-CCP−	157	172					
RF+	581	34	321.029	<0.01	0.1666	0.8311	0.8333
RF−	118	170					
AKA+	173	2	67.109	<0.01	0.015	0.3861	0.9849
AKA−	275	131					

Abbreviations: AKA, anti-keratin antibodies; OA, osteoarthritis; RA, rheumatoid arthritis; RACP, rheumatoid arthritis citrullinated peptide; RF, rheumatoid factor.

RACP, anti-CCP, and AKA maintain high discriminatory power for autoimmune RA versus degenerative OA.

Comparison between the RA and OA groups (Table 3) confirmed that all four biomarkers significantly distinguished autoimmune RA from degenerative OA (χ^2, $p < 0.01$). RACP, anti-CCP, and RF each demonstrated both high sensitivity and specificity in this context; whereas AKA—despite its low sensitivity—retained exceptionally high specificity, minimizing false positives in OA patients.

Analysis of biomarker positivity over the course of RA (Figure 2) revealed distinct temporal patterns. Within the first 6 months after symptom onset, RACP, anti-CCP, and RF each reached 100% positivity in confirmed RA cases. Thereafter, RF positivity remained above 90% at all subsequent disease durations (peaking again at 100% in those with ≥10 years' disease), while anti-CCP gradually rose from 67% in the 1–3 year subgroup to 89% in those 5–10 years post-onset. RACP oscillated between 70% and 84% over time. AKA showed the lowest early-phase detection (50% at <0.5 years) but increased to 64% in patients with long-standing disease (≥10 years), suggesting its potential role in identifying late-stage RA.

When we examined clinical correlations, there was no significant difference in swollen joint counts (SJC) based on any biomarker status. However, patients positive for RACP or anti-CCP had significantly higher tender joint counts (TJC) than seronegative individuals ($p < 0.05$), supporting an association between these autoantibodies and joint tenderness severity (Figure 3).

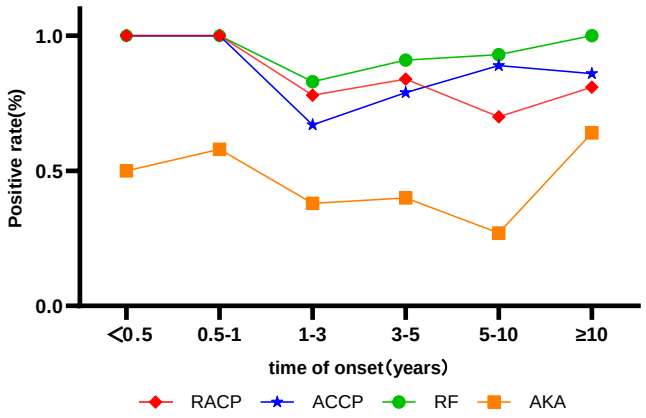

FIGURE 2 | Trend graph of positive rates for markers during the time of rheumatoid arthritis onset. AKA, anti-keratin antibodies; RA, rheumatoid arthritis; RACP, rheumatoid arthritis citrullinated peptide; RF, rheumatoid factor.

4 | Discussion

In real-world cohorts, RACP has emerged as a robust diagnostic marker for RA, particularly in cases with indeterminate clinical presentations. In our series, RACP achieved 76.86% sensitivity and 89.94% specificity, nearly matching the 89.92% specificity of the established anti-CCP assay. In the diagnosis of rheumatoid arthritis, RACP exhibits the highest AUC value among the four indicators (RACP, anti-CCP, RF, and AKA). This directly demonstrates that it possesses superior overall diagnostic efficacy in differentiating RA patients from healthy individuals (or those with other diseases), thereby standing out as the most reliable "discriminator" among the four indicators. These results align closely with other investigators' reports of RACP performance (sensitivity 65%–79.6%, specificity 96%–97.2%), and are corroborated by two recent large-scale studies [7, 11, 20–23]. Conversely, anti-CCP's sensitivity in our cohort was 70%, consistent with the 50%–80% range documented across multiple publications, thereby validating both our anti-CCP and RACP findings [24–29]. By leveraging one of the largest RACP-tested populations to date, our data reinforce the high diagnostic value of RACP in routine practice and support its role as an early warning sign in patients with equivocal arthritis symptoms.

However, this study has several limitations. As a single-center, retrospective analysis conducted in a rheumatology referral cohort, inflammatory conditions were overrepresented in the non-RA group, which may have inflated the false-positive rate and underestimated RACP's specificity. To enhance external validity, future investigations should employ prospective, multicenter designs that include healthy controls and non-rheumatologic populations, thereby reducing sample-composition bias.

Among the 1988 non-RA participants, 200 (10.1%) tested positive for RACP, and of these, 132 (66.0%) were diagnosed with undifferentiated arthritis. Given RACP's high sensitivity and specificity for RA, these patients may represent an early or preclinical stage of RA. Clinicians encountering patients with unexplained arthralgia or undifferentiated arthritis should consider RACP testing and longitudinal follow-up to facilitate early identification and intervention in individuals at risk of developing RA.

Combining RACP with established serological markers enhances RA diagnostic performance in distinct ways: pairing RACP with

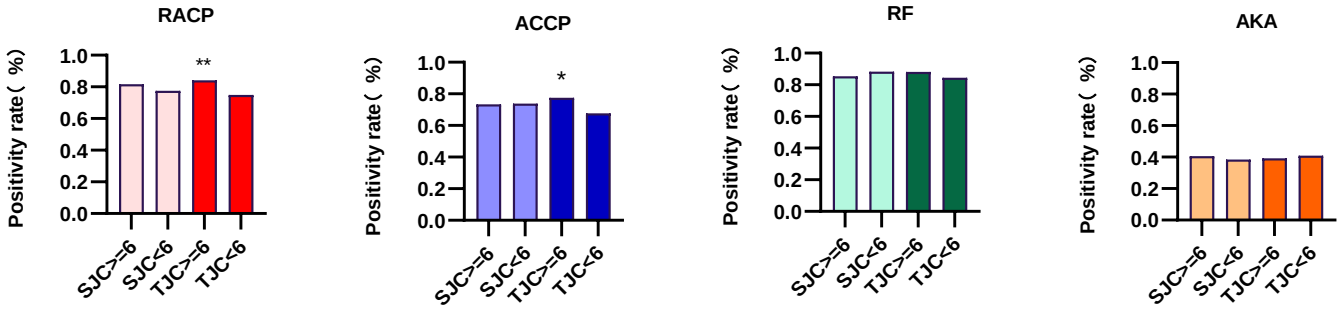

FIGURE 3 | Positive rate distribution of clinical symptoms and test results in patients with rheumatoid arthritis. AKA, anti-keratin antibodies; RA, rheumatoid arthritis; RACP, rheumatoid arthritis citrullinated peptide; RF, rheumatoid factor.

anti-CCP or RF increases sensitivity, whereas requiring dual negativity with AKA or anti-CCP improves specificity for ruling out RA. In our cohort, the "RACP+ or RF+" combination achieved 85.36% sensitivity—higher than the 77.92% seen with "RACP+ or anti-CCP+"—mirroring Zhao et al.'s [22] reported sensitivities of 93.2% versus 91.0% for these same combinations. Zhao et al. [22] also found that dual positivity for "RACP and AKA" conferred greater specificity (96.6%) than "RACP and RF" (90.1%) [22]. Because studies of "RACP and AKA" remain limited and given the antigenic overlap between RACP and anti-CCP, we looked to Liu et al.'s [30] work on combining anti-CCP, AKA, and RF, which demonstrated specificities of 98.9% for "Anti-CCP and AKA" and 98.1% for "Anti-CCP and RF". Consistent with these findings, RF alone in our analysis showed high sensitivity but low specificity; however, specificity rose substantially when RF was combined with RACP. Notably, dual-negative profiles, RACP–/AKA– and RACP–/anti-CCP–, excluded RA in 96% and 91% of cases, respectively, underscoring their utility as robust exclusion markers. Altogether, these results indicate that integrating RACP with anti-CCP or RF can optimize both sensitivity and specificity, offering a practical testing strategy for early identification and risk stratification of patients with non-specific arthritic symptoms.

RACP offers a robust means of distinguishing RA from other arthritides, particularly osteoarthritis (OA), which is characterized by degenerative rather than autoimmune pathology. In RA, neutrophil extracellular trap (NET) formation externalizes citrullinated autoantigens, driving local inflammation and ACPA production; this process is amplified by cytokines such as IL-17A and TNF-α, which are central to RA pathogenesis [31, 32]. By contrast, OA patients exhibit lower levels of NETosis and reduced citrullinated peptide burden—often even below that of healthy controls—reflecting a fundamentally different mechanism of joint damage [32]. Accordingly, we selected all 216 OA patients who underwent RACP testing as a non-autoimmune control cohort. In this group, RACP and anti-CCP positivity rates were only 2.31% and 1.14%, respectively, mirroring the low background reactivity seen in the broader non-RA population. The similarity of biomarker distributions when comparing RA vs. non-RA and RA vs. OA further underscores RACP's specificity in isolating autoimmune processes.

Beyond differential diagnosis, RACP demonstrates exceptional early-stage sensitivity, on par with established markers. In our cohort, individuals ultimately confirmed to have RA exhibited high RACP, anti-CCP, and RF positivity within the first year of symptom onset (Figure 2). This aligns with longitudinal studies showing that anti-CCP titers independently predict progression to RA—23 of 73 patients with early arthralgia developed RA over a 60-month follow-up—and that serological abnormalities (RF/anti-CCP) can precede clinical onset by a median of 3–4.5 years, and up to 14.5 years in some cases [24, 33, 34]. Because RACP directly detects citrullinated peptide antigens upstream of ACPA formation, it may become positive even earlier, offering a promising avenue for identifying Pre-RA and initiating intervention before irreversible joint damage occurs.

The capacity of RACP to reflect current RA severity warrants further exploration. Wigerblad et al. [35] demonstrated that intra-articular injection of ACPA into healthy mouse joints induces pain behaviors, implicating ACPA in symptom generation. Consistent with this, we observed that patients with TJC ≥ 6 had significantly higher RACP and anti-CCP positivity rates than those with TJC < 6, with the difference more pronounced for RACP. This parallels Maejima et al.'s [36] finding that anti-CCP titers increase in step with clinical disease activity scores, suggesting that RACP quantification may similarly correlate with inflammatory burden. Prospective studies linking longitudinal RACP measurements to standardized activity indices (e.g., DAS28-CRP: Disease Activity Score 28-Joint Count with C-Reactive Protein, SDAI: Simplified Disease Activity Index) are therefore needed to establish its utility as a dynamic biomarker of RA severity.

In conclusion, RACP demonstrates high sensitivity and specificity for RA diagnosis, and its combination with anti-CCP, RF, or AKA further enhances early diagnostic accuracy. RACP also effectively discriminates RA from degenerative OA and shows potential as an indicator of disease activity, providing a strong rationale for future research on its role in RA monitoring and prognosis, which may serve to bridge certain gaps in the extant research concerning RA biomarkers within this domain.

Author Contributions

Kun Yang: the study concept and design; acquisition of data; drafting of the manuscript; analysis and interpretation of data. Sixing Li, Leting Zheng, Fei Dong, Jing Wen, Zhendong He, and Jiale Wen: acquisition of data; administrative, technical, or material support. Fang Qin: the study concept and design; obtained funding; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; study supervision.

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Ethics Statement

This study was approved by the Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University (Approval No. 2025-E0117). As a retrospective analysis, the requirement for informed consent was waived by the ethics committee, and all patient data were anonymized.

Consent

The dataset employed in this study was manually curated from a hospital database following rigorous institutional review board (IRB) approval and adherence to all legal and ethical guidelines. Specifically, the dataset comprises anonymized patient information, including surnames, hospitalization numbers, gender, and selected laboratory test results. Data collection strictly complied with the hospital's privacy policies and data governance regulations, and access to the data was granted solely for the purposes of this study. To ensure transparency and reproducibility, inquiries regarding data acquisition or usage should be directed to the corresponding author.

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

Clinical Outcomes After SARS-CoV-2 Infection and Vaccination in Patients With Pediatric Rheumatic Diseases

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Dear Editor,

Pediatric rheumatic diseases, such as systemic juvenile idiopathic arthritis (s-JIA), are driven by underlying immune system abnormalities. Thus, it is known that various infectious diseases activate the immune system, leading to disease onset and flare-ups. Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic, there have been some reports of SARS-CoV-2 infection or vaccination triggering the onset or flare-ups of rheumatic diseases in adults. However, such reports in children remain limited [1–7]. Therefore, we investigated the clinical course of pediatric rheumatic patients after SARS-CoV-2 infection and vaccination at our hospital to identify which cases are at higher risk of developing diseases or experiencing flare-ups triggered by SARS-CoV-2 infection and vaccination.

The subjects of this study were pediatric rheumatic disease patients who visited our hospital and received treatment between April 2020 and March 2023. Medical records were retrospectively reviewed to examine the dates of SARS-CoV-2 infection and vaccination, clinical symptoms, laboratory findings, and treatment details. In this study, SARS-CoV-2 infection was defined as a positive result from an antigen test using a nasopharyngeal swab or a polymerase chain reaction test. The onset of disease was defined as the appearance of underlying diseases within 8 weeks of SARS-CoV-2 infection or vaccination, followed by the initiation of treatment. A flare-up was defined as a worsening of the underlying disease within 8 weeks of SARS-CoV-2 infection or vaccination, accompanied by an increase in the therapeutic dose or the addition of other medications. This study was conducted with approval from the Medical Ethics Review Committee of the Institute of Science Tokyo (approval number: M2023-096). Additionally, written consent was obtained from both the participating patients and their guardians.

This study included 130 participants, with 36 males and 94 females. Seventy of the 130 participants (53.8%) were infected with SARS-CoV-2, and 66 participants (50.8%) had received at least one vaccination. The underlying diseases of the 130 participants were as follows: 62 patients with JIA (19 with s-JIA, 25 with oligoarthritis, 9 with polyarthritis, 8 with enthesitis-related arthritis, and 1 with psoriatic arthritis); 19 with systemic lupus erythematosus (SLE); 10 with juvenile dermatomyositis (JDM); 4 with Sjogren's disease (SD); 3 with mixed connective tissue disease; 8 with Behçet's disease (BD); 3 with Takayasu's arteritis; 7 with chronic nonbacterial osteomyelitis; 9 with periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA)

Abbreviations: BD, Behçet's disease; JDM, juvenile dermatomyositis; MAS, macrophage activation syndrome; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, and adenitis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, Sjogren's disease; s-JIA, systemic juvenile idiopathic arthritis; SLE, systemic lupus erythematosus.

Summary

- Careful monitoring for flare-ups is necessary for the patients with s-JIA after SARS-CoV-2 infection.

syndrome; 3 with familial Mediterranean fever; and 2 with haploinsufficiency of A20.

After SARS-CoV-2 infection, disease onset was noted in 2 patients (2.9%) (1 with s-JIA and the other with PFAPA), and flare-ups were noted in 8 patients (11.8%) (3 with s-JIA, 1 with oligoarthritis JIA, 2 with SLE, 1 with SS, and 1 with BD) (Table 1). Disease flares were noted in 3 of 8 patients with s-JIA with SARS-CoV-2 infection (37.5%). Three patients with s-JIA developed macrophage activation syndrome (MAS) after SARS-CoV-2 infection, while 1 patient with SLE and 1 with BD required hospitalization (Table 2). On the other hand, flare-ups were noted in 19 of 60 patients without SARS-CoV-2 infection (31.7%). Disease flares were noted in 2 of 10 patients with s-JIA without SARS-CoV-2 infection (20.0%).

Seven patients (10.6%) had flare-ups of AIIRD after vaccination (1 with s-JIA, 2 with oligoarthritis JIA, 1 with ERA, 2 with SS, and 1 with BD) (Table 1). The symptoms of all patients who experienced flare-ups after vaccination improved with intensified treatment and did not require hospitalization (Table 2).

There are few reports on flare-ups of pediatric rheumatic diseases following SARS-CoV-2 infection or vaccination [1–5]. They report post-infection flare-ups in 5.5%–11.4% of patients [1, 2] and post-vaccination flare-ups in 4.4%–12.1% [2–4]. In this study, post-infection flare-ups were noted in 11.4% of patients, and post-vaccination flare-ups in 10.6%. These flare-up rates are consistent with previous reports. As previously reported, the post-vaccination flare-up cases showed only minor symptoms, and none required hospitalization [2–4]. Additionally, none of the patients in this study developed severe COVID-19 symptoms, which also aligns with previous reports [5].

Meanwhile, previous studies that used only biological agents reported no flare-ups following SARS-CoV-2 infection [6]. In contrast, this study noted flare-ups in patients receiving

TABLE 1 | Onset and flare-up following SARS-CoV-2 infection and vaccination.

				After infection		After vaccination	
	All patients	**Infection *n* (%)**	**Vaccination *n* (%)**	**Onset**	**Flare**	**Onset**	**Flare**
Number of patients	130	70 (47.4)	66 (50.8)	2 (2.9)	8 (11.4)	0 (0)	7 (10.6)
Sex							
Female	94			1	6		5
Male	36			1	2		2
Diagnosis							
Systemic JIA	19	9 (47.4)	10 (52.6)	1	3		1
Oligo JIA	25	14 (56.0)	10 (40.0)		1		
Poly JIA	9	5 (55.6)	6 (66.7)				2
ERA	8	3 (37.5)	5 (62.5)				1
PsA	1	1 (100)	0 (0)				
SLE	19	10 (52.6)	8 (42.1)		2		
JDM	10	4 (40.0)	5 (50.0)				
SS	4	2 (50.0)	4 (100)		1		2
MCTD	3	3 (100)	0 (0)				
BD	8	3 (37.5)	4 (50.0)		1		1
CNO	7	5 (71.4)	4 (57.1)				
PFAPA	9	8 (88.9)	5 (55.6)	1			
FMF	3	1 (33.3)	2 (66.7)				
TAK	3	2 (66.7)	2 (66.7)				
HA20	2	0 (0)	1 (50.0)				

Abbreviations: BD, Behçet's disease; CNO, chronic non-bacterial osteomyelitis; ERA, enthesitis related arthritis; FMF, familial Mediterranean fever; HA20, haploinsufficiency A20; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; MCTD, mixed connective tissue disease; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome; PsA, psoriatic arthritis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SLE; systemic lupus erythematosus; SS, Sjogren syndrome; TAK, Takayasu arteritis.

TABLE 2 | Clinical characteristics of the patients with flare-ups after SARS-CoV-2 infection and SARS-CoV-2 vaccination.

Case	Diagnosis	Sex	Age at infection	Age at onset	Treatment at the time of infection	Condition	Infection-related symptoms	Symptoms at flare-up	Treatment for the flare-up	Hospitalization
The patients with flare-ups after SARS-CoV-2 infection										
1	s-JIA	F	10 years	4 years	CAN, PSL, MTX, Tac	Active arthritis	Fever, arthralgia	Arthritis	PSL↑	N
2	s-JIA	M	5 years	0 year and 7 months	PSL	Remission	Fever	MAS	IVMP, PSL↑, CyA	Y
3	s-JIA	M	6 years	1 years	CAN	Remission	Fever, arthralgia	MAS	PSL	Y
4	Oligo JIA	F	12 years	12 years	MTX	Active arthritis	N.D	Arthritis	ADA	N
5	SLE	F	8 years	7 years	PSL, MMF, Tac HCQ, BEL	Induction of remission	Fever	Fever, thrombopenia, hypocomplementemia	MMF, Tac, IVMP	Y
6	SLE	F	13 years	7 years	PSL, MMF, HCQ, Bari	Induction of remission	Asymptomatic	Erythema, CLN swelling	MMF→MTX, Bari→Upa	N
7	SS	F	11 years	10 years	Topical CS	Persistent, erythema	N.D	Erythema	PSL	N
8	BD	F	11 years	10 years	PSL, COL, IFX	Recurrent stomatitis	Fever, cough	Genital ulcer, stomatitis	PSL↑	Y
The patients with flare-ups after SARS-CoV-2 vaccination										
1	s-JIA	F	19 years	1 years	PSL, MTX, Upa	Active arthritis	ARTHRITIS	PSL, Upa↑	N	1
2	Poly JIA	M	13 years	9 years	MTX, Tac, ADA	Remission	arthritis	MTX↑, NSAIDs	N	2
3	Poly JIA	F	11 years	6 years	MTX, Tac, ADA	Remission	Arthritis	Tac↑	N	3
4	ERA	M	18 years	14 years	SASP, Bari	Hip joint pain	Arthritis	Bari→ADA	N	4
5	SS	F	12 years	10 years	PSL	Parotitis	Parotitis, erythema	PSL↑, AZP	N	5
6	SS	F	13 years	11 years	PSL, AZP	Cervical adenopathy	Arthritis, erythema	PSL↑, NSAIDs	N	6
7	BD	F	12 years	9 years	COL, AZP, ADA	Remission	Stomatitis, folliculitis	ADA↑	N	7

Note: ↑ means dose up and → means changed.

biological agents as well as those treated with Janus Kinase inhibitors.

In this study, the rate of disease flares in patients with AIIRD other than s-JIA was higher in patients without SARS-CoV-2 infection compared to those with SARS-CoV-2 infection. These findings indicate that SARS-CoV-2 infection does not appear to contribute to increased relapse rates of AIIRD other than s-JIA.

One of the key findings of this study was that cases of s-JIA, which are common in Japan, had a higher flare-up rate (33%) compared to other diseases. Furthermore, the rate of disease flares in patients with s-JIA was higher in patients with SARS-CoV-2 infection compared to those without SARS-CoV-2 infection. These flare-ups occurred even after remission, and in some cases, infections triggered flare-ups that developed into MAS, suggesting the necessity of careful monitoring. Flare-ups of all other diseases were noted when the disease state was unstable. Therefore, careful monitoring seems particularly important when s-JIA patients who have not achieved remission are infected with SARS-CoV-2.

Limitations of this study include its retrospective approach, as well as its single-center design and small sample size. Large-scale studies with more cases across multiple centers should be conducted in the future.

In conclusion, this study suggested that the s-JIA patients who have not achieved remission require careful monitoring for flare-ups when infected with SARS-CoV-2. Since the s-JIA patients may experience flare-ups and concurrently develop MAS even while in remission with biological therapy, the utmost caution should be exercised to prevent infection. While flare-ups may occur after SARS-CoV-2 vaccination, no severe cases were noted in this study. Therefore, vaccination appears to be a reasonable recommendation for patients with pediatric rheumatic diseases.

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Author Contributions

The authors take full responsibility for this article.

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Conflicts of Interest

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Data Availability Statement

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

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LETTER TO THE EDITOR

Case Report: Daratumumab for a Patient With Refractory Systemic Lupus Erythematosus and Antiphospholipid Syndrome Presenting as Thrombocytopenia

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Dear Editor,

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder, often characterized by thrombocytopenia, particularly when accompanied by antiphospholipid syndrome (APS), which is frequently associated with elevated levels of antiphospholipid antibodies (aPL) [1]. Recent research has increasingly highlighted the role of long-circulating plasma cells, which secrete pathogenic antibodies, in the pathogenesis of SLE. Daratumumab, a monoclonal antibody targeting CD38, has emerged as a promising therapeutic option for autoimmune diseases, specifically by depleting malignant plasma cells. This case report discusses a patient with refractory SLE and APS, presenting with thrombocytopenia, with the aim of evaluating the clinical outcomes and therapeutic potential of daratumumab in this context.

A 54-year-old female patient presented with refractory thrombocytopenia for over a year despite being treated with high-dose steroids along with mycophenolate mofetil (MMF), cyclosporine A (CsA), and intravenous immunoglobulin (IVIG). She was diagnosed with SLE and APS 12 years ago. Previous therapies (Figure 1) failed to induce sustainable remission. Upon admission, the physical examination revealed scattered ecchymosis on both lower limbs. Laboratory tests showed a platelet count of 21×10^9/L, elevated antinuclear antibody (ANA) titers, and positive results for anti–double–stranded DNA (anti-dsDNA), anti-RO52, anti-SSA, anti-ribosomal P-protein antibodies, IgA anti-cardiolipin antibodies, IgM anti-β2 glycoprotein I antibodies, as well as IgG and IgM anti-phosphatidylserine/prothrombin antibodies. Additionally, platelet-associated antibodies, including anti-GP IIb/IIIa, anti-GP IX, and anti-GMP antibodies, were detected. Furthermore, the levels of lupus anticoagulant (LA), D-dimer, and fibrin degradation products (FDPs) were markedly elevated. These findings suggested that the thrombocytopenia may have resulted from a synergistic combination of immune-mediated platelet destruction and in vivo microthrombosis. Flow cytometry revealed a marked increase in CD38-positive plasma cells in peripheral blood. Following this, the patient received daratumumab at a dose of 4 mg/kg via intravenous infusion every 2 weeks for 1 month. After a 4-week treatment, the patient's platelet count increased from 21×10^9/L to 107×10^9/L. The SLE Disease Activity Index 2000 (SLEDAI-2K) score decreased from 18 to 6 and remained stable over the subsequent year. Furthermore, the patient's erythrocyte sedimentation rate (ESR), C3, C4, and immunoglobulin levels normalized (Figure 1A–E). Coagulation tests, including prothrombin time (PT), activated partial thromboplastin time (aPTT), D-Dimer, fibrin degradation products (FDPs), and lupus anticoagulant (LA) returned to the normal range. Daratumumab was well-tolerated, with no adverse reactions observed (Figure 1F). In addition, the ratio of lymphocyte subsets in the patient showed some changes after treatment (Figure 1G). The results indicate a slight decrease in the ratio of B lymphocyte subsets, which is consistent with the depletion of CD38-positive plasma cells (Figure 1H).

In this case, daratumumab was used for the first time at a low dose to treat thrombocytopenia secondary to refractory SLE and APS in a low dose, referred to as “mini-dara.” With “mini-dara,”

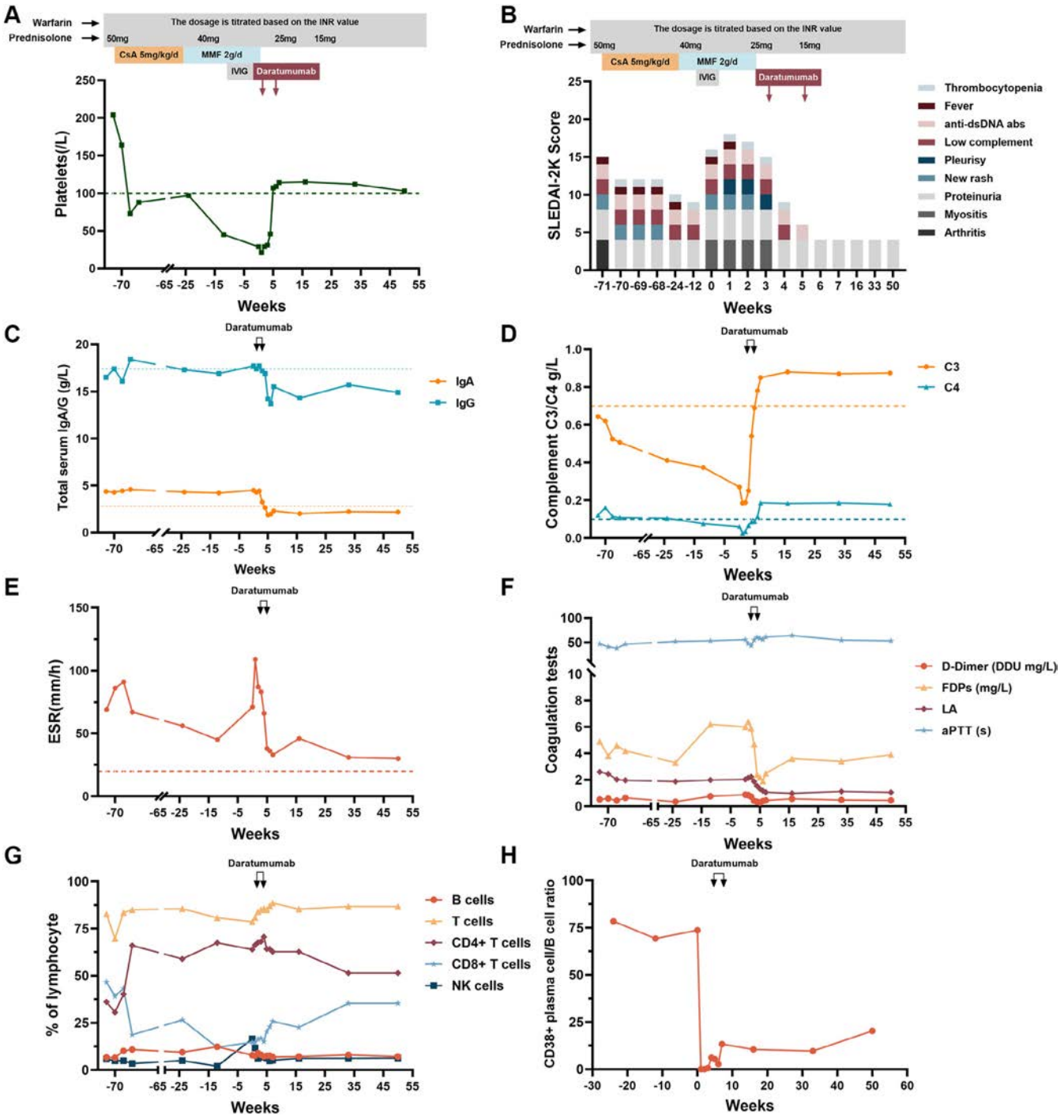

FIGURE 1 | The time course of the patient's immunosuppressive treatment and the clinical findings following these therapeutic interventions. (A–F) Platelet count, SLEDA-2K score, total serum IgG and IgA levels, serum complement C3 and C4 levels, ESR level, and coagulation tests before and after daratumumab treatment. Previous therapies with CsA, MMF, and IVIG did not lead to clinical remission. (G) The relative levels of lymphocyte subsets in peripheral blood under daratumumab treatment. (H) The relative proportion of CD38-positive plasma cells within B cells under daratumumab treatment. Arrows indicate daratumumab doses, and the dotted line indicates the upper normal limit for platelet counts, total serum IgG and IgA levels, serum complement C3 and C4 levels, and ESR level. CsA, cyclosporine A; INR, international normalized ratio; IVIg, intravenous immunoglobulin; MMF, mycophenolate mofetil; NK, natural killer; SLEDA-2K, Systemic lupus erythematosus disease activity index 2000.

thrombocytopenia caused by both immune-mediated destruction and microthrombosis was successfully corrected. Flow cytometry revealed a high proportion of CD38-positive plasma cells in peripheral blood (CD38+ plasma cell/B cell ratio of 73.64%). Following treatment with mini-dara, CD38+ plasma cells were undetectable, indicating that low-dose daratumumab effectively depleted these specific plasma cells expressing CD38. Notably, the patient maintained sustained remission during the subsequent 1-year follow-up period.

CD38 (Cluster of Differentiation 38) is a multifunctional transmembrane glycoprotein that is predominantly expressed on

TABLE 1 | Daratumumab for other autoimmune diseases.

Age (years)/ Sex	Diagnosis	Disease duration (months)	Previous treatment	Daratumumab regimens (mg/week)	Outcome	Duration of sustained remission (months)	References
50/F	SLE, active LN	216	GC, CsA, AZA, MMF, bortezomib, CYC, BEL	16 mg/kg qw*4w	Improved	36	Ostendorf et al. [6, 7]
32/F	SLE, anemia	204	CYC, MMF, AZA, MTX, IVIG, RTX; BEL, HCQ, plasmapheresis	16 mg/kg qw*4w	Improved	36	Ostendorf et al. [6, 7]
19/F	Primary APS	24	Acenocoumarol, HCQ, LMWH, aspirin, IVIG, plasmapheresis	8 mg/kg qw*1w; 16 mg/kg qw*3w	Improved	3	Pleguezu-elo et al. [3]
27/M	Refractory anti-SRP+ IMNM	8	GC, RTX, MTX, TAC, IVIG	16 mg/kg qw*8w; 16 mg/kg q2w*7w; 16 mg/kg qm*1m	Improved	6	Océane et al. [8]
20/M	AAV	2	GC, RTX, CYC, avacopan, plasmapheresis	16 mg/kg qw*8w	Improved	6	Rixecker et al. [9]
57/M	AAV (MPO-ANCA+)	12	GC, CYC, RTX	1800 mg qw*8w	Improved	8	Ostendorf et al. [10]
41/M	AAV (PR3-ANCA+)	4	GC, CYC, RTX, plasmapheresis	1800 mg qw*4w	Improved	4	Ostendorf et al. [10]
18/F	Refractory primary SjD	60	RTX	1800 mg qw*4w	Improved	6	Nocturne et al. [11]
68/F	Refractory primary SjD	NA	RTX, CYC, BEL	1800 mg qw*4w	Improved	6	Nocturne et al. [11]
19/M	MDA5+ dermatomy-ositis	Onset	GC, CYC, tofacitinib, MMF, CsA, RTX, IVIG, anakinra	1800 mg qw*4w	Improved	6	Holzer et al. [12]

Abbreviations: AAV, ANCA-associated vasculitis; APS, anti-phospholipid syndrome; AZA, azathioprine; BEL, azathioprine; CsA, cyclosporine A; CYC, cyclophosphamide; GC, glucocorticoids; HCQ, hydroxychloroquine; IMNM, immune-mediated necrotising myopathy; IVIG, intravenous immunoglobulin; LMWH, low molecular weight heparin; LN, lupus nephritis; MDA5, melanoma differentiation-associated gene 5; MMF, mycophenolate mofetil; MTX, methotrexate; RTX, rituximab; SjD, Sjögren disease; TAC, tacrolimus.

the surface of plasma cells [2]. Daratumumab, the first monoclonal antibody targeting CD38, was initially approved for the treatment of multiple myeloma. Due to its capacity to induce plasma cell depletion, it has since been investigated for reducing pathogenic autoantibodies by targeting and depleting long-lived plasma cells, thereby offering a potential strategy for the management or treatment of refractory autoimmune disorders. Daratumumab has demonstrated efficacy in reducing venous thromboembolic events in patients with APS by lowering antiphospholipid antibody titers [3], and it also shows promise as a therapeutic option for refractory immune thrombocytopenia (ITP) through the reduction of anti-GP IIb/IIIa antibody levels [4] In the treatment of SLE, the depletion of circulating plasma cells by daratumumab underscores its therapeutic potential in targeting antibody-producing plasma cells. Additionally, SLE patients with elevated levels of interferon (IFN)-alpha show significant defects in hematopoiesis [5]. In two reported cases of SLE treated with daratumumab, the treatment effectively led to significant symptom amelioration in patients [6] and a sustained remission in the subsequent 3-year follow-up [7], which was associated with a reduction in type I IFN activity. In addition, daratumumab has demonstrated promising therapeutic potential in other antibody-mediated autoimmune diseases, such as refractory primary Sjögren disease, refractory ANCA-associated vasculitis, and myositis. Clinical reports indicate that after one or two cycles of daratumumab, patients achieved remission of clinical symptoms, with stabilization observed during follow-up periods ranging from 3 months to 3 years. Here we summarize the characteristics and outcomes of these patients (Table 1). Regarding its impact on other lymphocyte subsets, daratumumab may exert therapeutic effects through interactions with T or NK cells, in addition to its primary action on plasma cells, as indicated by these case reports. In the context of APS treatment, no significant changes were observed in B-cell and overall lymphocyte levels following daratumumab administration. However, a transient decrease in NK-cell levels was noted, which mirrors findings observed in our case. While most cases demonstrated significant disease remission after treatment, complete recovery was not achieved, which may be attributed to the course and dosage of daratumumab. Future prospective clinical trials are necessary to evaluate the safety and efficacy of daratumumab in patients with autoimmune diseases, to define optimal treatment regimens, and to identify the most suitable patient populations.

Author Contributions

Xinyu Li: writing – original draft, methodology, investigation, data curation. **Xuesong Liu:** writing – review and editing, formal analysis, visualization. **Hanlin Yin:** conceptualization, validation, writing – review and editing, corresponding author. **Liangjing Lu:** writing – review and editing, supervision, project administration, resources, corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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LETTER TO THE EDITOR

Successful Treatment of Pediatric Generalized Pustular Psoriasis With Adalimumab: Case Report and Literature Review

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Dear Editor,

Generalized pustular psoriasis is a rare, life-threatening form of psoriasis. It was first discovered in 1910 by von Zumbusch. As is well documented, while it can affect any age, the median age of onset is under 50 years old. In patients with a family history of psoriasis or homozygous IL36RN mutation, the age of onset is generally earlier. In the pediatric population, the prevalence of GPP ranges from 0.6% to 7%, with a male predominance and onset between 3 and 16 years [1]. The pathophysiology of pustular psoriasis has not been fully elucidated. GPP is frequently managed with systemic therapy, which is challenging due to the lack of randomized controlled trials and standardized guidelines. Traditional systemic therapies are associated with significant shortcomings, such as slow onset of action, limited efficiency, and poor tolerability. In recent years, the introduction of biologics has significantly improved the treatment outcomes of GPP patients.

The present article outlines a case of a GPP patient aged 3 years and 6 months who had a poor response to glucocorticoids and antibiotics, and was unable to use spesolimab due to social factors. After treatment with adalimumab, the body temperature normalized, while the rash gradually resolved. This study aimed to summarize the efficacy and safety of adalimumab in the treatment of pediatric GPP.

1 | Case Report

A 3-year and-6-month-old Chinese girl presented to the Children's Hospital, Zhejiang University School of Medicine, on October 9, 2023, with a rash that lasted for more than 20 days and a fever for 1 week as chief complaints. The rash initially appeared on the abdomen and waist before spreading to the whole body. It was irregular in shape, accompanied by itching and pain. A week prior to admission, the patient experienced recurrent high fever. The child was diagnosed with eczema due to a history of recurrent rash and intermittently applied hormone ointments. The birth and family history of the patient were unremarkable. On admission, physical examination revealed a generalized erythematous rash, with small pustules visible over the affected areas (Figure 1A–C). No other abnormalities were detected on systemic examinations. However, the white blood cell count (WBC count), neutrophil count (NEU count), CRP, and ESR were significantly increased. On the other hand, the levels of cytokines, procalcitonin, ferritin, rheumatoid factor, and Anti-Streptolysin O were within the normal range. Infections with Epstein–Barr virus, cytomegalovirus, and other viruses and tuberculosis were excluded. Concurrently, hematologic malignancies and solid tumors were excluded. Moreover, no evidence of bacterial infection was detected in blood cultures. Cardiac ultrasound displayed no signs of coronary artery dilation. Moreover, no evidence of immunodeficiency was identified.

Abbreviations: CRP, C-reactive protein; EMA, European Medicines Agency; ESR, erythrocyte sedimentation rate; FDA, Food and Drug Administration; GPP, generalized pustular psoriasis; GPPASI, Generalized Pustular Psoriasis Area and Severity Index; GPPGA, Generalized Pustular Psoriasis Physician Global Assessment; MTX, methotrexate; NEU count, neutrophil count; WBC count, white blood cell count.

Yandie Li and Yiping Xu contributed equally to this work.

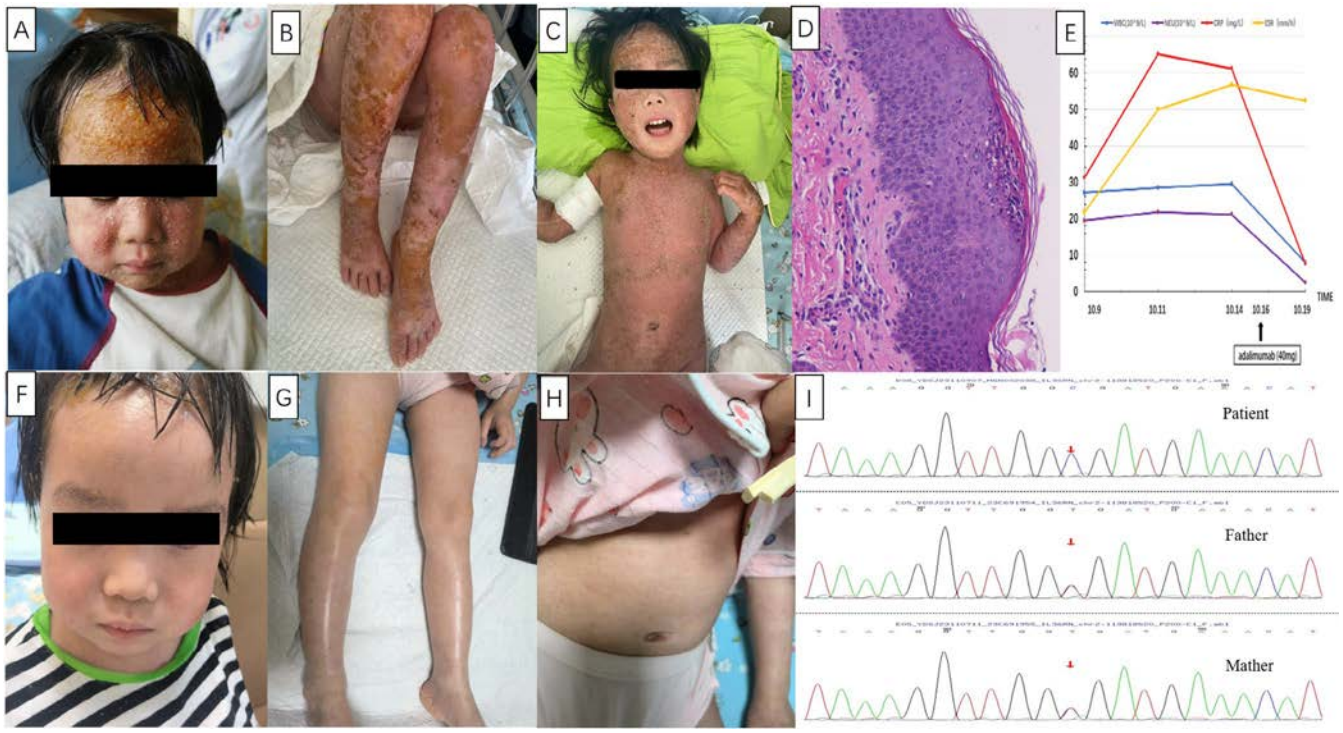

FIGURE 1 | Changes in rash before and after adalimumab treatment in generalized pustular psoriasis patient. (A–C) Prior to adalimumab treatment, there were systemic sterile pustules based on erythema, accompanied by some scales. (F–H) After treatment with adalimumab, the rash disappeared and there was no recurrence during a 1-year follow-up. (D) Skin biopsy showed a red background, with focal distribution of dotted, linear, and irregular blood vessels covered with white scales, and formation of small pustules on the epidermis, with abundant infiltration of neutrophils throughout the epidermis and dermis (Hematoxylin–Eosin). (E) WBC count, NEU count, CRP and ESR levels before and after treatment of adalimumab. Arrow indicates the first administration of adalimumab (40 mg in week 0, and 20 mg Q2W). After treatment of adalimumab, WBC count, NEU count and CRP return to normal and ESR has decreased. (I) The mutation in the IL-36RN gene (NM012275.3; intron3): Chr2:113818520 c.115 + 6 T>C homozygous mutation.

Despite treatment with mometasone furoate cream, dexamethasone cream, antibiotics (including cefotaxime, meropenem, linezolid and vancomycin), intravenous immunoglobulin, and intravenous methylprednisolone (2 mg/kg, twice daily), the patient still experienced recurrent fever and worsening rash. On October 11, 2023, a skin biopsy was performed. Immunofluorescence analysis was performed, which was all negative for IgG, IgA, IgM, and C3. Of note, histopathological analysis revealed a red background with a focal distribution of dotted, linear, and irregular blood vessels covered with white scales and small pustules on the epidermis, with abundant infiltration of neutrophils throughout the epidermis and dermis (Figure 1D). The patient was eventually diagnosed with GPP based on the clinical manifestations and laboratory findings.

Next, the patient was assessed using a Generalized Pustular Psoriasis Physician Global Assessment (GPPGA), which yielded a score of 3 (moderate), while the Generalized Pustular Psoriasis Area and Severity Index (GPPASI) yielded a score of 48.6. Given the young age and urgency of her condition, timely symptom relief was crucial. Currently, spesolimab is the preferred first-line biologic for GPP treatment, which targets IL-36R, blocking the IL-36 signaling pathway to suppress inflammatory responses. However, in Chinese Mainland, spesolimab is expensive, and patients' families could not afford it. Finally, we chose adalimumab. Fortunately, after the administration of adalimumab (40 mg) with the informed consent of her parents, the body temperature immediately normalized, and the rash gradually resolved (Figure 1F–H). More importantly, the levels of inflammatory markers returned within the normal range (Figure 1E). Similarly, after treatment with adalimumab, WBC count, NEU count, and CRP returned to within normal levels, accompanied by a significant decrease in ESR. Finally, the patient and his parents were recommended to undergo genetic testing, which uncovered a pathogenic homozygous mutation in the IL-36RN gene (NM012275.3; intron3): chr2:113818520 c.115 + 6 T>C homozygous mutation (Figure 1I). Subcutaneous injection of 20 mg adalimumab every 2 weeks achieved satisfactory disease control.

Subsequently, the patient was administered adalimumab for 3 months without experiencing rash or fever recurrence. However, due to economic considerations of the parents, adalimumab was discontinued. We have been following up with the child for over a year, monitoring the adverse reactions, including infection, injection site reactions, and liver and kidney function tests. No adverse events occurred, and GPP has not recurred. At the latest follow-up visit, the child's GPPGA score was 1, and the GPPASI score was 0.6. The complete clinical and follow-up dataset is shown in Table S1.

This study was approved by the Research Ethical Committee of the Children's Hospital, Zhejiang University School of Medicine (2024-IRB-0355). Informed consent for the collection and publication of data and images was obtained from the patient's parents.

2 | Literature Review

Furthermore, a literature review was performed using PubMed, Web of Science, Wanfang, and CNKI by searching for the terms "generalized pustular psoriasis" or "GPP" and "adalimumab." Previously reported cases of adalimumab use in treating GPP patients are summarized in Table 1. In previous studies, a total of 15 GPP patients, including eight males and seven females aged 3–17 years, were treated with adalimumab [2–9]. These patients had undergone various treatments. Nevertheless, their conditions remained inadequately controlled. Noteworthily, following the administration of adalimumab, the majority of patients achieved complete remission.

3 | Discussion

GPP is an uncommon and severe form of psoriasis characterized by small sterile pustules on an erythematous base, some of which may partially merge into a lake of pus. To date, its pathogenesis remains to be elucidated. Multiple cytokines, including IL-36, IL-1, IL-17, and TNF-α, have been reported to be implicated in its pathogenesis. For instance, Johnston et al. described that the IL-1/IL-36 chemokine neutrophil axis plays a central role in the pathogenesis of GPP [10]. TNF-α is associated with increased production of IL-36R agonists, which stimulate the IL-36 pathway, inducing more TNF-α production in the sustained inflammatory cycle. TNF-α inhibitors indirectly inhibit the expression of IL-36γ, leading to reduced activation of the pro-inflammatory IL-36 pathway [11]. Three gene mutations were identified in GPP patients, namely IL36RN, CARD14, and AP1S3. The incidence of IL-36RN mutations ranges between 23% and 37% across GPP patients. It is worthwhile emphasizing that IL-36RN mutations are associated with more severe clinical phenotypes and earlier disease onset [12]. An earlier study concluded that the mutation rate of IL36RN in patients with pustular psoriasis can be as high as 75% [13]. Herein, the child harbored an IL-36RN mutation, indicating a severe condition that warrants timely treatment.

GPP is a potentially life-threatening condition that presents significant therapeutic challenges. Herein, topical glucocorticoids, systemic glucocorticoids, and antibiotics did not delay disease progression. An optimal therapy should be highly efficacious, fast-acting, easily administered, and safe for the patient. First-line treatment for children with GPP is similar to that for adults, including acitretin, cyclosporine, MTX, etanercept, and spesolimab. Considering that retinoids can cause premature epiphyseal closure, it may not be an ideal first-line therapy in children. Meanwhile, MTX and cyclosporine have long-term oncogenic potential [14], with the latter also associated with hypertrichosis [15]. Second-line therapy includes adalimumab, infliximab, and UVB phototherapy in combination with systemic therapy [16]. Long-term use of corticosteroids has been linked to multiple side effects. At the same time, PUVA is contraindicated in children under 12 years [17]. The patient's parents were reluctant to use drugs that could negatively impact the mental and physical development of the child and preferred the use of biologics. A pathogenic mutation was detected in the IL36RN gene of the patient. Spesolimab is a humanized IgG1 monoclonal antibody that selectively binds to IL-36R with high affinity and inhibits the IL-36-mediated signaling pathway. However, Spesolimab is exclusively indicated for the treatment of GPP in adolescents aged 12 years and above (weight ≥ 40 kg) and adults in China [18]. In addition to the age factor of the patient, the most important thing is that the girl's parents cannot afford it. Therefore, we searched through literature to find better second-line biologics, including infliximab and adalimumab. From the perspective of medication routes, infliximab belongs to intravenous injection, while adalimumab belongs to subcutaneous injection. Adalimumab was ultimately selected, and the decision is also supported by literature. Successful switching to adalimumab treatment in GPP patients who have failed treatment with infliximab may be ascribed to adalimumab being a completely human monoclonal antibody with a lower probability of generating autoantibodies [19]. A phase 3, multicenter, open-label, 52-week study investigated the efficacy and safety of adalimumab 80 mg at week 0, followed by adalimumab 40 mg every other week, with an option to escalate to 80 mg when necessary, in Japanese patients with GPP aged between 15 and 75 years. Of the 10 enrolled patients (mean disease duration, 10.6 years), seven patients, including three with the dose escalation to 80 mg every other week before week 15, achieved clinical response at week 16, whilst five achieved clinical response at week 52. Besides, nine patients experienced one or more adverse events, and three experienced severe adverse events. In conclusion, adalimumab was effective and well tolerated for up to 52 weeks in the treatment of Japanese patients with GPP [20]. In our patient, body temperature normalized, and the rash progressively resolved within 24 h of initiating adalimumab, indicating successful treatment outcomes.

Considering the convenience of adalimumab and its favorable efficacy, the patient received adalimumab treatment for 3 months without recurrence. If the subsequent effect was not satisfactory, a switch to Spesolimab might have been considered, given the age of the patient. Triggering factors for GPP, such as infections (e.g., streptococcal infections), topical irritants, medication reactions, or withdrawal from systemic psoriatic therapy, have been reported in previous studies. However, no triggering factors were identified in our case.

In another case, a 35-year-old male diagnosed with psoriasis and psoriatic arthritis was initiated on adalimumab (80 mg). However, significant aggravation of psoriatic lesions was observed after 5 days. Pathological examination of the rash displayed neutrophil infiltration between epidermal cells and the formation of spongiform pustules (Kogoj). Direct and indirect immunofluorescence test results were negative. The occurrence of GPP-like skin lesions is an established adverse reaction to adalimumab [21]. However, current literature on the role of adalimumab in GPP is limited. Therefore, caution is warranted when using adalimumab for the treatment of GPP.

4 | Conclusion

For pediatric GPP cases where first-line biologics cannot be used, adalimumab may be a viable treatment option. Nevertheless, long-term follow-up is required to monitor GPP recurrence and potential adverse reactions.

TABLE 1 | Summary of previous reports in patients with pediatric GPP treated with adalimumab.

Case	Age (Y)	Gender	IL36RN gene mutation	Diagnosis	Treatment prior to adalimumab	Dose of adalimumab	Adverse events	Outcome clinical	References
1	13	F	ND	GPP	MTX, ACI, CsA, ETA, IFL	40 mg in week 0 and 1, and 40 mg Q2W	No	CR	[2]
2	17	F	ND	GPP, PA	MTX, ETA, CsA, ACI	40 mg Q2W	No	CR	[3]
3	8	M	ND	GPP	ACI, CsA	ND	No	CR	[4]
4	16	F	ND	GPP	ACI, CsA	80 mg, 40 mg after 1 week and then Q2W	No	CR	[5]
5	13	M	ND	GPP	Glycyrrhizin	40 mg in week 0 and 1, 40 mg Q2W	No	CR	[6]
6	11	F	ND	GPP	Topical steroids	40 mg in week 0 and 1, 40 mg Q2W	No	CR	[6]
7	9	M	ND	GPP	Topical steroids	20 mg in week 0 and 1, 20 mg Q2W	Flu-like symptoms	CR	[6]
8	11	F	ND	GPP	Glycyrrhizin, topical steroids	40 mg in week 0 and 1, 40 mg Q2W	No	CR	[6]
9	8	F	ND	GPP	Topical steroids	40 mg in week 0 and 1, 40 mg Q2W	No	CR	[6]
10	7	M	ND	GPP	ACI, topical steroids	40 mg in week 0 and 1, 40 mg Q2W	No	CR	[6]
11	2	F	ND	GPP	Topical steroids	20 mg in week 0 and 1, 20 mg Q2W	No	CR	[6]
12	3	M	ND	GPP	Topical steroids, antibiotics, CsA, MTX	20 mg Q2W	No	CR	[7]
13	3	M	c.227C>T	GPP	Systematic CS, ACI, TCM, topical steroids	20 mg in week 0 and 1, 20 mg Q2W	No	CR	[8]
14	5	M	c.227C>T; c.338C>T	GPP, DITRA	MTX, PRED	20 mg weekly	No	CR	[9]
15	3	M	c.115 + 6 T>C	GPP	Systematic CS, topical steroids	40 mg in week 0, and 20 mg Q2W	No	CR	This case

Abbreviations: ACI, acitretin; CR, complete response; CS, corticosteroids, CsA, cyclosporine A; ETA, etanercept; F, female; GPP, generalized pustular psoriasis; IFL, infliximab; M, male; MTX, methotrexate; ND, not described; PA, psoriatic arthritis; PRED, prednisolone; TCM, traditional Chinese medicine; Y, years.

Author Contributions

All authors contributed to the study conception and design. M.L. conceived, designed, and guided the study, and revised the manuscript. Y.L. provided the clinical data of patients and data analysis. The first draft of the manuscript was written by Y.L. and Y.X. Y.L. and Y.X. contributed equally. Q.Z. and L.Z. revised the manuscript. All authors read and approved the final manuscript.

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Ethics Statement

This study was approved by the Ethical Committee, Children's Hospital, Zhejiang University School of Medicine (2024-IRB-0355).

Consent

Written informed consent was obtained from the patient.

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:** apl70396-sup-0001-TableS1.tif.

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ORIGINAL ARTICLE

Effects of Traditional Chinese Medicine Rehabilitation Program on Knee Osteoarthritis in Aging Population: A Multicenter Randomized Controlled Trial

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Keywords: aging | knee | osteoarthritis | rehabilitation | traditional Chinese medicine

ABSTRACT

Introduction: Knee osteoarthritis (KOA) is a prevalent degenerative disease that causes pain and disability in older individuals. This study aimed to examine the effects of the traditional Chinese medicine (TCM) rehabilitation program for aging populations with KOA.

Methods: A total of 101 participants with KOA were randomly assigned to either a TCM rehabilitation group ($n = 49$) or a conventional physical therapy group ($n = 52$) with a 1:1 allocation ratio for this randomized controlled trial. Participants in the TCM group received acupuncture, massage, and South Shaolin exercise training for 4 weeks, with three sessions per week lasting 50 min per session. Participants in the control group received conventional physical therapies of equal duration and frequency.

Results: Outcomes were Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Visual Analogue Scale (VAS), Knee Outcome Survey Activities of Daily Living Scale, the 6-min Walking test (6MWT), the Time Up and Go (TUG) Test, and the Stair-climbing Test. Significant improvements were observed in the WOMAC, VAS, 6MWT, TUG, and Stair-climbing test after a 4-week TCM rehabilitation intervention ($p < 0.05$). The WOMAC and VAS were found to be decreased at the 4-week follow-up assessments compared to baseline scores ($p < 0.05$). Only the TUG test showed significant changes in the control group compared with the TCM rehabilitation group ($p = 0.043$) after 4 weeks post-intervention.

Conclusion: The TCM rehabilitation program improved knee function and reduced pain intensity in aging populations with knee osteoarthritis. Well-designed randomized controlled trials with long-term follow-up assessments are needed to draw more definitive conclusions. Trial registration Chinese Clinical Trial Register ChiCTR2000033351, date of registration: May 29, 2020.

Weihong Zhong and Jiajia Ye contributed equally to this work.

1 | Introduction

Knee osteoarthritis (KOA) is a common joint disorder that leads to significant pain and disability, especially in the aging population worldwide. The initial damage of KOA is primarily to articular cartilage, with progressive deterioration in the membrane, subchondral bone, ligaments, joint capsule, and periarticular muscles in the later stage [1]. It is estimated that approximately 17% of the global population with ages ranging from 50 to 75 years have symptoms of KOA [2, 3]. The prevalence of KOA is around 13% in women and 10% in men among individuals aged 60 years and older. It increases with age and obesity, imposing a substantial health and socioeconomic burden [4].

Despite the significant impact of KOA, it has been observed that most older adults with KOA have insufficient treatments [3]. The current mainstream treatment for KOA is taking nonsteroidal anti-inflammatory drugs (NSAIDs), but the affordability of NSAIDs remains a challenge for economically disadvantaged older adults [5]. Physical exercise has been shown to have beneficial effects for KOA, but its acceptance has not been widespread among the aging Chinese population due to cultural factors and the lack of culturally tailored exercise programs [6].

Traditional Chinese medicine (TCM) has been practiced for over 2000 years in China, with its practices primarily grounded in clinical experience rather than scientific evidence [7]. TCM therapies, including Chinese herbs, acupuncture, massage, Tai chi, Qigong, and moxibustion, are embraced by various age groups within the Chinese community, particularly among older adults due to their minimal side effects and perceived clinical effectiveness. A specialized training program combining acupuncture, massage, and South Shaolin exercise, developed by the Osteoarthritis Rehabilitation Research Center at the Fujian Rehabilitation Hospital in China, has been previously reported to demonstrate considerable clinical efficiency and safety [8]. However, the comparative data on the effectiveness of this TCM training program versus other conventional therapies remain limited. Thus, there is an urgent need to conduct an experimental study to examine the therapeutic effects of this training program among the aging Chinese population afflicted with KOA. Therefore, a multicenter randomized controlled trial study was proposed to assess the effects of a 4-week TCM training program among the aging Chinese population with KOA. This study aimed to provide valuable insights into the efficacy of the TCM training program. Specifically, the objective was to examine whether there would be significant improvements in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale, Visual analogue scale (VAS), Time up and go test (TUG), Knee Outcome Survey Activities of Daily Living Scale (KOS-ADLS) score, 6-min walking test (6MWT), and Stair test after a 4-week TCM rehabilitation program. The findings of this study may offer an evidence-based alternative for the management of KOA. It is, therefore, hypothesized that the TCM rehabilitation program would enhance knee function and alleviate pain in individuals with knee osteoarthritis.

2 | Methods and Materials

2.1 | Study Design

This multicenter, randomized, single-blind, two-arm parallel assignment, controlled trial was approved by the Ethics Institutional Review Boards of Fujian Rehabilitation Hospital and Gansu Provincial Hospital of Chinese Medicine (Trial Registration No. ChiCTR2000033351). The study was carried out according to the Declaration of Helsinki, and the procedures and results were reported in accordance with the CONSORT checklist.

2.2 | Participants

Participants were recruited through advertisements and referrals from their doctors at the Rehabilitation Hospitals (Fujian, China) and Gansu Provincial Hospital of Chinese Medicine (Gansu, China) from June 1, 2020 to April 2, 2022. Eligibility criteria included: (1) aged 50 to 80 years; (2) KOA diagnosis as per the *Diagnosis and Treatment of Osteoarthritis guidelines* [9]; (3) radiological diagnostic grade between 2 and 3 based on the Kellgren–Lawrence (K-L) criteria [5]; and (4) voluntary participation with signed informed consent. Participants were excluded if they: (1) have comorbidities such as psoriasis, syphilitic neuropathy, Charcot's arthropathy, brown-yellow disease, metabolic osteopathy, and acute trauma [8]; (2) have secondary or traumatic KOA; (3) have received intra-articular steroid injection or joint replacement in the past 3 months; (4) have total knee replacement in the past year; (5) were unable to walk independently; and (6) were not able to communicate.

2.3 | Sample Size Calculation

The sample size calculation for this RCT was based on the author's previous study [5]. The previous study found that the mean and standard deviation of WOMAC among the mind–body intervention group was 20.08 ± 9.95, and 30.16 ± 10.95 for the control group. The effect size measured by Cohen's d was calculated to be 0.45. A total of 124 participants were required to achieve sufficient statistical power ($1\text{-}\beta = 0.8$). To account for a potential drop-out rate (estimated to be 10%), a total of 138 participants were needed for the 2 groups.

2.4 | Procedure

A qualified research assistant who received diagnostic training from a medical doctor explained the research and conducted an initial screening for eligibility. All eligible participants were randomly assigned to either the TCM rehabilitation group or the physiotherapy control group in a 1:1 ratio using computer-generated numbers. Participants were informed about their group allocation (TCM intervention or physiotherapy) after baseline assessments through phone calls by a research assistant. Two blinded research assistants conducted all outcome assessments. All participants provided signed informed consent before participation. The protocol for this study has been published in the Journal of Annals of Palliative Medicine https://doi.org/10.21037/apm-21-1179.

2.5 | Intervention Program and Control Condition

2.5.1 | Traditional Chinese Medicine (TCM) Rehabilitation Program for the Treatment Group

The TCM rehabilitation program comprised acupuncture, massage, and South Shaolin exercise. Selected acupoints on the affected side of the leg included calf nose (ST35), inner knee eye (EX-LE4), Liangqiu (ST34), Xuehai (SP10), Weizhong (BL40), Zusanli (ST36), Yanglingquan (GB34), Yinlingquan (SP9), and Ashi points. The participants were in a supine position, and disposable needles (0.3×40 mm, Hautuo, Wuhan, China) were injected 10–25 mm into the skin by 2 professional TCM practitioners with the "Deqi" sensation confirmed by participants. A 15-min massage was administered after acupuncture, followed by a 5-min South Shaolin exercise performed 4 times a day, with a 5-min interval between each session, over the course of 4 weeks [8].

2.5.2 | Conventional Physiotherapy Program for the Control Group

Participants in the control group received a comprehensive physiotherapy program based on the guidelines recommended by the International Osteoarthritis Research Society. This program comprised multiple therapeutic components: isokinetic training to improve muscle support around affected knee joints, aerobic exercises to boost cardiovascular health, dynamic balance exercises to reduce fall risk and enhance stability, and active range of motion exercises to promote joint flexibility. Low frequency electrotherapy at 10 Hz and 30 mA (No. XY-K-SISS-A, Henan, China) was applied to relieve pain and reduce muscle tension, while neuromuscular training focused on enhancing muscle control and coordination. Each session lasted 40 min, conducted three times per week over a four-week period, with two professional physiotherapists providing precise guidance and supervision to maximize therapeutic benefits [10].

2.6 | Outcome Measures

Clinical assessments were conducted at baseline, 4-week post-intervention, and 4-week follow-up intervals. All these assessments were consistently conducted in the same rooms by 2 research assistants.

2.6.1 | Visual Analogue Scale (VAS)

The VAS was widely used for assessing pain severity. Participants were asked to indicate their subjective pain feeling on a 10-cm line, with 0 representing "no pain" and 10 suggesting "unbearable pain."

2.6.2 | Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Scale

The WOMAC was used to assess participants' physical function (with 17 questions), pain (with 5 questions), and stiffness (with 2 questions). The score ranges from 0 to 96. A higher total score indicates more severe knee dysfunction.

2.6.3 | Knee Outcome Survey Activities of Daily Living Scale (KOS-ADLS) Score

The KOS-ADLS was a patient-report scale used to assess the symptoms and functional limitations in daily activities for individuals with knee disorders [11]. It comprises 17 items, with lower scores indicating a greater degree of disability [12].

2.6.4 | Time Up and Go Test (TUG)

The TUG was a test that basically assesses mobility skills, including balance and agility. The time taken for a participant to rise from a chair, walk 3 m, turn around, walk back, and sit down is recorded in seconds. A longer test completion time indicates greater mobility impairments [13].

2.6.5 | Six- Minute Walking Test (6MWT)

The 6MWT was used to assess endurance and the ability to walk longer distances [13]. Participants were asked to walk as far as possible in 6 min. The distance covered was recorded in meters.

TABLE 1 | Baseline value of all variables in two groups.

Characteristics	TCM ($n=49$)	Con ($n=52$)	P
Age, year	66.24 ± 10.79	63.92 ± 6.96	0.199
Gender: females, %	42, 93%	45, 87%	0.905
Height, cm	160.12 ± 6.15	161.87 ± 6.10	0.156
Weight, kg	61.86 ± 8.59	64.69 ± 7.50	0.080
Body mass index, kg/m2	24.13 ± 3.28	24.70 ± 2.61	0.337
Smoking (yes), %	3, 6%	4, 7%	0.759
Drinking (yes), %	3, 6%	4, 7%	0.759
Exercise (no), %	4, 8%	1, 2%	0.151
WOMAC	16.92 ± 7.76	20.15 ± 10.82	0.089
VAS	4.20 ± 1.66	4.54 ± 1.84	0.341
KOS-ADLS, %	0.74 ± 0.14	0.74 ± 0.13	0.766
6MWT(m)	3.86 ± 0.86	3.95 ± 0.94	0.608
TUG(s)	10.46 ± 2.43	10.00 ± 1.81	0.303
Stair(s)	12.78 ± 3.13	14.12 ± 5.86	0.159

Abbreviations: 6MWT, the 6-min walking test; KOS-ADLS, Knee Outcome Survey Activities of Daily Living Scale; Stair, Stair-climbing test; TCM, Traditional Chinese Medicine group; Con, Control group. TUG, Time up and go test; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

2.6.6 | Stair Test

The stair test was a way to assess fitness function. Participants were instructed to ascend and descend a flight of 9 stairs in their usual manner. The time taken to complete this task was measured and recorded in seconds [8].

FIGURE 1 | A posture demonstration of South Shaolin exercise.

2.7 | Safety Measures

Participants were required to complete a form after each intervention session to document any adverse events, health status, and their participation throughout this study. The adherence and the incidence of adverse events were analyzed by the research team to determine the relationship with the intervention.

2.8 | Data Analysis

The demographic data of baseline characteristics are presented using descriptive statistics. The Shapiro–Wilk test was used to assess the normality of the data. Individual outcome measures were assessed using Mixed Factor Analysis of Variance (Mixed factor ANOVA; group × time) to identify both interaction and main effects. If a significant interaction effect emerged, a separate subgroup analysis with post hoc pairwise comparison between timepoints with the Bonferroni adjustment was conducted. Also, independent t-tests and paired sample t-tests were used to analyze data on the 6-min walking test (6MWT), Time Up and Go test (TUG), and Stair-Climbing test (Stair) with their effect sizes (Cohen's d). An intention-to-treat (ITT) analysis with the last observation carried forward (LOCF) method was employed for missing data. The ITT analysis was performed only on participants who completed at least 70% of the total intervention sessions. All statistical analyses were performed using SPSS 25 (IBM, New York, U.S.) using an alpha level of 0.05 to indicate a significant level.

3 | Results

The baseline characteristics of participants in both groups were comparable, as detailed in Table 1. The mean age was

TABLE 2 | Mean and SD performance value of WOMAC, VAS, and KOS-ADLS.

	Mean ± SD		Condition effect	Time effect	Condition × time effect		
Variable Group	**Week 4**	**Week 8**	***p***	***p***	***F***	***p***	η^2
WOMAC			0.224	0.001*	1.588	0.209	0.016
TCM	11.18 ± 7.19	7.10 ± 5.12					
Con	12.02 ± 9.39	8.33 ± 8.53					
VAS			0.318	0.001*	0.197	0.783	0.002
TCM	2.78 ± 1.25	2.07 ± 1.18					
Con	2.92 ± 1.41	2.29 ± 1.17					
KOS-ADLS			0.676	0.346	0.065	0.848	0.001
TCM	77% ± 23.4	76% ± 25.2					
Con	75% ± 23.4	72% ± 23.6					

Note: Knee Outcome Survey Activities of Daily Living Scale.
Abbreviations: Con: Control group, TCM: Traditional Chinese Medicine group.
*Denotes a significant effect ($p < 0.05$).

TABLE 3 | Within-group differences for WOMAC, VAS, and KOS-ADLS in two groups.

Variable		Pre vs. Week 4		Week 4 vs. Week 8		Pre vs. Week 8	
		TCM	Con	TCM	Con	TCM	Con
WOMAC	*p*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
	95% CI	4.13 to 7.33	6.00 to 10.27	2.15 to 6.02	1.90 to 5.49	7.46 to 12.17	9.48 to 14,19
VAS	*p*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
	95% CI	0.93 to 1.77	1.06 to 1.94	0.33 to 1.00	0.37 to 0.88	1.55 to 2.45	1.70 to 2.55
KOS-ADLS	*p*	0.456	0.560	0.105	0.026*	0.907	0.757
	95% CI	−0.09 to 0.04	−0.09 to 0.05	−0.01 to 0.05	0.01 to 0.06	−0.07 to 0.07	−0.06 to 0.08

Abbreviations: Con, Control group; KOS-ADLS, Knee Outcome Survey Activities of Daily Living Scale; TCM, Traditional Chinese Medicine group; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
*Denotes a significant effect ($p < 0.05$).

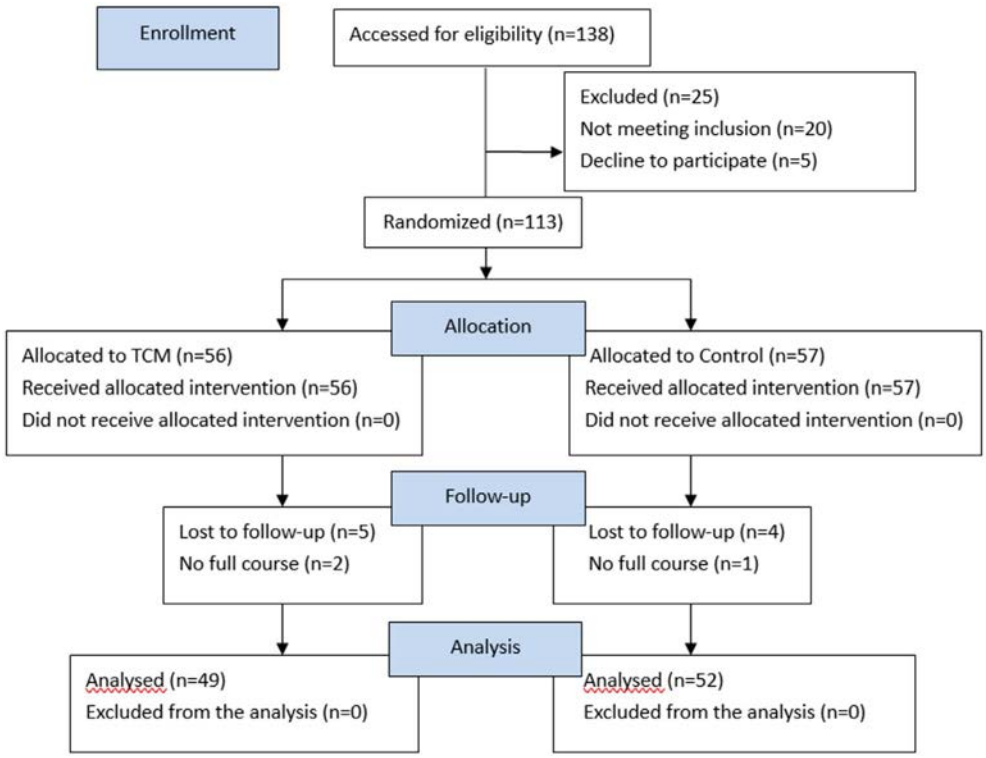

FIGURE 2 | Flowchart of this study.

66.24 years in the TCM rehabilitation group and 63.92 years in the control group, with females constituting approximately 90% of the participants. Initially, 113 participants were involved in this study. Nine of them did not attend the follow-up assessment, and three of them did not receive the full course of treatment. Consequently, the data of 101 participants were ultimately analyzed, as illustrated in Figure 1. No adverse effects were reported throughout the study.

The WOMAC, VAS, and KOS-ADLS assessments were conducted at three timepoints: baseline, 4 weeks post-intervention, and at a 4-week follow-up (Tables 2 and 3) (Figure 2). Due to pandemic-related restrictions, the 4-week follow-up assessments were conducted via phone. Participants completed the 6MWT, TUG, and Stair tests at baseline and 4 weeks post-intervention. However, these assessments were not conducted at the 4-week follow-up due to the same restrictions. Significant time effects were observed for both WOMAC and VAS scores ($p = 0.001$) (Table 3). Additionally, significant within-group differences were observed in the 6MWT, TUG, and Stair tests in the two groups between baseline and 4 weeks post-intervention (*ps* < 0.05) (Tables 4 and 5). However, there were no significant differences between groups across all outcome measures except the TUG test ($p = 0.043$). The details are shown in (Tables 4, 5 and 6).

4 | Discussion

The present study examined the effects of a TCM rehabilitation program on physical functioning and pain-related outcomes

TABLE 4 | The differences in 6MWT, TUG, and Stair between pre-and post-4 weeks intervention.

Variable	6MWT(m)				TUG(s)				Stair(s)			
	Baseline	Week 4	*p*	*d*	baseline	Week 4	*p*	*d*	baseline	Week 4	*p*	*d*
TCM	3.86 ± 0.86	4.15 ± 0.88	0.005*	−0.33	10.46 ± 2.43	4.76 ± 0.44	0.001*	3.27	12.78 ± 3.13	11.87 ± 3.07	0.005*	0.29
Con	3.95 ± 0.94	4.24 ± 0.93	0.005*	−0.31	10.00 ± 1.81	4.52 ± 0.65	0.001*	4.03	14.12 ± 5.86	12.60 ± 4.56	0.001*	0.29
P	0.608	0.647			0.303	0.043*			0.159	0.364		

Abbreviations: 6MWT, the 6-min walking test; Con, Control group. Stair, Stair-climbing test; CM, Traditional Chinese Medicine group; TUG, Time up and go test.
*Denotes a significant effect ($p < 0.05$).

TABLE 5 | Between-group differences for WOMAC, VAS, and KOA-ADLS.

Variable		Week 4	Week 8
WOMAC	*P*	0.618	0.387
	95% CI	−4.15 to 2.48	−4.02 to 1.57
VAS	*P*	0.412	0.258
	95% CI	−0.68 to 0.41	−0.71 to 0.27
KOA-ADLS	*P*	0.109	0.115
	95% CI	0.05 to −0.08	0.05 to −0.07

Abbreviations: KOS-ADLS, Knee Outcome Survey Activities of Daily Living Scale; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

among Chinese aging populations with KOA. Results indicated that a 4-week TCM rehabilitation program improved pain sensation, physical function, strength, mobility, endurance, and fitness function similar to those who went through a comprehensive physiotherapy program based on the guidelines recommended by the International Osteoarthritis Research Society. No significant between-group differences were observed across all outcome measures except the TUG test.

Significant improvements in the WOMAC and VAS scores are consistent with the existing literature, suggesting that both TCM rehabilitation practices and conventional therapy can effectively reduce pain and improve physical functioning in patients with KOA [6, 7, 14, 15]. Similarly, the improvements in the 6MWT, TUG, and Stair Tests in the TCM rehabilitation group were aligned with previous findings, indicating that the TCM rehabilitation program significantly enhanced mobility, endurance, and fitness function similar to those who go through a comprehensive physiotherapy program [16]. However, those who went through a comprehensive physiotherapy program seem to have significant mobility skills compared to those who went through the TCM rehabilitation program.

Several factors contributed to understanding the present findings. Firstly, acupuncture, a key component of the TCM rehabilitation program, may facilitate the release of endogenous opioids and serotonin in the central nervous system, playing a vital role in analgesic and anti-inflammatory effects [17, 18]. This led to improved joint function and reduced pain intensity, as reflected in WOMAC and VAS scores [5, 6].

Secondly, massage therapy, another important component of this TCM rehabilitation program, has been shown to have beneficial effects on blood circulation and lymphatic flow, contributing to reducing muscular tension and promoting relaxation [19, 20]. These physiological responses may increase circulation, facilitate the removal of inflammatory mediators, and increase the flow of nutrients to reduce pain intensity.

Thirdly, South Shaolin exercise, a form of Qigong that combines mind, body movement, and breathing techniques, has been associated with improvements in physical function and pain reduction [21]. This exercise emphasized the mind–body integration, which promoted muscle strength, endurance, and

TABLE 6 | Mean change and MCID for outcome variables.

Variables	Pre-Week 4 Mean change (SD)	Week 4-Week 8 Mean change (SD)	Pre-Week 8 Mean change (SD)	MCID
WOMAC	5.73(5.56)	4.08(6.74)	9.82(8.19)	5.5[15]
TCM	8.13(7.65)	3.69(6.45)	11.83(8.47)	
Con				
VAS	1.36(1.41)	0.64(1.12)	2.0(1.50)	2.0[16]
TCM	1.49(1.50)	0.64(0.89)	2.13(1.43)	
Con				
KOA-ADLS	−2.54(23.64)	2.13(9.01)	−0.41(24.21)	2.2[15]
TCM	−2.03(25.0)	3.08(9.67)	1.04(24.17)	
Con				
6MWT(m)	−25.69(58.52)	----—	----—	61[17]
TCM	−18.56(43.87)			
Con				
TUG(s)	0.65(1.57)	----—	----—	2.82[17]
TCM	0.75(1.44)			
Con				
Stair	1.67(3.54)	----—	----—	5.5[18]
TCM	1.32(2.39)			
Con				

Abbreviations: MCID, Minimal Clinically Important Difference; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; VAS, visual analogue scale; KOS-ADLS, Knee Outcome Survey Activities of Daily Living Scale; Stair, Stair-climbing test; TUG, Time up and go test; TCM, Traditional Chinese Medicine group; Con, Control group.

physical function [22]. These benefits may lead to better performance in functional tests such as the 6MWT, TUG, and Stair test. Moreover, the mediative component of this exercise also played a vital role in pain management.

The observed enhancements in pain relief, physical function, endurance, strength, balance, and overall fitness can be attributed to the multifaceted modalities of this Traditional Chinese Medicine (TCM) rehabilitation program. Specifically, acupuncture played a considerable role in diminishing pain and reducing inflammation. Massage therapy, on the other hand, enhanced blood circulation and facilitated muscle relaxation. Moreover, the incorporation of South Shaolin exercises strengthened muscles and improved balance, mobility, and fitness function. These benefits were reflected not only in pain and functional capacity but also in enhanced mobility, endurance, balance, and physical fitness.

Although these noticeable improvements were observed after the 4-week TCM rehabilitation program, there was no significant enhancement in daily activities, as measured by the KOA-ADLS. This observation aligned with prior studies [23], suggesting that daily activities involved a broad spectrum of motions that were not entirely covered by the general practices of acupuncture, massage, and South Shaolin exercise. Additionally, South Shaolin exercise was only practiced for 5 min. This may not be sufficient to significantly impact daily activities. Further studies might require more personalized or activity-specific interventions to explain this finding.

Interestingly, the control group showed a significantly greater improvement in the Timed Up and Go (TUG) test compared to the TCM rehabilitation group. Chun et al. [24] reported that muscle strength was the primary modifiable factor associated with enhanced physical performance. This finding was supported by Qiu et al. [25] who observed that TUG performance improved more in the exercise group than in the general treatment group for patients with knee osteoarthritis (KOA). In this study, participants in the control group received isokinetic muscle training targeting the affected knee joints, which may have significantly improved knee muscle strength compared to the TCM group, who only performed static and semi-squatting exercises (South Shaolin exercises). These TCM exercises may comparatively lack sufficient intensity to effectively strengthen the muscles surrounding the knee joints. Overall, conventional physical therapy perhaps has a more direct impact on muscle strength, balance, speed, and agility necessary for better TUG test outcomes compared to TCM rehabilitation interventions [26]. However, few studies have directly compared TCM rehabilitation programs with conventional physical therapy in KOA patients. Further research with specific outcome measures to confirm these findings is highly needed [14].

Despite the interesting findings of this study, several limitations should be noted. First, participants in the control group received conventional therapy for KOA, which may have minimized the observed differences between the TCM rehabilitation and control groups. Waitlist controls in future studies should be considered to better isolate the therapeutic effects of the TCM rehabilitation program. Second, the recruitment of participants exclusively from hospitals may limit the generalizability of the results. Expanding the study to include more medical centers and diverse community settings would enhance the applicability of the findings. Third, the majority of participants were

female in this study. This may have introduced heterogeneity into the results. Therefore, the findings of this study should be interpreted with caution. Fourth, lockdown measures significantly shortened the data collection period, reducing the total number of participants. Finally, due to constraints related to the COVID-19 pandemic, certain outcome measures could not be fully implemented as initially planned, as some participants were unable to complete specific assessments. Future studies are encouraged to include more comprehensive follow-up assessments to evaluate the long-term impacts of the TCM rehabilitation program.

5 | Conclusion

This RCT demonstrated that the TCM rehabilitation program, comprising acupuncture, massage, and South Shaolin exercise, was effective in relieving pain intensity and enhancing physical function, endurance, strength, mobility, and fitness function among Chinese aging populations with KOA. It indicated that a 4-week regimen involving 3 sessions per week with each session lasting 50 min can be designed for KOA patients to gain positive effects on the knee joint among the elderly with KOA.

Author Contributions

Weihong Zhong, **Xiaoqin Chen**, and **Shanli Yang** conceived the experiment and collected data. **Jiajia Ye** and **Daniel Kwasi Ahorsu** analyzed the data and drafted the manuscript. **Jiajia Ye**, **Daniel Kwasi Ahorsu**, **Xiaoqin Chen**, and **Shanli Yang** critically reviewed and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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EDITORIAL

Rheumatic Diseases and Sinusitis: Epidemiological Features, Immune Mechanisms, and Treatment Challenges

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1 | Background

Rheumatic diseases are a diverse group of chronic disorders primarily affecting the joints, bones, muscles, and connective tissues, often involving internal organs, blood vessels, and the nervous system. They encompass systemic autoimmune diseases (e.g., systemic lupus erythematosus), inflammatory arthritides (e.g., rheumatoid arthritis), metabolic arthropathies (e.g., gout), degenerative conditions (e.g., osteoarthritis), and infection-related arthritis. In recent years, with the deepening understanding of immune-related diseases, researchers have gradually noticed a possible comorbid relationship between sinusitis and various rheumatic diseases. Sinusitis is considered a disease characterized by mucosal barrier dysfunction, chronic inflammation, and immune dysregulation. These features are also reflected in rheumatic diseases. Moreover, research has shown that patients with rheumatic diseases have a significantly increased risk of developing sinusitis [1]. Based on this epidemiological characteristic, our study delves deeply into the association between RD and sinusitis regarding common immune pathways and molecular mechanisms cross-talk as well as drug treatment aspects, aiming to provide guiding insights for the future diagnosis and treatment of comorbidities between the two diseases, thereby improving the quality of life for patients.

2 | Challenges and Future Research Directions in the Field of Rheumatism and Sinusitis

Although the potential link between RD and sinusitis has garnered growing attention, relevant research still faces significant limitations. We reviewed research from PubMed, Web of Science, Embase, Cochrane, and Scope databases and summarized current findings (Figure S1; Table 1; Table S1). The results show new research advances in this field every year (Figure S2A). The published literature predominantly focuses on drug treatment ($n = 27$), clinical correlation ($n = 22$), molecular mechanisms ($n = 6$), diagnostic management ($n = 4$), physical therapy ($n = 2$), and spa therapy ($n = 1$) (Figure S2B). Research on drug treatment and clinical correlation is abundant, yet exploration of molecular mechanisms and drug development remains limited.

Further keyword clustering analysis of the literature in the Web of Science Core Collection (WOSCC) database (Figure S3) revealed that the research hotspots in this field focus on molecular mechanisms (blue cluster), drug treatment and pharmacological mechanisms (green, yellow, and red clusters), and clinical management (purple cluster). Research on molecular mechanisms has received considerable attention.

Ping-Ting Zhou and Zi-Hui Xie have contributed equally to this work and share the first authorship.

TABLE 1 | Characteristics of the included studies.

Rank	Title	PMID	First author	Journal	Create date
1	Pulsed magnetotherapy in Czechoslovakia—a review	8047671	Jerabek J	Rev Environ Health	April 1994
2	Bronchiolar disease in rheumatoid arthritis	8912776	Hayakawa H	Am J Respir Crit Care Med	November 1996
3	Increased frequency of cystic fibrosis deltaF508 mutation in bronchiectasis associated with rheumatoid arthritis	10445602	Puechal, X	The European respiratory journal	Jun 1999
4	Antibiotics for sore throat	10796471	Del Mar, C B	The Cochrane database of systematic reviews	June 1905
5	Infliximab: an updated review of its use in Crohn's disease and rheumatoid arthritis	11985485	Keating GM	BioDrugs	May 2002
6	Use of complementary and alternative therapies by patients self-reporting arthritis or rheumatism: results from a nationwide canadian survey	12415605	Fautrel, Bruno	J Rheumatol	Nov 2002
7	How does Chlamydia cause arthritis?	12951871	Kuipers, Jens G	Rheumatic diseases clinics of North America	Aug 2003
8	[Experiences in differential agglutination tests in rheumatic diseases]	13179732	JACOB, W	Zeitschrift fur Immunitatsforschung und experimentelle Therapie	Mar 1954
9	Cause of rheumatic fever-chronic sinusitis	14895240	BROWN, E E	Archives of pediatrics	Dec 1951
10	Experimental production of characteristic pneumonitis with a granuloma resembling Masson body in rabbits following focal infection of paranasal sinuses with hemolytic streptococcus	14907194	OKABAYASHI, A	Japanese journal of medicine	Aug 1951
11	Antibiotics for sore throat	15106140	Del Mar, C B	The Cochrane database of systematic reviews	June 1905
12	Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys	16225695	Tirkey, Naveen	BMC pharmacology	Oct 2005
13	Clinical and laboratory characteristics of 75 patients with specific polysaccharide antibody deficiency syndrome	17042135	Cheng, Yew Kuang	Annals of Allergy Asthma & Immunology	SEP 2006
14	The association of rheumatoid arthritis and its treatment with sinus disease	17143978	Michaud K	J Rheumatol	December 2006
15	Modulation of transcription factors by curcumin	17569208	Shishodia S	Adv Exp Med Biol	June 2007
16	Chronic rhinosinusitis and psoriasis: do mutually exclusive systemic Th1 and Th2 disease patterns exist?	1773576	Rashid RM	Acta Otolaryngol	June 2007

(Continues)

TABLE 1 | (Continued)

Rank	Title	PMID	First author	Journal	Create date
17	Fibromyalgia and chronic rhinosinusitis: outcomes after endoscopic sinus surgery	18702911	Soler ZM	Am J Rhinol	August 2008
18	Conundrum and therapeutic potential of curcumin in drug delivery	20932240	Kumar A	Crit Rev. Ther Drug Carrier Syst	October 2010
19	Sodium oxybate reduces pain, fatigue, and sleep disturbance and improves functionality in fibromyalgia: results from a 14-week, randomized, double-blind, placebo-controlled study	21397402	Russell JI	Pain	March 2011
20	Antioxidant, antinociceptive, and anti-inflammatory activities of Xanthii Fructus extract	21466841	Huang MH	J Ethnopharmacol	April 2011
21	Advances in homeopathy and immunology: a review of clinical research	21622275	Bellavite, Paolo	Frontiers in bioscience (Scholar edition)	Jun 2011
22	Molecular mechanisms of curcumin action: gene expression	22996381	Shishodia S	Biofactors	September 2012
23	Is there a relationship between fibromyalgia and rhinitis?	23394501	Frieri M	Allergy Asthma Proc	February 2013
24	Urinary metabonomics study on toxicity biomarker discovery in rats treated with Xanthii Fructus	23831081	Lu F	J Ethnopharmacol	July 2013
25	Nasal and paranasal involvement in primary Sjogren's syndrome	23943735	Midilli R	Rhinology	August 2013
26	A PPARγ, NF-κB and AMPK-dependent mechanism may be involved in the beneficial effects of curcumin in the diabetic db/db mice liver	24945581	Jiménez-Flores LM	Molecules	June 2014
27	Prevalence of sinonasal disease in children with Juvenile idiopathic arthritis	25125135	Pipolo C	Laryngoscope	August 2014
28	Curcumin as a wound healing agent	25200875	Akbik D	Life Sci	September 2014
29	Recent infections are associated with decreased risk of rheumatoid arthritis: a population-based case–control study	25656683	Sandberg ME	Ann Rheum Dis	February 2015
30	Recent developments in curcumin and curcumin based polymeric materials for biomedical applications: A review	26391597	Mahmood K	Int J Biol Macromol	September 2015

(Continues)

TABLE 1 | (Continued)

Rank	Title	PMID	First author	Journal	Create date
31	Otolaryngologic Manifestations of Various Rheumatic Diseases: Awareness and Practice Among Otolaryngologists.	26693453	Gera, Chanchal	Indian journal of otolaryngology and head and neck surgery: official publication of the Association of Otolaryngologists of India	Dec 2015
32	Frankincense—therapeutic properties	27117114	Al-Yasiry AR	Postepy Hig Med Dosw (Online)	April 2016
33	Anti-tumor necrosis factor therapy is associated with certain subtypes of chronic rhinosinusitis	27121598	Leonard CG	J Laryngol Otol	April 2016
34	Phytochemistry and pharmacology of the genus Drypetes: A review	27353868	Wansi JD	J Ethnopharmacol	June 2016
35	Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections	28928442	Tian C	Nat Commun	September 2017
36	Radon-enriched hot spring water therapy for upper and lower respiratory tract inflammation	29116046	Passali, Desiderio	Otolaryngologia polska = The Polish otolaryngology	Aug 2017
37	Head and Neck Manifestations of Systemic Disease	30342611	Moroco AE	Med Clin North Am	October 2018
38	Traditional Uses, Botany, Phytochemistry, Pharmacology, Pharmacokinetics and Toxicology of *Xanthium strumarium* L.: A Review	30669496	Fan W	Molecules	January 2019
39	Should nasal biopsy inevitably be performed for classifying granulomatosis with polyangiitis in patients with rhinosinusitis? A retrospective chart review study	30887162	Yoo, Juyoung	Rheumatology International	MAY 2019
40	Safety and Effectiveness of Adalimumab in Patients With Polyarticular Course of Juvenile Idiopathic Arthritis: STRIVE Registry Seven-Year Interim Results	31421019	Brunner HI	Arthritis Care Res (Hoboken)	August 2019
41	Ethno-medicinal study of Artemisia ordosica Krasch. (traditional Chinese/Mongolian medicine) extracts for the treatment of allergic rhinitis and nasosinusitis	31585162	Xiao, Bin	Journal of Ethnopharmacology	FEB 2020
42	A Comprehensive Review on Physiological Effects of Curcumin	32746480	Ahsan, Rabiya	Drug research	Oct 2020
43	Screening of dental and sinus infections in rheumatoid arthritis	33089506	Descamps E	Eur J Clin Invest	October 2020
44	Chronic adenoiditis	33251901	Wang, Hai	Journal of International Medical Research	NOV 2020

(Continues)

TABLE 1 | (Continued)

Rank	Title	PMID	First author	Journal	Create date
45	Factor XIII-A in Diseases: Role Beyond Blood Coagulation	33535700	Dull K	Int J Mol Sci	February 2021
46	Sjogren's Syndrome and Pulmonary Disease	33788195	Peredo RA	Adv Exp Med Biol	March 2021
47	Ethnomedicinal use, phytochemistry, pharmacology, and toxicology of Daphne gnidium: A review	33865924	Khouchlaa, Aya	Journal of Ethnopharmacology	JUL 2021
48	Curcumin, the active substance of turmeric: its effects on health and ways to improve its bioavailability	34143894	Abd El-Hack ME	J Sci Food Agric	June 2021
49	Association of Sinusitis and Upper Respiratory Tract Diseases With Incident Rheumatoid Arthritis: A Case–control Study	34654732	Kronzer VL	J Rheumatol	October 2021
50	Timing of sinusitis and other respiratory tract diseases and risk of rheumatoid arthritis	35042150	Kronzer VL	Semin Arthritis Rheum	January 2022
51	The difference in pathogenic bacteria between chronic rhinosinusitis in patients with and without Sjogren's syndrome: a retrospective case–control study	35915401	Yang PR	BMC Infect Dis	August 2022
52	Paranasal sinus occupancy assessed from magnetic resonance images-associations with clinical indicators in patients with systemic lupus erythematosus	37086435	Valdés Hernández MDC	Rheumatology (Oxford)	April 2023
53	Higher Risk of Rheumatoid Arthritis in Patients With Chronic Rhinosinusitis: Prospective Association in the U.K. Biobank and Genetic Evidence by Mendelian Randomization Analysis	38225197	Dong Y	Am J Rhinol Allergy	January 2024
54	Association between sinusitis and incident rheumatic diseases: a population-based study	38388169	Kronzer VL	RMD Open	February 2024
55	Rheumatic adverse events associated with biologic therapy for chronic rhinosinusitis: A systematic review and meta-analysis	39302201	Xiao JB	Int Forum Allergy Rhinol	September 2024
56	Causal relationships between allergic and autoimmune diseases with chronic rhinosinusitis	39455747	Tu J	Sci Rep	October 2024
57	Management of Upper Respiratory Tract Infection	—	Tiewsoh, Karalanglin	Indian Journal of Pediatrics	June 1905

(Continues)

TABLE 1 | (Continued)

Rank	Title	PMID	First author	Journal	Create date
58	Turmeric and curcumin: Biological actions and medicinal applications	—	Chattopadhyay, I	Current Science	Jul 2004
59	THE CLINICAL IMPLICATION OF NASAL BIOPSY FOR CLASSIFYING GRANULOMATOSIS WITH POLYANGIITIS IN PATIENTS WITH RHINOSINUSITIS: A SINGLE CENTRE RETROSPECTIVE STUDY	—	Yoo, Juyoung	Annals of the Rheumatic Diseases	Jun 2019
60	Infections of the upper respiratory airways: The opinion of a general practitioner	—	Pappalepore, V.	Journal of Chemotherapy	Mar 2006
61	CHARACTERIZATION AND WOUND REPAIR POTENTIAL OF ESSENTIAL OIL EUCALYPTUS GLOBULUS LABILL	—	Tomen, Ibrahim	Fresenius Environmental Bulletin	July 1905
62	Antioxidant Activity of Methanol Extract of Tinospora crispa and Tabernaemontana corymbosa	—	Zulkefli, Heida Nadia	Sains Malaysiana	Jun 2013

Although some studies indicate that immune dysregulation and chronic inflammation may be common pathological features of RD and sinusitis [2], the specific relationship between their onset and development remains unclear. Identification of specific immune pathways and key molecular markers remains limited.

Drug therapy is the primary focus of this field. Current research examines the effects of traditional Chinese medicine, immunosuppressants, biological agents, and other drugs on sinusitis occurrence in RD patients. However, most research is in its early stages, and the mechanisms by which drugs contribute to comorbidities remain unclear. Additionally, balancing drug therapeutic effects with side-effect-induced comorbidities is a pressing clinical challenge in RD treatment. While current research shows various traditional Chinese medicines' significant efficacy in treating RD and sinusitis, insufficient evidence-based research currently supports this strategy, necessitating further in-depth, systematic studies for stronger scientific evidence. Additionally, developing more specific biological agents is a key research direction for treating RD and sinusitis.

Research on the diagnosis and management in the co-occurrence of RD and sinusitis remains limited. Under immunosuppressive therapy, changes in the immune system of RD patients often lead to atypical clinical manifestations of sinusitis, complicating early diagnosis and timely intervention. Therefore, developing professional diagnostic tools and standards to establish comprehensive management strategies is urgently needed. This study aims to investigate the epidemiological characteristics, immune mechanisms, and therapeutic associations in the comorbidity between rhinosinusitis and rheumatic diseases.

3 | Potential Association Between Rheumatism and Sinusitis

3.1 | Molecular Mechanism and Immunological Intersection

Functional enrichment analysis of shared susceptibility genes in sinusitis and RD revealed predominant enrichment in immune response, cell activation, and related biological processes (Table S2). The molecular mechanism primarily involves inflammatory pathways, such as NF-κB and TNF signaling, as well as immune regulatory pathways, including T cell receptor signaling and Th1/Th2 cell differentiation. Enrichment analysis also highlights that “cytokine response” and “cytokine-cytokine receptor interaction” are pivotal in the functional mechanism of cross-genes (Figure S4). In addition, we have analyzed and summarized the cross-over of immune mechanisms and molecular mechanisms in rheumatic diseases that are prone to concurrent sinusitis (Table S3).

RD are often accompanied by systemic immune disorders, manifested as dysfunction of T cells and B cells and cytokine imbalance [3]. This immune disorder leads to chronic inflammation in joints and other tissues, and may impair the immune defense of the upper respiratory tract, promoting

chronic sinus inflammation. For instance, abnormally activated dendritic cells in RD patients overproduce proinflammatory cytokines (e.g., IL-1β, IL-18, IL-1α) [3]. In patients with chronic rhinosinusitis (CRS), IL-1β expression in nasal mucosal tissues is significantly elevated. As key inflammatory mediators in the IL-1 family, IL-1β, IL-18, and IL-1α activate signaling pathways like NF-κB, triggering the release of other inflammatory factors and chemokines. This cascade response results in extensive mucosal infiltration by inflammatory cells, hyperplasia of mucous glands, and disruption of epithelial barriers—collectively forming the pathological basis of sinusitis [4]. Other studies show that abnormally activated immune cells, such as T cells, in psoriasis patients secrete excessive IL-17. IL-17 enhances chemokine expression in intestinal epithelial cells, promotes antimicrobial responses, and aids in epithelial barrier repair [5]. However, excessive IL-17 activity may lead to persistent immune activation, exacerbating inflammation, damaging the epithelium, and promoting sinusitis [6].

Further analysis revealed that key immune cells and inflammatory factors involved in sinusitis share significant similarities with those in RD. In sinusitis, activation of macrophages and $CD4^+$ T cells, along with overexpression of pro-inflammatory cytokines (e.g., IFN-γ, IL-6, IL-17), disrupts mucosal barrier permeability and immune homeostasis, promoting chronic pathological changes in the sinuses. These factors can penetrate the damaged mucosal barrier, inducing systemic inflammatory responses [7].

The evidence above indicates that there is a common immune pathway and molecular mechanism crossover between RD and sinusitis, which may lead to patients with RD being more susceptible to sinusitis.

3.2 | Drugs Affect the Occurrence of Comorbidities

Drug treatment for RD may significantly affect the pathogenesis of sinusitis. Traditional disease-modifying antirheumatic drugs (DMARDs) and biologics reduce inflammatory responses by targeting specific immune pathways. However, the local immune microenvironment regulation of nasal mucosa has not been considered in drug design, increasing susceptibility to upper respiratory tract infections. This may lead to inadequate regulation of comorbidity. Long-term use of glucocorticoids, a common immunotherapy for RD, may cause direct damage to nasal and sinus mucosa [8].

3.3 | Exploration of the Potential and Mechanism of Traditional Chinese Medicine (TCM) in Treating Rheumatism and Sinusitis

As noted, RD and sinusitis share similar pathological features. Traditional Chinese medicine (TCM), through its unique pharmacological mechanisms, can regulate the body's internal environment and inhibit excessive immune responses. Modern research confirms that traditional Chinese medicines like Cang-Er-Zi [9] demonstrate therapeutic potential for sinusitis. Tripterygium wilfordii Hook (TWH) has been shown to be effective in treating RA, and its multi-target mechanism has been confirmed by a large number of studies [10]. TCM may have therapeutic potential in both conditions through shared anti-inflammatory and immune regulation mechanisms. But further research is needed to clarify the effectiveness and broad applicability of TCM in treating comorbidities.

Although there is currently no research evidence to show that a single TCM can simultaneously treat RD and sinusitis, based on the shared pathological basis and cross-over of immune mechanisms between the two diseases, TCMs rely on their unique advantages and still have great potential to treat both RD and sinusitis at the same time.

4 | Discussion

We have explored the multifaceted relationship between RD and sinusitis. Given that RD patients often require immunosuppressive therapy, which may increase their risk of respiratory infections like sinusitis clinicians must closely monitor their conditions and ensure balanced drug selection. Our research shows that investigating common pathogenic and therapeutic pathways offers instructive insights for clinical practice and targeted therapies. Future research should actively explore immunomodulatory therapies targeting specific immune pathways and biomarkers. Furthermore, we recommend a multidisciplinary team (MDT) approach involving otolaryngologists, rheumatologists, and immunologists to develop comprehensive diagnostic and treatment plans. Lastly, traditional Chinese medicine has potential in regulating the internal environment. In the future, there should be a call for enhanced clinical research and collaboration to fill research gaps and better guide clinical practice.

However, this study integrated diverse research types, which introduced some heterogeneity in design due to differences in sample size, subject characteristics, and statistical methods. Potential publication bias may also have impacted the bibliometric analysis, as studies with positive findings are more likely to be published. Additionally, variations in database inclusion criteria may affect the comprehensiveness and accuracy of the analysis. Despite these challenges, rigorous literature screening and quality assessment methods were employed to minimize bias and enhance result reliability. These limitations should be considered in future research to improve homogeneity and reliability in exploring the association between rheumatism and sinusitis.

5 | Conclusion

Our study demonstrates shared epidemiological patterns and common immune mechanisms between RD and sinusitis. Future research should focus on elucidating underlying immune pathways, identifying biomarkers, and developing targeted therapies. Enhanced interdisciplinary collaboration will optimize comprehensive management strategies. Through more in-depth clinical and basic research, we will explore and improve treatment strategies, develop drugs with fewer side effects

and more targeted effects, and ultimately improve the quality of life of patients.

Author Contributions

Ping-Ting Zhou, Zi-Hui Xie: conceptualization, data curation, formal analysis, investigation, methodology, resources, software, supervision, validation, visualization, writing – original draft, writing – review and editing. **Jian-Peng Wang:** data curation, formal analysis, investigation, software, supervision. **Hai-Feng Pan, Yu-Chen Liu:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft and writing – review.

Acknowledgments

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Studies inclusion and analysis process. **Figure S2:** Overall analysis of the research field of rheumatism and sinusitis comorbidity. (A) Annual number of published papers in this field; (B) Subject classification of published research in this field. **Figure S3:** Analysis of key studies in the field of rheumatism and sinusitis comorbidity. (A) Keyword clustering analysis of studies published in this field; (B) Keyword timeline of studies published in this field. **Figure S4:** Analysis of molecular mechanisms of co-morbidity between rheumatism and sinusitis. (A) Cross-gene acquisition and network interaction diagram of co-morbidity genes; (B) KEGG enrichment analysis of cross-genes; (C) GO enrichment analysis of cross-genes. **Data S1:** Studies inclusion and analysis process. **Data S2:** Analysis of the molecular mechanism of comorbidity between rheumatic diseases and sinusitis. **Table S1:** Retrieval methods for bibliometric research in the field of rheumatism and sinusitis comorbidity. **Table S2:** Summary of disease-related genes. **Table S3:** Types of rheumatic diseases with concurrent sinusitis and their immune mechanisms.

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LETTER TO THE EDITOR

Clinician Counseling for Weight Loss and Physical Activity Among US Adults With Arthritis, 2019 and 2023: A Cross-Sectional Study

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Dear Editor,

Arthritis is one of the most common chronic conditions in the United States (US) [1]. According to the registers of the Centers for Disease Control and Prevention, 58.5 million (23.7%) US adults reported arthritis, and 25.7 million (43.9% among those with arthritis) reported arthritis-attributable activity limitations [2]. The American College of Rheumatology recommends weight loss for arthritis patients with overweight/obesity, which can improve function and mobility [3]. Physical activity is also recommended as a first-line, nonpharmacologic strategy to manage arthritis symptoms [3]. In 2014, 45.5% (target 45.3%) and 61.0% (target 57.4%) of the corresponding population received clinician counseling from their health care providers, respectively, meeting the Healthy People 2020 (HP2020) objectives [4, 5].

Understanding the current clinical counseling rates is crucial to assess management guidelines for arthritis patients in clinical practice and public health policy changes.

This cross-sectional, observational study updated the national estimates of clinician counseling for weight loss and physical activity among US adults with arthritis; it also monitored the progress toward a HP2030 objective (A-04: increase the proportion of adults with arthritis who get counseling for physical activity, target of 57.70%).

We used data on adults (aged ≥ 18 years) with self-reported arthritis and available questionnaires of clinician counseling for weight loss (only in 2019) or physical activity (in 2019 and 2023) from the latest National Health Interview Survey (NHIS) (Figure S1). NHIS is an ongoing, annual, in-person survey designed to be representative of the civilian, noninstitutionalized US population that gathers data on various health topics.

Arthritis was defined as a "yes" response to the question, "Have you ever been told by a doctor or other health care professional that you had some form of arthritis, rheumatoid arthritis, gout, lupus or fibromyalgia?" Those answering yes were then asked about weight loss counseling, and physical activity counseling. Clinician counseling for weight loss or physical activity was defined as a "yes" response to the question "Has a doctor or other health professional ever suggested losing weight or physical activity/exercise to help your arthritis or joint symptoms?" Information about age, sex, education level, race/ethnicity, ratio of family income to poverty, body mass index (BMI), and arthritis-attributable symptoms were collected through established questionnaires.

Age-standardized prevalence estimates with 95% confidence intervals (CIs) of clinician counseling for weight loss (among those overweight/obese) or physical activity were calculated

TABLE 1 | Age-standardized prevalence of clinician counseling for weight loss and physical activity among adults with arthritis, by sociodemographic characteristics: NHIS, 2019 and 2023.

Characteristic	Clinician weight loss counseling, 2019[a]		Clinician physical activity counseling, 2019		Clinician physical activity counseling, 2023	
	Unweighted no./ sample size	Weighted prevalence (95% CI)[b]	Unweighted no./ sample size	Weighted prevalence (95% CI)[b]	Unweighted no./ sample size	Weighted prevalence (95% CI)[b]
Overall	2333/5782	40.70 (38.10, 43.35)	4295/7994	52.15 (49.72, 54.57)	4091/7460	54.60 (51.99, 57.18)
Sex						
Female	1477/3378	45.13 (41.83, 48.47)[c]	2835/4890	58.73 (55.71, 61.68)[c]	2687/4597	58.83 (55.60, 61.98)[c]
Male	856/2404	35.35 (31.30, 39.62)	1460/3104	43.74 (40.21, 47.34)	1404/2863	48.40 (44.29, 52.52)
Age group, years						
18–44	253/593	40.80 (36.27, 45.50)[c]	415/790	49.69 (45.55, 53.82)[c]	338/628	54.88 (50.23, 59.46)
45–64	971/2165	43.14 (40.58, 45.73)	1620/2824	57.10 (54.64, 59.53)	1277/2329	54.12 (51.73, 56.48)
≥65	1109/3024	36.10 (34.02, 38.23)	2260/4380	51.11 (49.22, 53.01)	2476/4503	54.55 (52.75, 56.34)
Race/ethnicity						
Hispanic	193/446	37.57 (30.05, 45.74)	324/565	49.62 (42.33, 56.92)[c]	373/635	59.64 (51.67, 67.13)[c]
Black, non-Hispanic	336/713	46.41 (39.15, 53.82)	528/869	56.20 (49.19, 62.97)	509/806	62.01 (54.22, 69.22)
White, non-Hispanic	1713/4396	39.91 (36.78, 43.11)	3234/6187	51.72 (48.97, 54.46)	3017/5659	52.72 (49.67, 55.74)
Other	91/227	44.07 (31.79, 57.12)	209/373	53.54 (42.82, 63.95)	192/360	52.96 (43.15, 62.55)
Education level						
Less than high school	257/706	31.73 (25.78, 38.34)[c]	467/967	37.38 (32.04, 43.06)[c]	409/816	56.42 (49.21, 63.38)[c]
High school	624/1689	38.05 (32.97, 43.40)	1162/2307	50.48 (45.98, 54.96)	1032/2066	49.82 (44.84, 54.80)
Some college	805/1874	44.84 (40.35, 49.41)	1410/2493	57.61 (53.48, 61.62)	1273/2254	56.78 (51.80, 61.63)
4-year college degree or more	639/1486	43.59 (38.64, 48.67)	1234/2186	55.27 (51.03, 59.44)	1361/2292	57.16 (53.01, 61.21)
Income-to-poverty ratio						
Poor (<100)	377/813	44.94 (38.17, 51.91)	596/1109	51.62 (45.57, 57.63)[c]	503/933	54.40 (47.50, 61.14)
Low income (100 to <200)	522/1298	40.48 (34.98, 46.22)	896/1723	51.61 (46.86, 56.33)	878/1664	53.31 (47.18, 59.35)
Middle income (200 to <400)	674/1759	40.13 (35.75, 44.68)	1282/2447	49.72 (45.37, 54.07)	1296/2336	58.34 (53.69, 62.84)
High income (≥400)	760/1912	39.16 (34.77, 43.75)	1521/2715	55.25 (51.32, 59.12)	1414/2527	52.08 (48.17, 55.96)

(Continues)

TABLE 1 | (Continued)

Characteristic	Clinician weight loss counseling, 2019[a]		Clinician physical activity counseling, 2019		Clinician physical activity counseling, 2023	
	Unweighted no./ sample size	Weighted prevalence (95% CI)[b]	Unweighted no./ sample size	Weighted prevalence (95% CI)[b]	Unweighted no./ sample size	Weighted prevalence (95% CI)[b]
Body mass index						
Underweight & healthy weight (< 25)	/	/	894/2011	40.79 (36.36, 45.38)[c]	923/1847	53.46 (48.15, 58.68)[c]
Overweight (25 to < 30)	503/2576	20.74 (17.62, 24.25)[c]	1258/2571	47.21 (43.18, 51.28)	1310/2448	52.18 (47.70, 56.63)
Obese (≥ 30)	1830/3206	54.80 (51.13, 58.43)	2023/3198	61.24 (57.58, 64.79)	1782/3020	56.92 (53.05, 60.71)

Abbreviations: CI, confidence interval; NHIS, National Health Interview Survey.
[a]Includes only adults with arthritis with a body mass index of ≥ 25.0 (calculated as weight in kilograms divided by height in meters squared). Clinician weight loss counseling was only surveyed in NHIS 2019.
[b]Nationally representative estimates of outcomes are weighted and age-standardized (excepted age category) to the direct method using the (standard) projected 2000 US population.
[c]Between-group comparisons yielded $p < 0.05$.

overall and by selected sociodemographic and arthritis-related characteristics. Sampling weights were applied to account for the complex survey design, to generate nationally representative estimates, and to adjust for nonresponse. The χ^2 test was used to calculate differences between groups. Statistical significance was defined as 2-sided $p < 0.05$. All analyses were conducted using R (Version 4.2.2). This study followed the strengthening the reporting of observational studies in epidemiology (STROBE) checklist (Appendix S1).

The unweighted study subsamples included 5782 to 7994 adults with arthritis. The overall age-standardized prevalence of clinician weight loss counseling in 2019 was 40.70% (95% CI, 38.10%–43.35%), and the overall age-standardized prevalence of clinician physical activity counseling increased from 52.15% (95% CI, 49.72%–54.57%) in 2019 to 54.60% (95% CI, 51.99%–57.18%) in 2023, but had yet to reach the HP2030 target (Table 1).

Females (45.13%), middle-aged adults (43.14%), adults with some college (44.84%), and obese adults (54.80%) had a higher age-standardized prevalence of clinician weight loss counseling than their corresponding comparators in 2019. Females (58.83%), non-Hispanic Blacks (62.01%), and obese adults (56.92%) had a higher age-standardized prevalence of clinician physical activity counseling than their corresponding comparators in 2023 (Table 1).

In both 2019 and 2023, adults with arthritis-attributable symptoms had a higher age-standardized prevalence of receiving clinician counseling for weight loss and physical activity (Figure 1). However, adults with no/mild pain or without arthritis-attributable symptoms did not meet the HP2030 target.

From 2019 to 2023, the prevalence of clinician physical activity counseling among adults with arthritis increased by 2.45 percentage points. This improvement is encouraging; however, it has not reached the HP2030 target. This gap was more pronounced among males and those without arthritis-attributable symptoms. Although it was found that patients of female sex, younger age, obesity, and severe symptoms appear to benefit more from exercise than their counterparts, early physical activity management would prevent the development of more severe symptoms [6, 7]. Considering the underlying reasons of specific groups, such as the low consultation rates among males in primary care and patients' insufficient awareness of arthritis (fear of exercise due to symptoms), the proactive reminders of clinicians are critical [8].

The HP2030 did not set a specific target for adults with arthritis and overweight/obesity who get counseling for weight loss; however, this proportion decreased from 45.5% in 2014 to 40.7% in 2019 [5]. In the current context of rising rates of overweight and obesity, this finding highlights the importance of weight monitoring and management in this population, as high BMI contributed to the increased burden of arthritis [9, 10].

While arthritis encompasses diverse pathophysiology and subtypes (e.g., osteoarthritis, rheumatoid arthritis), obesity and physical inactivity contribute to disease progression through convergent mechanisms. In osteoarthritis, excess weight imposes

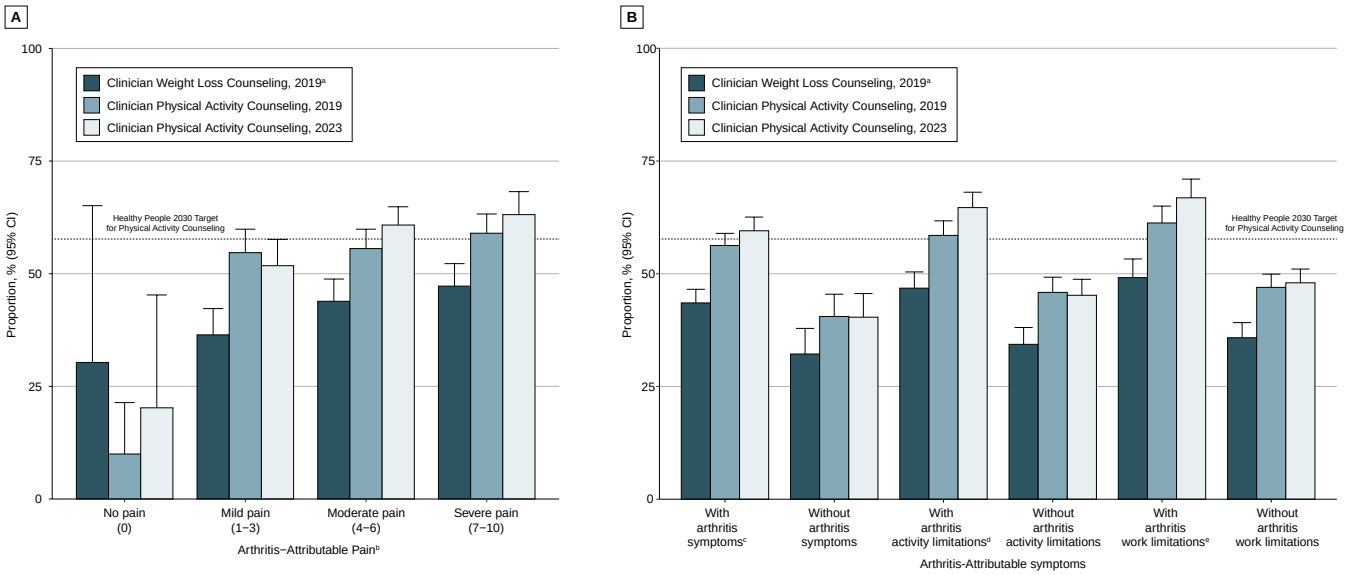

FIGURE 1 | Age-standardized prevalence of clinician counseling for weight loss and physical activity among adults with arthritis, by arthritis-attributable pain and symptoms: NHIS, 2019 and 2023. [a]Includes only adults with arthritis with a body mass index of ≥ 25.0 (calculated as weight in kilograms divided by height in meters squared). Clinician weight loss counseling was only surveyed in NHIS 2019. [b]Arthritis-attributable pain was answered to the question, "Please think about the past 30 days, keeping in mind all of your joint pain or aching and whether or not you have taken medication. During the past 30 days, how bad was your joint pain on average? Please answer on a scale of 0 to 10 where 0 is no pain or aching and 10 is pain or aching as bad as it can be.", among adults with self-report arthritis. The ratings were then translated into the World Health Organization (WHO) severity stages of mild (1–3), moderate (4–6), and severe (7–10). [c]Arthritis-attributable symptoms were defined as a "yes" response to the question, "The next questions refer to your joints. Please do not include the back or neck. During the past 30 days, have you had any symptoms of pain, aching, or stiffness in or around a joint?", among adults with self-report arthritis. [d]Arthritis-attributable activity limitations were defined as a "yes" response to the question, "Are you now limited in any way in any of your usual activities because of arthritis or joint symptoms?", among adults with self-report arthritis. [e]Arthritis-attributable work limitations were defined as a "yes" response to the question, "Do arthritis or joint symptoms now affect whether you work, the type of work you do, or the amount of work you do?", among adults with self-report arthritis. Error bars indicate 95% confidence intervals. NHIS, National Health Interview Survey.

biomechanical stress and promotes low-grade inflammation via adipokines [6]. In inflammatory arthritis, obesity worsens outcomes by amplifying systemic inflammation and reducing drug efficacy [5]. Conversely, physical activity benefits all subtypes by improving both disease-related and systemic manifestations [6, 11]. Thus, clinician counseling on weight loss/physical activity remains a universally endorsed non-pharmacological cornerstone for arthritis management, irrespective of subtype.

Our findings are subject to several limitations. First, the response rates in the sample adult interview decreased from 59.1% in 2019 to 47.0% in 2023; although the sampling weights at least partially adjust for this potential bias. Second, all the NHIS data are self-reported and might be susceptible to recall and social desirability biases. In addition, the counseling question does not address the quality or frequency as well as the acceptance rate of the counseling. Third, this study did not analyze the subtypes of arthritis due to the absence of relevant questionnaires in NHIS. Finally, because of the cross-sectional design, a causal relationship between the study outcomes and the characteristics examined cannot be inferred.

In conclusion, these findings underscore the need for targeted interventions to bridge counseling gaps. Clinicians should prioritize early, proactive counseling on physical activity and weight management for all adults with arthritis, especially in males and those without symptoms, to mitigate and prevent the disease progression. Health care provider education and training in standardized counseling protocols (e.g., "Health Care Provider Toolkit: Physical Activity Counseling for Adults with Arthritis" from the New York State), electronic medical record prompts, and connections to community programs are potential strategies to facilitate clinician counseling that can help adults manage their arthritis and achieve the HP2030 targets.

Author Contributions

Dr. Haowei Chen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design, acquisition, analysis, or interpretation of data, and critical review of the manuscript for important intellectual content: All authors. Drafting of the manuscript, statistical analysis, and administrative, technical, or material support: Haowei Chen and Zhaohua Zhu. Obtained funding: Changhai Ding. Supervision: David J. Hunter and Changhai Ding.

Conflicts of Interest

Prof. David J. Hunter is the editor of the osteoarthritis section for UpToDate, and co-Editor-in-Chief of Osteoarthritis and Cartilage; and provides consulting advice on scientific advisory boards for Pfizer, Lilly, TLCBio, Novartis, Tissuegene, and Biobone. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in the National Health Interview Survey at https://www.cdc.gov/nchs/nhis/documentation/index.html.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Flowchart of study participants. **Appendix S1:** apl70401-sup-0002-AppendixS1.doc.

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ORIGINAL ARTICLE

2024 Update of Chinese Guidelines for Management of Hyperuricemia and Gout Part II: Recommendations for Patients With Common Comorbidities

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Keywords: comorbidity | gout | guideline | hyperuricemia | management

ABSTRACT

Objective: The aim of this updated guideline is to provide comprehensive recommendations for the management of gout in patients with common comorbidities, such as chronic kidney disease (CKD), cardiovascular disease (CVD), diabetes, osteoarthritis (OA), and gastrointestinal disorders.

Methods: This guideline was developed by a multidisciplinary expert panel consisting of specialists in endocrinology, rheumatology, nephrology, cardiology, gastroenterology, and methodology. The development process adhered to standard methodologies, including PICO (population, intervention, comparator, and outcomes) question deconstruction, systematic literature review, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) for evidence and recommendation evaluation, Delphi voting, and expert consensus.

Changgui Li and Mingshu Sun contributed equally to this work.

Results: The guideline presents 26 evidence-based recommendations addressing seven clinical questions for patients with hyperuricemia and gout in the context of comorbidities. Key recommendations include the maintenance of strict serum urate targets, particularly for patients with CKD stage ≥ 3, chronic gouty arthritis, and OA, in order to prevent disease progression. In patients with CVD or diabetes, intra-articular triamcinolone is preferred over systemic glucocorticoids. Prioritized anti-inflammatory treatments for patients with CKD, gastrointestinal diseases, and OA are recommended. The guideline also introduces emerging therapies, such as interleukin-1 inhibitors and selective urate transport inhibitors, as potential treatment options for refractory cases.
Conclusion: The update offers a comprehensive, patient-centered approach to managing gout, particularly in individuals with associated comorbidities. Multidisciplinary collaboration and emerging new treatments and evidence ensure the optimization of the recommendations.

1 | Introduction

Gout is the most prevalent inflammatory arthritis in adults, especially in men. Biomedical research and clinical studies have focused on gouty arthritis for decades, as have the clinical concerns and guidelines for gout management [1, 2]. Recent evidence underscores that gout is a heterogeneous disease, with about 80% of the gout population having one or more comorbid conditions like metabolic syndrome (MetS), chronic kidney disease (CKD), cardiovascular disease (CVD), hypertension, gastrointestinal disease, and osteoarthritis (OA) [3, 4]. The relationship between gout and those comorbidities remains uncertain, as shared pathogenetic mechanisms, causal effects, and reciprocal interactions have been identified. This evolvement of gout complicates management strategies and requires tailored approaches.

The clinical management of gout in patients with comorbidities presents unique challenges, as many of the comorbidities are chronic and require long-term treatment strategies. How to control gout flares in patients with moderate-to-severe CKD, peptic ulcer, and hemorrhage or acute myocardial infarction? Will urate-lowering therapy (ULT) in those patients differ from isolated gouty arthritis? And how to manage gout overlapping OA? These clinical scenarios advocate management strategies and specific medications that may offer mutual benefits to gout and those associated conditions.

The 2019 Chinese Guidelines for the Diagnosis and Treatment of Hyperuricemia and Gout (shorted as 2019 Guidelines) [2], endorsing available evidence by that time, accomplished several weak recommendations addressing ULT in patients with CKD ≥ stage 2, and prioritized medications for hypertension, dyslipidemia, and diabetes mellitus. During the past 4 years, emerging novel evidence prompts the possibility for an update of the 2019 Guidelines. This guideline is launched by the Chinese Society of Endocrinology, supported by a multidisciplinary expert group, focusing on both gout and those complicated conditions, aimed to provide optimized options in sophisticated scenarios for clinical practitioners.

2 | Methods

This guideline was developed by the Chinese Society of Endocrinology, following standard procedures and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approaches. A steering committee of five senior specialists oversaw the process, supported by a multidisciplinary Expert Panel (EP) consisting of 20 endocrinologists, 12 rheumatologists, 7 experts in nephrology, gastroenterology, and cardiology, and 2 methodologists. A Working Group (WG) of 11 junior endocrinologists assisted in guideline development. All participants signed conflict of interest disclosures. The guideline is registered in the International Practice Guidelines Registry Platform under number PREPARE-2024CN563.

In brief, two rounds of surveys were conducted to address key clinical issues, leading to the selection of seven key clinical questions for this guideline. The WG applied the population, intervention, comparison, and outcome (PICO) framework to systematically assess the evidence and prioritize outcomes (Tables S1 and S2). Systematic Literature Reviews (SLRs) were conducted to address each PICO question, focusing on relevant literature published between January 2019 and January 2024. The evidence panel also conducted a rapid review to incorporate the most recent data prior to the guideline publication in March 2025. Major databases such as the Cochrane Library, PubMed, Embase, CNKI, and Wanfang Data were searched for relevant studies, alongside additional searches on resources like Guidelines International Network, National Guideline Clearinghouse, National Institute for Health and Care Excellence, and WHO. The qualities of evidence were assessed and graded using the GRADE system. In total, 2598 records were retrieved, 999 abstracts reviewed, and 103 full-text articles analyzed. The evidence collected was used to prepare evidence bodies for the PICO questions, which were presented to the EP (Table 1). The recommendations were drafted and modified by the EP during the following two meetings and two rounds of Delphi voting. A 10-point visual analog scale (VAS) was used to rate the level of agreement. Consensus was achieved when over 80% of the EP members rated a recommendation with a VAS of ≥ 7. Figure S1 shows the overall protocol of guideline development. One patient representative was invited to participate in the development process, who fully affirmed the importance of the clinical questions and the shared decision-making model of the recommendation. The final version of recommendations will be distributed through publications, academic meetings, etc.

3 | Results

Seven clinical questions were finalized, and consensus was achieved on 26 recommendations (Table 2 and Figure 1).

TABLE 1 | Seven clinical questions and the literature distribution of each evidence body.

		Distribution of evidence (Chapter)			
	Questions	**Systematic review/ Meta-analysis**	**Original research**	**Guidelines/Consensus**	**Others**
1	Gout flare management in patients with chronic kidney disease stage ≥ 3	1 Systematic review 1 Meta-analysis	3 Randomized controlled trials 4 Cohort studies	5 Guidelines 1 Consensus	1 Case report 1 Retrospective study 1 Experimental study
2	Urate-lowering therapy in patients with hyperuricemia/gout combined chronic kidney disease stage ≥ 3	3 Systematic reviews 1 Meta-analysis	3 Randomized controlled trials 4 Cohort studies 4 Cross-sectional studies	0	1 Conference abstract
3	Gout flare management in patients with heart failure or acute myocardial infarction	3 Meta-analyses	7 Randomized controlled trials 6 Cohort studies 1 Case–control study	1 Guideline	1 Case series study
4	Heart failure management in patients with hyperuricemia/gout combined chronic heart failure	1 Systematic review 1 Meta-analysis	12 Randomized controlled trials 1 Cohort study 1 Case–control study 1 Cross-sectional study	0	1 Conference abstract
5	Gout flare management in patients with peptic ulcer or hemorrhage	2 Systematic reviews 2 Meta-analyses	4 Randomized controlled trials 1 Observational study	1 Guideline 1 Consensus	0
6	Glucocorticoid therapy for gout flares in patients with type 2 diabetes	0	1 Cohort study	5 Guidelines 2 Consensuses	1 Experimental study
7	Management for patients with both gout and osteoarthritis	1 Systematic review 3 Meta-analyses	2 Randomized controlled trials 2 Cohort studies 1 Cross-sectional study	4 Guidelines 3 Consensuses	1 Experimental study

TABLE 2 | Recommendations with grades (GoR), levels of evidence (LoE) and levels of agreement (LoA).

Recommendation	PICO question	GoR	LoE	LoA (%)
Gout flare management in patients with CKD stage ≥ 3				
1. In patients with CKD stage ≥ 3 who are experiencing gout flare, short-term systemic or intra-articular glucocorticoid therapy is recommended	1	2	A	98.4
2. Colchicine and nonsteroidal anti-inflammatory drugs (NSAIDs) should be used with caution	2	2	B	98.4
3. Interleukin-1 (IL-1) inhibitors can be considered as alternative options	2	2	C	98.4
4. In dialysis patients with gout flare, colchicine is contraindicated, NSAIDs or low-dose Anakinra may be considered	3	2	C	98.4
ULT in patients with CKD stage ≥ 3				
5. ULT is recommended in asymptomatic hyperuricemia/gout patients with CKD stage ≥ 3 when SU levels exceed 7 mg/dL (420 μmol/L), aiming to maintain SU levels below 5 mg/dL (300 μmol/L)	4,5	2	B	100
6. Febuxostat is recommended as the first-line treatment, and allopurinol as the second-line treatment. Both medications should be initiated at low doses and adjusted based on SU levels and kidney function	6	2	B	100
7. In patients with CKD stage 4–5, febuxostat should not exceed 40 mg/day	7	2	B	100
8. In patients with CKD stage 3–4, allopurinol should be initiated at 50 mg/day, with gradual increases of 50 mg/day every 4 weeks, up to a maximum of 200 mg/day. In patients with CKD stage 5, allopurinol is contraindicated	7	2	C	100
9. Pegloticase can be considered where both drugs are inadequate	6	2	C	100
Gout flare management in patients with heart failure or acute myocardial infarction				
10. In addition to standard therapy for heart failure/myocardial infarction, colchicine may be considered as a preferred treatment for gout flare, administered with regular dosages	8,9	2	C	98.4
11. NSAIDs should be avoided with low-dose aspirin retained	8,9	2	B	98.4
12. In patients who are intolerant or unresponsive to colchicine, short-term glucocorticoids may be considered, as well as IL-1 inhibitors, if available	8,9	2	C	100
Heart failure management in patients with hyperuricemia/gout				
13. In managing heart failure, alongside appropriate ULT, angiotensin receptor-neprilysin inhibitors (ARNI) and sodium-glucose co-transporter 2 (SGLT-2) inhibitors (e.g., dapagliflozin, empagliflozin) are prioritized	10,11	2	B	100
14. Losartan potassium is recommended for initiating hypertension treatment, while switching from other antihypertensives is not advised	10,11	2	C	100
15. Thiazide diuretics should be minimized, and loop diuretics use only with close monitoring and control of SU levels	10,11	2	C	98.4
Gout flare management in patients with peptic ulcer or hemorrhage				
16. In patients at risk of gastrointestinal hemorrhage, selective cyclooxygenase-2 inhibitors are recommended as the preferred treatment over non selective NSAIDs	12	2	B	100
17. In patients with a history of peptic ulcer or hemorrhage, or those with controlled hemorrhage, short-term glucocorticoids are recommended if colchicine is ineffective or intolerant, provided that proton pump inhibitors (PPIs) or mucosal protectants are used concurrently	13	2	B	100

(Continues)

TABLE 2 | (Continued)

Recommendation	PICO question	GoR	LoE	LoA (%)
18. In the case of active hemorrhage, where standard therapy is in the place, short-term parenteral glucocorticoids may be considered, along with Anakinra if deemed appropriate	14	2	C	100
Glucocorticoid therapy for gout flares in patients with type 2 diabetes				
19. In patients with type 2 diabetes experiencing a gout flare, where blood glucose levels are rigorously monitored and controlled, intra-articular triamcinolone acetonide is recommended as the first-line treatment over equivalent systemic glucocorticoid	15	2	C	95.2
Management for patients with comorbid osteoarthritis (OA)				
20. In patients with both gout and OA, appropriate exercise during periods of arthritis relief is recommended	16	2	A	100
21. Additional weight management should be emphasized in those who are overweight or obese	17	1	A	100
22. The initiation of ULT is recommended, irrespective of baseline SU levels, with a target SU range between 180 and 300 μmol/L	18	2	B	98.4
23. NSAIDs are preferred analgesic treatment, with a course of 7–10 days	19	1	A	100
24. Long-term use of NSAIDs, exceeding 4 weeks, is not suggested	19	2	B	100
25. Intra-articular glucocorticoid injections are recommended in patients with hip or knee involvements	20	2	C	100
26. Surgical interventions should be considered for refractory pain or disability in large joints when conservative measures fail	21	2	C	98.4

Abbreviation: PICO, population, intervention, comparison, outcome.

3.1 | Recommendations and Explanations

3.1.1 | Gout Flare Management in Patients With CKD Stage ≥ 3

In patients with CKD stage ≥ 3 experiencing gout flare, short-term systemic or intra-articular glucocorticoid therapy is recommended; colchicine and nonsteroidal anti-inflammatory drugs (NSAIDs) should be used with caution; and interleukin-1 (IL-1) inhibitors can be considered as alternative options. In dialysis patients with gout flare, colchicine is contraindicated, while NSAIDs or low-dose Anakinra may be considered.

Western guidelines commonly recommend systemic glucocorticoids as one of the first-line treatments for acute gout, especially in patients with systemic symptoms, or when colchicine or NSAIDs are ineffective or contraindicated, or in those with renal dysfunction, given their comparable pain relief effects during acute gout. Evidence suggests glucocorticoids have minimal impact on kidney function and may even promote renal urate excretion. Despite limited RCT evidence, the EP supports glucocorticoids as the first choice for patients with CKD stage ≥ 3, without requiring dosage adjustments.

Studies found that colchicine exposure was up to twice as high in patients with moderate or severe renal impairment compared to those with normal kidney function, with excessive use increasing bone marrow suppression and causing gastrointestinal side effects, which can worsen with higher doses and be fatal. The EP therefore agreed that colchicine use is contraindicated in dialysis patients and should be dosed cautiously in those with renal impairment, with a maximum dose of 0.5 mg/day for moderate impairment (eGFR 30–59 mL/min/1.73 m^2). All NSAIDs carry risks in CKD, particularly for renal ischemia, and their use should be avoided long-term in CKD stage ≥ 3. In dialysis patients, NSAIDs may be considered with close monitoring for adverse effects.

IL-1 inhibitors, such as Anakinra (IL-1 receptor antagonist), Canakinumab (anti-IL-1β monoclonal antibody), and Rilonacept (soluble IL-1 decoy receptor), are recommended when other therapies are insufficient [1]. Anakinra has shown non-inferior to conventional treatments, with safety profiles comparable to those of glucocorticoids. A multicenter retrospective study has reported that Anakinra may be a safe option for gout patients with CKD stage 4–5 [5]. Further evidence is needed to confirm the efficacy and safety of the other two drugs in patients with CKD stage ≥ 3 [6]. Therefore, IL-1 inhibitors require careful consideration in patients with severe renal impairment (eGFR < 30 mL/min/1.73 m^2) or those on dialysis, with reduced dosing (e.g., Anakinra 100 mg every other day) recommended. Long-term use requires monitoring for neutropenia, and IL-1 antagonists are contraindicated in patients with active infections.

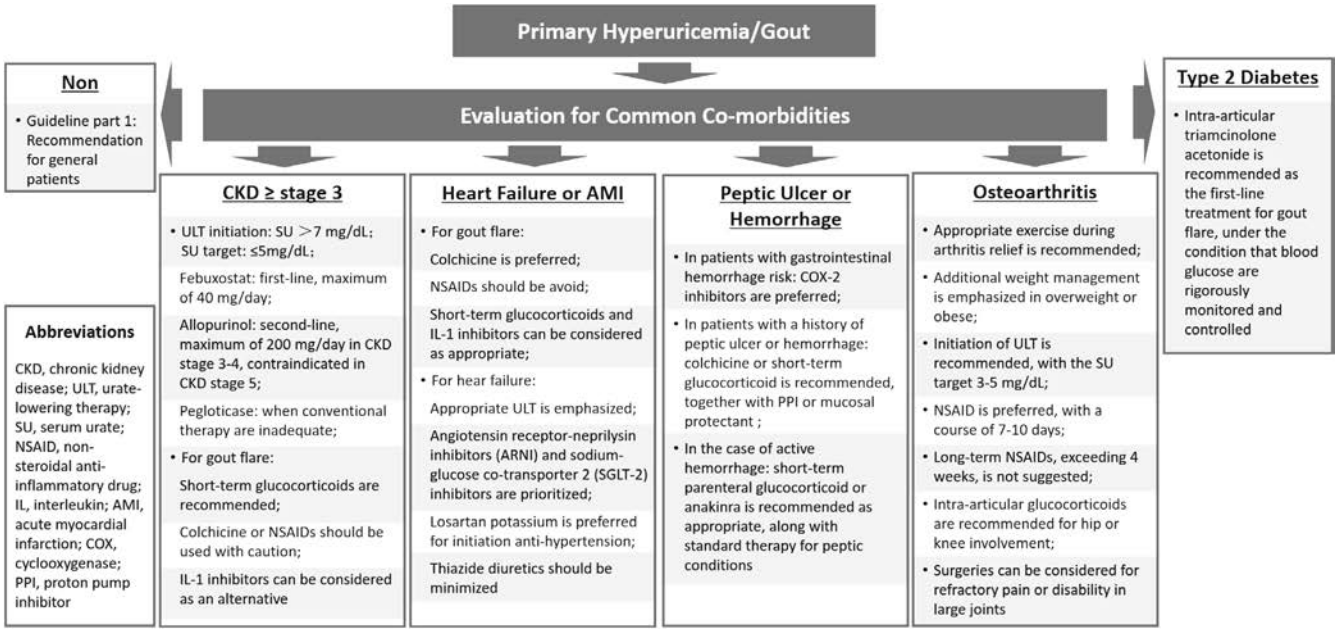

FIGURE 1 | Flow chart of recommendations.

3.1.2 | ULT in Patients With CKD Stage ≥3

ULT is recommended in patients with CKD stage ≥3 when SU levels exceed 7 mg/dL (420 μmol/L), aiming to maintain SU levels below 5 mg/dL (300 μmol/L). Febuxostat is recommended as the first-line treatment, and allopurinol as the second-line treatment. Both medications should be initiated at low doses and adjusted based on SU levels and kidney function. Febuxostat should not exceed 40 mg/day in patients with CKD stage 4–5. Allopurinol should be initiated at 50 mg/day, with gradual increases of 50 mg/day every 4 weeks, up to a maximum of 200 mg/day in patients with CKD stage 3–4, and is contraindicated in patients with CKD stage 5. Pegloticase can be considered where both drugs are inadequate.

Hyperuricemia is an independent risk factor for the development and progression of CKD. Systemic reviews and RCTs show that ULT, particularly with febuxostat, can slow CKD progression in patients with asymptomatic hyperuricemia and CKD stage 3–4 [7]. Studies have shown that the prevalence of CKD stage ≥3 increases with the higher SU levels, and maintaining SU levels below 300 μmol/L has an 11% lower risk of CKD deterioration [8]. Based on current evidence, the EP recommended maintaining sUA levels below 300 μmol/L in patients with hyperuricemia and CKD stage 3, to prevent further kidney deterioration.

Febuxostat, eliminated via renal and biliary pathways, requires no dose adjustment for mild to moderate renal impairment, making it the preferred xanthine oxidase inhibitor in CKD patients. Allopurinol is associated with an increased risk of severe skin reactions in renal failure. Topiroxostat, primarily metabolized by the liver, offers potential benefits for patients with renal insufficiency. Both febuxostat and topiroxostat effectively prevent eGFR decline in CKD patients with hyperuricemia, outperforming other urate-lowering drugs [9, 10]. For eGFR <30 mL/min/1.73 m^2, febuxostat's safety and efficacy at 20–40 mg/day are well-established [11]. Uricosurics like benzbromarone should be avoided in patients with CKD stage >3 or urolithiasis. Pegloticase has proved its efficacy and safety in patients with CKD stage 3–5 who were refractory to conventional ULT [12, 13]. A study in gout patients receiving hemodialysis showed no significant effect of hemodialysis on pegloticase administration, indicating its feasibility in these patients [14]. Although it does not have market access in China, this updated guideline provides an option for gout patients with severe renal diseases based on the clinical evidence presented here.

3.1.3 | Gout Flare Management in Patients With Heart Failure or Acute Myocardial Infarction

In addition to standard therapy for heart failure/myocardial infarction, colchicine may be considered as a preferred treatment for gout flare, administered with regular dosages; NSAIDs should be avoided with low-dose aspirin retained. In patients who are intolerant or unresponsive to colchicine, short-term glucocorticoids may be considered, as well as IL-1 inhibitors, if available.

Several studies indicated that NSAIDs increase the risk of adverse cardiovascular events, such as acute myocardial infarction and heart failure. Guidelines recommended avoiding NSAIDs in heart failure due to their potential to cause water and sodium retention, increase systemic vascular resistance, and reduce diuretic efficacy, all of which may worsen heart failure [15]. Based on this evidence, the EP agreed that NSAIDs should be avoided for gout flare in patients with heart failure or acute myocardial infarction.

Colchicine has demonstrated potential cardiovascular protective effects, reducing inflammation and stabilizing plaques, which may prevent myocardial infarction [16, 17]. A cohort study showed that colchicine reduced cardiovascular events in

gout patients, making it safe and effective for pain relief during gout flares in patients with heart failure or myocardial infarction, while potentially reducing cardiovascular risks.

The safety of glucocorticoids for gout flares in these patients is less clear. Short-term (<4 weeks) glucocorticoid therapy has shown benefit in patients with heart failure, but long-term use may increase cardiovascular risk, mainly due to fluid retention.

Anakinra using in short term has shown efficacy in gout patients with heart failure who were intolerant or resistant to colchicine, without raising infection risk [18], though its high cost and long-term infection risk limit its use. Canakinumab [19, 20] and rilonacept [21] may reduce cardiovascular events but have not been studied for gout flares in heart failure or myocardial infarction patients.

3.1.4 | Heart Failure Management in Patients With Hyperuricemia/Gout

In managing heart failure, alongside appropriate ULT, angiotensin receptor-neprilysin inhibitors (ARNI) and sodium-glucose co-transporter 2 (SGLT-2) inhibitors (e.g., dapagliflozin, empagliflozin) are prioritized; losartan potassium is recommended for initiating hypertension treatment, while switching from other antihypertensives is not advised; thiazide diuretics should be minimized, and loop diuretics used only with close monitoring and control of SU levels.

In patients with asymptomatic hyperuricemia or gout combined with heart failure, strict control of SU levels has been recommended. Febuxostat has been suggested to be used with caution in patients with CVD. In recent years, ARNI [22] and SGLT2 inhibitors [23–25] have become key therapies for heart failure. Small studies suggest that ARNI can lower SU levels in heart failure patients, regardless of baseline levels [26]. A randomized controlled study [27] showed that ARNI reduced cardiovascular mortality and heart failure-related hospitalizations, alongside lowering SU levels, compared to valsartan in patients with heart failure and hyperuricemia.

SGLT2 inhibitors, initially developed for diabetes, reduce renal glucose reabsorption and promote urinary excretion. These drugs have been shown to lower the risks of heart failure hospitalization and cardiovascular mortality in both diabetic and nondiabetic patients. Studies confirmed their urate-lowering effect, with dapagliflozin reducing SU by 0.84 mg/dL (50 μmol/L) in heart failure patients [28]. The EMPEROR-Reduced trial [29] showed that empagliflozin reduced SU by 1.12 mg/dL (67 μmol/L) and decreased gout flare frequency. A multicenter trial found that a high dose of losartan potassium (150 mg/day) reduced SU levels by 0.27 mg/dL (16 μmol/L) and hyperuricemia incidence in patients with heart failure.

Diuretics, commonly used in heart failure, can elevate SU levels and trigger gout, possibly through reduced blood volume, increased renal urate reabsorption, and potassium-depleting effects. Potassium-sparing diuretics, like spironolactone, should be used cautiously. The TOPcAT study showed that spironolactone had no significant effect on SU but improved heart failure outcomes [30], and a case–control study found it reduced gout flares in heart failure patients.

3.1.5 | Gout Flare Management in Patients With Peptic Ulcer or Hemorrhage

In patients at risk of gastrointestinal hemorrhage, selective cyclooxygenase (COX)-2 inhibitors are recommended as the preferred treatment over nonselective NSAIDs. In patients with a history of peptic ulcer or hemorrhage, or those with controlled hemorrhage, short-term glucocorticoids are recommended if colchicine is ineffective or intolerant, provided that proton pump inhibitors (PPIs) or mucosal protectants are used concurrently. In the case of active hemorrhage, where standard therapy is in place, short-term parenteral glucocorticoids may be considered, along with Anakinra, if deemed appropriate.

NSAIDs remain the first-line treatment for gout flares. In elderly patients and those with a history of peptic ulcer, hemorrhage, or perforation, drugs with rapid action and fewer gastrointestinal side effects are preferred. Selective COX-2 inhibitors, such as etoricoxib and celecoxib, have demonstrated similar efficacy in pain relief with fewer gastrointestinal side effects compared to nonselective NSAIDs like indomethacin and diclofenac.

In patients with a history of peptic ulcer or hemorrhage, colchicine may be considered, with short-term glucocorticoids serving as alternatives when colchicine is ineffective or poorly tolerated. Glucocorticoids offer comparable pain relief to NSAIDs but with fewer gastrointestinal adverse events, including indigestion, nausea, vomiting, and gastrointestinal hemorrhage [31]. A RCT indicated that for patients with peptic ulcer hemorrhage and gout flare, short-term use of methylprednisolone (with PPI) was more effective and safer than celecoxib [32]. Furthermore, a single intramuscular injection of mixed betamethasone (7 mg) demonstrated better outcomes and fewer adverse reactions than a diclofenac sodium regimen.

Anakinra has proven effective and safe for treating gouty arthritis, especially in patients with severe or complicated conditions where traditional treatments are inadequate [33]. Anakinra (100 mg/day, subcutaneous injection for 5 days) is comparable in efficacy to conventional treatments, with fewer gastrointestinal side effects. The pain relief effects are similar between the Anakinra 100 mg/day and the 200 mg/day groups during the study period of 5 days. However, agents like Canakinumab and Rilonacept are not recommended for gout patients with gastrointestinal hemorrhage due to insufficient evidence supporting their safety in this context.

3.1.6 | Glucocorticoid Therapy for Gout Flares in Patients With Type 2 Diabetes

In patients with type 2 diabetes experiencing a gout flare, where blood glucose levels are rigorously monitored and controlled, intra-articular triamcinolone acetonide is recommended as the first-line treatment over equivalent systemic glucocorticoid.

This approach is preferred over equivalent systemic glucocorticoids, such as oral prednisone, intramuscular mixed betamethasone, or intravenous dexamethasone sodium phosphate.

Cohort studies indicated consistently that systemic glucocorticoids were related to an increased risk of diabetes mellitus [34]. Blood glucose levels might rise at 24–72 h after the administration of glucocorticoid [35], leading to a recommendation for limited use of glucocorticoids in diabetic patients. Synovial fluid aspiration and intra-articular glucocorticoid injection are prioritized for acute gouty arthritis, especially in large joints. Triamcinolone acetonide has low solubility in synovial fluid, providing prolonged anti-inflammatory effects, making it superior to systemic glucocorticoids when administered with an appropriate dose [2]. However, joint infection must be ruled out, and repeated injections should be avoided.

If systemic glucocorticoids are necessary, oral prednisone or equivalent is preferred at 0.5 mg/kg/day, with discontinuation after 3–5 days for the sake of blood glucose control. Betamethasone and dexamethasone both have high anti-inflammatory potency, making them better substitutes for short-term systemic glucocorticoids.

3.1.7 | Management for Patients With Comorbid OA

In patients with both gout and OA, appropriate exercise during periods of arthritis relief is recommended; additional weight management should be emphasized in those who are overweight or obese; the initiation of ULT is recommended, irrespective of baseline SU levels, with a target SU range between 180 and 300 μmol/L; NSAIDs are the preferred analgesic treatment, with a course of 7–10 days; long-term use of NSAIDs, exceeding 4 weeks, is not suggested; intra-articular glucocorticoid injections are recommended in patients with hip or knee involvement; surgical interventions should be considered for refractory pain or disability in large joints when conservative measures fail.

A meta-analysis underscores the significant benefits of therapeutic exercise in improving pain and physical function in patients with OA [36]. The 2023 NICE guideline for OA management recommends informing overweight or obese patients that weight loss can enhance physical function, reduce pain, and improve quality of life [37]. Regular, moderate physical activity has been shown to reduce CRP levels, decrease gout flares, and alleviate pain. Overweight and obesity are strongly associated with elevated SU levels and increased risk of gout flares, and weight loss can lower SU levels by 30–168 μmol/L, reducing recurrent flares [38]. In obese men, weight loss not only reduced SU levels but also prevented the development of gout. The OPPORTUNITY study demonstrated that adherence to nonsurgical interventions including exercise and weight loss significantly improved the quality of life in OA patients [39]. Therefore, the EP recommends moderate exercise and weight management for patients with both gout and OA.

A retrospective study found that SU level correlated with synovitis and soft tissue swelling in knee OA patients, as measured by MRI [40]. As a result, this guideline suggests a more aggressive ULT strategy, aiming for a target SU range of 180–300 μmol/L to prevent disease progression.

A systematic review and meta-analysis found no benefit of colchicine in reducing pain in OA patients [41]. In contrast, a study showed that etoricoxib significantly improved pain relief, joint function, and quality of life in elderly OA patients [42]. However, due to the risk of gastrointestinal, cardiovascular, and other adverse events, long-term NSAID use is not recommended [43]. Chronic NSAID use has also been linked to increased joint pain, stiffness, and disability, accelerating the need for knee replacement in OA patients [44]. Therefore, NSAID use should be limited to no more than 4 weeks; alternative treatments should be considered beyond this duration.

Intra-articular glucocorticoid injections are effective for managing hip or knee OA, especially in patients with moderate-to-severe pain and joint effusion. The Multidisciplinary Italian Consensus advises their use for short-term pain relief, with caution regarding repeated injections. A RCT showed that intra-articular triamcinolone acetonide (40 mg, every 3 months for 2 years) increased cartilage loss compared to saline injections [45]. Repeated glucocorticoid injections should generally not exceed four per year [46].

When nonsurgical treatments fail and quality of life is severely impacted, the NICE Clinical Practice Guideline for OA suggests considering joint replacement surgery [37]. The Malaysian consensus recommends knee replacement surgery for advanced knee OA patients, with joint replacement considered for those with persistent pain and functional decline.

4 | Discussion

Gout has evolved from a rare inflammatory arthritis to a common disorder worldwide, frequently accompanying a range of diseases that are typically associated with a severe socioeconomic burden [47]. Some comorbidities are considered with shared mechanisms, like hypertension, CVD, stroke, obesity, hyperlipidemia, type 2 diabetes mellitus, and CKD, which are now clustered as cardiometabolic-renal (CMR) conditions. Studies on gout populations from Britain, France, China, and the US showed consistently a high prevalence of those conditions [3, 48]. OA is another common arthritis, which has long been linked to gout due to the epidemiological consistency. The prevalence of knee OA in a gout cohort was reported as high as 68%, and the disease outcomes tend to be more severe in such patients [4]. Besides, some complications, like peptic ulcer disease, hyperglycemia, hypertension, and cardiac dysfunction, are unavoidable in a population with repeated or long-term NSAIDs and glucocorticoids [49]. These comorbidities and complications have made gout management a clinical challenge in these patients who are usually grouped as difficult to treat [50]. In the past decade, novel drugs in the related fields have become commercially available, producing new comprehensive management approaches that may serve as evidence addressing those problems.

The auto-inflammatory nature of gout has gradually been recognized. However, MSU crystal deposition and phagocytosis are

always the first signals of a gout flare. Long-term ULT to maintain SU at target levels to avoid MSU crystal formation or promote its resolution is the core of gout management, as indicated by previous guidelines [1, 2]. In addition to symptomatic gout, asymptomatic hyperuricemia has been associated with injuries to the kidneys, vascular intima, as well as joints, visualized by advanced imaging measures in recent years, like dual-energy CT [4]. According to the 2019 FREED study, lowering SU to below 5 mg/dL with febuxostat delayed the progression of kidney dysfunction in patients with hyperuricemia at risk for cerebral, cardiovascular, or renal disease (Supplementary Reference 86). Based on these updates, the stricter SU goal of < 5 mg/dL in hyperuricemia/gout patients with CKD stage ≥ 3 was ultimately agreed by the EP. Furthermore, this guideline recommends febuxostat as the preferred choice, due to the higher risk of hypersensitivity to allopurinol in Asian populations, particularly in cases of impaired renal function.

Heart failure can be the consequence of both the CMR condition and side effects from NSAIDs and glucocorticoids. Diuretics are the key medication for heart failure, which are also the most common drugs associated with hyperuricemia and gout. Fortunately, two novel categories of anti-heart failure medications, the angiotensin receptor-neprilysin inhibitors (ARNI) and the sodium-glucose cotransporter-2 (SGLT-2) inhibitors, have been proved to be effective not only for heart failure but also for lowering SU levels [27–29]. SGLT-2 inhibitors (dapagliflozin or empagliflozin), in particular, improve several components of metabolic syndrome, while also slowing CKD progression and reducing the risk of major cardiovascular events and premature death. Therefore, SGLT-2 inhibitors may offer multiple benefits in treatment of heart failure patients with comorbid hyperuricemia.

Gout flare management tends to be sophisticated when patients are complicated with conditions like CKD, acute myocardial infarction, heart failure, diabetes, and peptic ulcer or hemorrhage. On the other hand, recurrent gouty arthritis may have adverse impacts on the prognosis of CVDs. In these patients, treatments with colchicine, NSAIDs, or glucocorticoids are restricted to some conditions that are hard to systemically acquire. This guideline accomplishes detailed recommendations addressing these clinical dilemmas, combining evidence from relevant fields and new drugs. For example, colchicine is prioritized for the prophylaxis or termination of gout flare in patients with heart failure or acute myocardial infarction due to the benefits of both gout and heart disease from low-dose colchicine confirmed by multiple studies [16, 17]. Biotherapy is the main progression in clinical therapy in recent years, with piled evidence in gout management. The efficacy and safety of IL-1 inhibitors for gout flares in patients inappropriate for traditional anti-inflammatory medicine have been verified by several RCTs [5, 18, 20, 21]. One novel IL-1β inhibitor, firsekibart, is now commercially available in China, which encourages the recommendations of IL-1 inhibitors in this guideline.

Although patients with gout are frequently complicated with OA, gout overlapping OA is seldom considered in previous management guidelines. OA is primarily a condition of chondrocyte degeneration, resulting from the "wear-and-tear" mechanism. The alteration of synovial lining permeability and synovial fluid physiochemical environment, and the cartilage fibrillation in OA may facilitate urate supersaturation, promote urate crystallization and deposition, while the latter in turn activates synovial lining macrophages and results in the generation of inflammatory mediators, particularly IL-1β, which may contribute to further OA cartilage degradation [47]. These clinical and experimental studies suggest that gout and OA exacerbate reciprocally when coexisting in the same joints. Giving full consideration to these studies and integrating the monotherapy approaches for both conditions, this guideline provides comprehensive recommendations for managing gout combined with OA, including lifestyle interventions, stricter SU control, appropriate anti-inflammatory therapy, and surgical intervention.

In summary, this updated guideline provides comprehensive management recommendations for gout with comorbidities, guaranteed by multidisciplinary collaboration. This guideline echoed the 2018 EULAR recommendations for evaluation of comorbidities once gout is diagnosed, while focused on factors related to the Chinese population and traits of gout in China. Novel drugs (dotinurad, topiroxostat, tigulixostat, AR882, rizinurad/SHR4640, etc.) are emerging, promising the next update in the future (Supplementary Reference 96).

Author Contributions

Changgui Li conceived the idea and submitted the project to the Chinese Association of Endocrinology Executive Committee. Jiajun Zhao co-chaired the meetings. Changgui Li was in charge of the whole process of the guideline development. Mingshu Sun wrote the manuscript with the help from Zhen Liu. Yuwei He, Xinde Li, Fei Yan, Lin Han, Xufei Luo, and Can Wang conducted most of the data collection, literature searching, and evidence retrieving. Ronald ML Yip provided opinions as an external reviewer. All authors have contributed to this work and have approved the final version of the manuscript.

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Ethics Statement

This study does not involve human participants.

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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Additional references are presented in Supplementary Reference.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Flow chart of guideline development. **Table S1:** Clinical questions and PICO deconstruction. **Table S2:** Importance of outcome indicator.

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ORIGINAL ARTICLE

Association Between Traditional Chinese Medicine Use and Sepsis Risk and Glucocorticoid Exposure in Patients With Systemic Lupus Erythematosus: A Retrospective Cohort Study

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ABSTRACT

Aim: Sepsis greatly increases morbidity and mortality in patients with systemic lupus erythematosus (SLE). Conventional treatments, particularly glucocorticoids (GCs), raise infection risk. This retrospective cohort study aims to investigate the association of Traditional Chinese Medicine (TCM) use with sepsis incidence, mortality, and GC dependence in patients with SLE, based on data from the Chang Gung Research Database (CGRD).

Methods: Patients in Taiwan with newly diagnosed SLE were enrolled from the CGRD between 2005 and 2020. They were stratified into groups based on TCM treatment post-diagnosis. Outcomes included sepsis incidence and the dose and duration of GC usage. Data were analyzed using Cox proportional hazard models and Kaplan–Meier analysis.

Results: The study included 10 846 newly diagnosed patients with SLE, of whom 1801 received at least 28 days of TCM treatment, while 8302 did not. After propensity score matching, 5403 and 1801 individuals were included in the non-TCM and TCM groups, respectively, with no significant baseline differences in age, sex, biochemical profiles, and comorbidities between the groups. Integrative TCM usage was associated with a significantly lower risk of sepsis (adjusted hazard ratio [aHR]: 0.49; 95% confidence interval [CI]: 0.40–0.60, $p < 0.001$) and mortality rate (aHR 0.52, 95% CI 0.44–0.60, $p < 0.001$) over an 18-year period. Additionally, the TCM group had a significantly lower daily GC dose (1.74 vs. 2.47 units/day; $p = 0.02$).

Conclusion: TCM use was significantly associated with lower risks of sepsis and lower GC dosage in patients with SLE, suggesting its potential as an integrative therapy.

1 | Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects various organs and systems. It has a multifactorial etiology, involving several identified genetic and environmental factors [1, 2]. The management of SLE varies depending on the affected organs and the disease severity. The current standard treatment for active SLE includes nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), antimalarials, and immunosuppressants [3]. While GCs are essential for suppressing inflammatory and immune responses, their long-term use increases the risk of serious infections [4, 5]. Infections are a primary cause of morbidity and mortality in patients with SLE, accounting for 14%–82% of related ICU admissions and often progressing to sepsis—a life-threatening organ dysfunction caused by a dysregulated host response to infection [6, 7]. Given these treatment-related risks, identifying safer complementary therapies is crucial. In the recent decades, Traditional Chinese Medicine (TCM) has been increasingly acknowledged globally as a complementary or alternative therapeutic approach for managing SLE [8]. A systematic review has demonstrated that TCM has a statistically significant impact on reducing the SLE Disease Activity Index score, decreasing the erythrocyte sedimentation rate, and elevating serum complement C4 levels, as compared to a placebo [9]. Recent studies have also shown that TCM is associated with a lower risk of pneumonia and hospitalization for infection in patients with SLE [10, 11]. Despite these promising findings, the specific role of TCM in preventing the progression of infection to sepsis has not been thoroughly investigated.

This retrospective study, therefore, aimed to investigate the association between integrative TCM use and the risk of sepsis and mortality in patients with SLE. Additionally, we examined the association between TCM usage and GC dosage to assess its potential role in lowering long-term steroid exposure.

2 | Methods

2.1 | Data Sources and Ethics Statement

The data used in this study were retrieved from the Chang Gung Research Database (CGRD). The CGRD contains the primary electronic health records of patients attending the Chang Gung Memorial Hospital (CGMH), including details such as sex, age, diagnoses for each outpatient/emergency visit or admission, prescriptions, comorbidities, procedures, and laboratory results [12]. The CGRD also contains detailed records of TCM use among patients at CGMH, with all TCMs classified into two main categories: single herbs (SH) and herbal formulas (HF). Herbal formulas are pre-prepared mixtures of several single herbs, blended in specific proportions according to traditional Chinese medicine literature prior to commercial production. This study was approved by the Institutional Review Board (IRB) of Chang Gung Medical Foundation (IRB Approval No.: 202202074B0). The need for obtaining informed patient consent was waived as the identification number of each participant was securely encrypted, ensuring patient anonymity.

2.2 | Study Design

Figure 1 demonstrates the flow diagram of this study. We initially identified 14 530 patients who were newly diagnosed with SLE between January 1, 2005 and December 31, 2020, from the CGRD. The diagnosis of SLE was based on the catastrophic illness record using code 710 from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) or code M32 from the International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM). Subsequently, patients aged < 20 years or who were diagnosed with SLE in 2004 were excluded from the analysis. For the purpose of this study, our definition of TCM treatment was restricted to the prescription of Chinese herbal medicine. Other TCM modalities, such as acupuncture, cupping, or massage, were not included in our exposure definition. Subsequently, the remaining eligible individuals were divided into two groups based on whether they received TCM treatment after being diagnosed with SLE. The TCM group was defined as individuals who received at least 28 days of TCM treatment during the study period, while those who did not receive TCM were classified under the non-TCM group. This 28-day threshold was chosen to distinguish sustained from incidental users and was aligned with the typical 28-day prescription cycle for chronic diseases under Taiwan's healthcare system. The index date was determined as the first day on which TCM was prescribed. Propensity score matching (PSM) was conducted between the TCM and non-TCM groups, with a 1:3 ratio, with the matching based on baseline characteristics (age, sex, year of diagnosis) in the sepsis analysis. For the analysis of GC dosage, additional PSM was performed using a 1:2 matching ratio, taking into account additional characteristics such as comorbidities, medications, and laboratory data (Appendix A: Figure A1).

2.3 | Study Variables

Demographic variables, including sex, age, comorbidities, year of diagnosis, baseline renal function (creatinine and proteinuria), and pretreatment medications, such as NSAIDs, were sourced from the CGRD. Comorbidities were defined by either two outpatient visits or one inpatient record for any of the following conditions within the year prior to the index date. The ICD-9-CM and ICD-10-CM codes were used to identify comorbidities (hypertension: 401.xx–405.xx, I10–I15; diabetes mellitus: 250.x, E08–E13; CKD: 580.x–588.x, 403.xx, 404.xx, 095.4x, 236.9x, 250.4x, 274.1x, 442.1x, 447.3x, 440.1x, 572.4x, 642.1x, 646.2x, 753.1x, 283.11, 446.21, N18; gout: 274.xx, M10). The use of conventional medication was defined as having a prescription for at least 90 days during the year prior to the index date. Vaccination was defined as receipt of herpes zoster, human papillomavirus, influenza, COVID-19, or pneumococcal vaccines after the index date. Additionally, SLE-specific variables, such as biochemical profiles (C3, C4, and anti-double stranded DNA antibodies), and disease-modifying antirheumatic drug (DMARD) usage, were

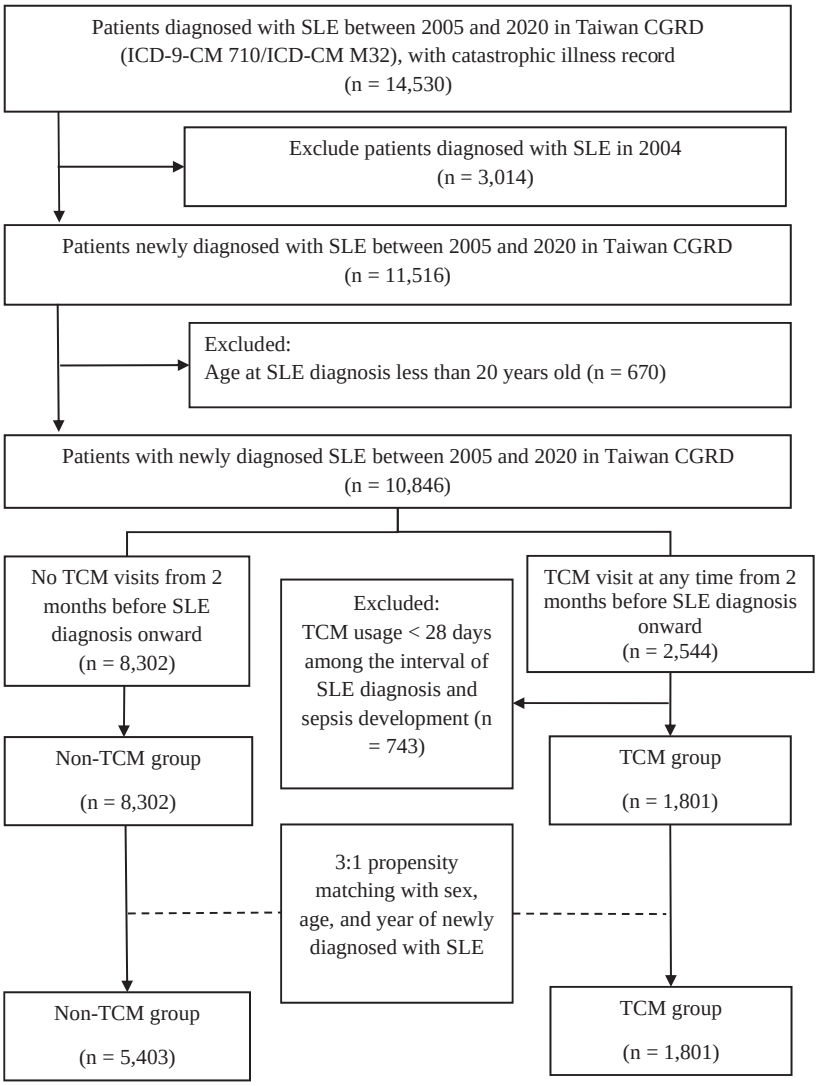

FIGURE 1 | Flow diagram of the study for identifying the effect of Traditional Chinese Medicine (TCM) use on the risk of sepsis in patients with systemic lupus erythematosus (SLE).

collected. The laboratory data were retrieved from the period closest to the index date within the preceding year.

2.4 | Outcome Assessment

The outcomes of our study were categorized into two main categories corresponding to the study designs. The first group focused on the occurrence of sepsis, identified using ICD-10-CM (A40, A41, A021, A027, A227, A267, A327, A483), and ICD-9-CM codes (0031, 0202, 0223, 0270, 0271, 0380, 03810, 03811, 03819, 0382, 0383, 03840–038044, 03849, 0388, 0389, 04089), with the onset of sepsis determined by the date of diagnosis. Additionally, we assessed all-cause mortality as part of this category. The second category involved the duration or reduction of GC usage, with doses standardized to prednisone-equivalents (each unit corresponding to 5 mg of prednisone, Table A1) [13, 14]. Each eligible individual was followed from their respective index date until the occurrence of an outcome event (sepsis or death), loss to follow-up, or the end of 2022, whichever occurred first.

2.5 | Data Analysis

Continuous data, such as age and baseline laboratory data, are presented as medians and interquartile ranges (IQRs), and were compared between TCM and non-TCM groups using the Mann–Whitney *U* test (except for the outcome of GC dose). Categorical data, including sex, comorbidities, and baseline conventional medications, were presented as numbers and percentages, and the chi-squared test was used for group comparisons. The Cox proportional hazards regression model was employed to analyze sepsis rates and mortality, with adjustment for the following factors: age, sex, comorbidities, baseline medications, and baseline laboratory data. Cumulative incidence was calculated using the Kaplan–Meier approach. A competing-risk regression model, with adjustment for covariates, was also applied for group comparisons. Statistical analyses were conducted using SAS v9.4 software (SAS Institute, Cary, NC, USA). Two-sided statistical tests were employed, with significance defined as a p value < 0.05.

3 | Results

3.1 | Demographic Features

Between January 1, 2005, and December 31, 2020, 10846 individuals newly diagnosed with SLE were identified and analyzed using PSM, resulting in the inclusion of 1801 TCM users and 5403 nonusers in the final cohort. Table 1 presents the baseline demographic characteristics of all analyzed individuals. No significant differences were observed between groups in terms of sex, age, associated comorbidities, and the prevalence of DMARD use. The medium age of the individuals was 51 (IQR 39, 60) years in the TCM group and 50 (IQR 38, 59) years in the non-TCM group, with a female predominance of 88.84% in both groups. The use of GCs was higher in the non-TCM group (27.69% vs. 23.54% in the TCM group, p value <0.001). However, differences

TABLE 1 | Demographics, physiological profiles, and laboratory findings of patients with systemic lupus erythematosus among Traditional Chinese Medicine (TCM) users and non-TCM users.

	Non-TCM group, $n=5403$	TCM group, $n=1801$	p[a]	Absolute standardized mean difference[b]
Age (years), median (IQR)	50 (38, 59)	51 (39, 60)	0.55	0.01
Male	603 (11.16%)	201 (11.16%)	1	0
Comorbidities				
Hypertension	723 (13.38%)	255 (14.16%)	0.40	0.02
Diabetes mellitus	265 (4.9%)	81 (4.5%)	0.48	0.02
Chronic kidney disease	450 (8.33%)	119 (6.61%)	0.02	0.07
Gout	80 (1.48%)	24 (1.33%)	0.65	0.01
Baseline medications				
NSAID	657 (12.16%)	232 (12.88%)	0.42	0.02
Glucocorticoids	1496 (27.69%)	424 (23.54%)	<0.001	0.10
DMARDS				
Hydroxychloroquine	1542 (28.54%)	557 (30.93%)	0.05	0.05
Azathioprine	283 (5.24%)	100 (5.55%)	0.61	0.01
Methotrexate	369 (6.83%)	101 (5.61%)	0.07	0.05
Leflunomide	48 (0.89%)	9 (0.5%)	0.17	0.05
Mycophenolate mofetil	67 (1.24%)	23 (1.28%)	0.90	<0.01
Cyclosporin	65 (1.2%)	19 (1.05%)	0.61	0.01
Cyclophosphamide	23 (0.43%)	10 (0.56%)	0.48	0.02
Rituximab	0 (0%)	0 (0%)	—	—
Biochemical profiles				
Creatinine (mg/dL), median (IQR)	0.71 (0.60, 0.90)	0.70 (0.60, 0.85)	0.02	0.15
Proteinuria (mg), median (IQR)	533.87 (134.33, 2151.13)	252.10 (105.54, 1816.13)	<0.001	0.31
C3 (mg/dL), median (IQR)	92.20 (72.50, 111.00)	93.40 (78.30, 110.00)	0.13	0.08
C4 (mg/dL), median (IQR)	17.60 (11.90, 23.60)	18.20 (12.40, 23.70)	0.27	0.05
Anti-dsDNA (U/mL), median (IQR)	71.00 (40.50, 217.65)	72.85 (40.50, 244.00)	0.86	0.03
Anti-dsDNA positive	177 (3.28%)	50 (2.78%)	0.49	0.03

Abbreviations: Anti-dsDNA, anti-double-stranded deoxyribonucleic acid; C3, complement C3; C4, complement C4; DMARDs, disease-modifying antirheumatic drugs; IQR, interquartile range; TCM, Traditional Chinese Medicine.
[a]Chi-squared test for categorical data, Mann–Whitney U test for continuous data.
[b]Calculation of effect size: mean for continuous data and percentage for categorical data.

between the two groups were negligible, as indicated by an absolute standardized mean difference (MD) < 0.1. Additionally, the biochemical parameters, including baseline creatinine (0.71 [IQR 0.60, 0.90] mg/dL in the non-TCM group vs. 0.70 [IQR 0.60, 0.85] mg/dL in the TCM group, p value = 0.02) and proteinuria (533.87 [IQR 134.33, 2151.13] mg in the non-TCM group vs. 252.10 [IQR 105.54, 1816.13] mg in the TCM group, p value < 0.001), were notably higher in the non-TCM group. Other disease activity profiles, such as C3, C4, and anti-dsDNA levels, showed no significant differences between the two groups.

In the analysis of GC dosage, baseline demographic characteristics are shown in Table A2. Although baseline creatinine and proteinuria levels were higher in the non-TCM group, no significant differences were seen between the groups regarding sex, age, comorbidities, or the prevalence of baseline medication use.

3.2 | Association Between TCM Usage and Sepsis Incidence

The TCM group had a sepsis rate of 0.65 per 100 person-years, which was lower than that of the non-TCM group, in which the rate was 1.46 per 100 person-years (Table 2). After adjusting for confounding covariates, the adjusted hazard ratio (aHR) for sepsis in TCM users was 0.49 (95% confidence interval [CI] 0.40–0.60, p value < 0.001), compared to non-TCM users. A competing-risk analysis was performed, with death as the competing event, which yielded a sub-distribution HR of 0.69 (95% CI: 0.55–0.85, p value < 0.001). When the cumulative days of TCM use were stratified, the aHR for sepsis was 0.47 (95% CI: 0.31–0.72) for 28–90 days of use and 0.50 (95% CI: 0.39–0.63) for > 90 days, compared to non-TCM users. Furthermore, utilizing survival analysis to validate this result, TCM users demonstrated a significantly lower cumulative incidence of sepsis, as evidenced by the log-rank test result (p value < 0.001) (Figure 2).

A stratified analysis by vaccination status was conducted to assess potential confounding by health-seeking behavior. Among the non-vaccinated group (n = 9903), TCM use was associated with a significantly lower risk of sepsis (aHR: 0.49, 95% CI: 0.40–0.61). In the vaccinated group (n = 200), which included 33 sepsis events, TCM use showed a nonsignificant trend toward lower sepsis risk (aHR: 0.38, 95% CI: 0.10–1.45).

3.3 | Association Between TCM Usage and 18-Year Mortality Rate

TCM users had a significantly lower mortality rate of 1.20 per 100 person-years than did the non-TCM group, with a rate of 2.44 per 100 person-years (Table 2). After adjusting for confounding factors, the TCM group displayed an aHR for mortality of 0.52 (95% CI 0.44–0.60, p value < 0.001), indicating a significant reduction in the risk of mortality. Furthermore, survival analysis confirmed that the TCM group demonstrated notably higher survival rates, as supported by the log-rank test (p value < 0.001) (Figure 2).

TABLE 2 | Association between the use of Traditional Chinese Medicine and sepsis risk and 18-year mortality rate.

	Non-TCM group, n = 5403	TCM group, n = 1801	p
Sepsis-related outcomes			
Number of events	691	107	—
Person years, mean ± SD	8.73 ± 4.63	9.19 ± 4.52	< 0.001
Total person years	47 190.22	16 550.03	—
Incidence rate: per 100 person-years	1.46	0.65	—
Adjusted[a] HR, 95% CI	as reference	0.49 (0.40–0.60)	< 0.001
Competing-risk regression model[b] Adjusted[a] HR, 95% CI	as reference	0.69 (0.55–0.85)	< 0.001
Mortality-related outcomes			
Number of events	1185	203	—
Person years, mean ± SD	8.99 ± 4.58	9.38 ± 4.43	0.002
Total person years	48 599.52	16 887.07	—
Incidence rate: per 100 person-years	2.44	1.20	—
Adjusted[a] HR (95% CI)	as reference	0.52 (0.44–0.60)	< 0.001

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation; TCM, Traditional Chinese Medicine.
[a]Adjusted for all the parameters in Table 1.
[b]Death as a competing event.

3.4 | Association Between TCM Usage and the GC Dosage

The independent t-test showed a significantly lower daily prednisone equivalent dose of GC exposure in the TCM group (Table 3; 2.47 ± 16.75 units/day in the non-TCM group vs. 1.74 ± 5.19 units/day in the TCM group; p value = 0.02). Additionally, the duration of GC usage was non-significantly shorter in the TCM than in the non-TCM group (580.78 days in non-TCM group vs. 550.69 days in TCM group; p value = 0.31).

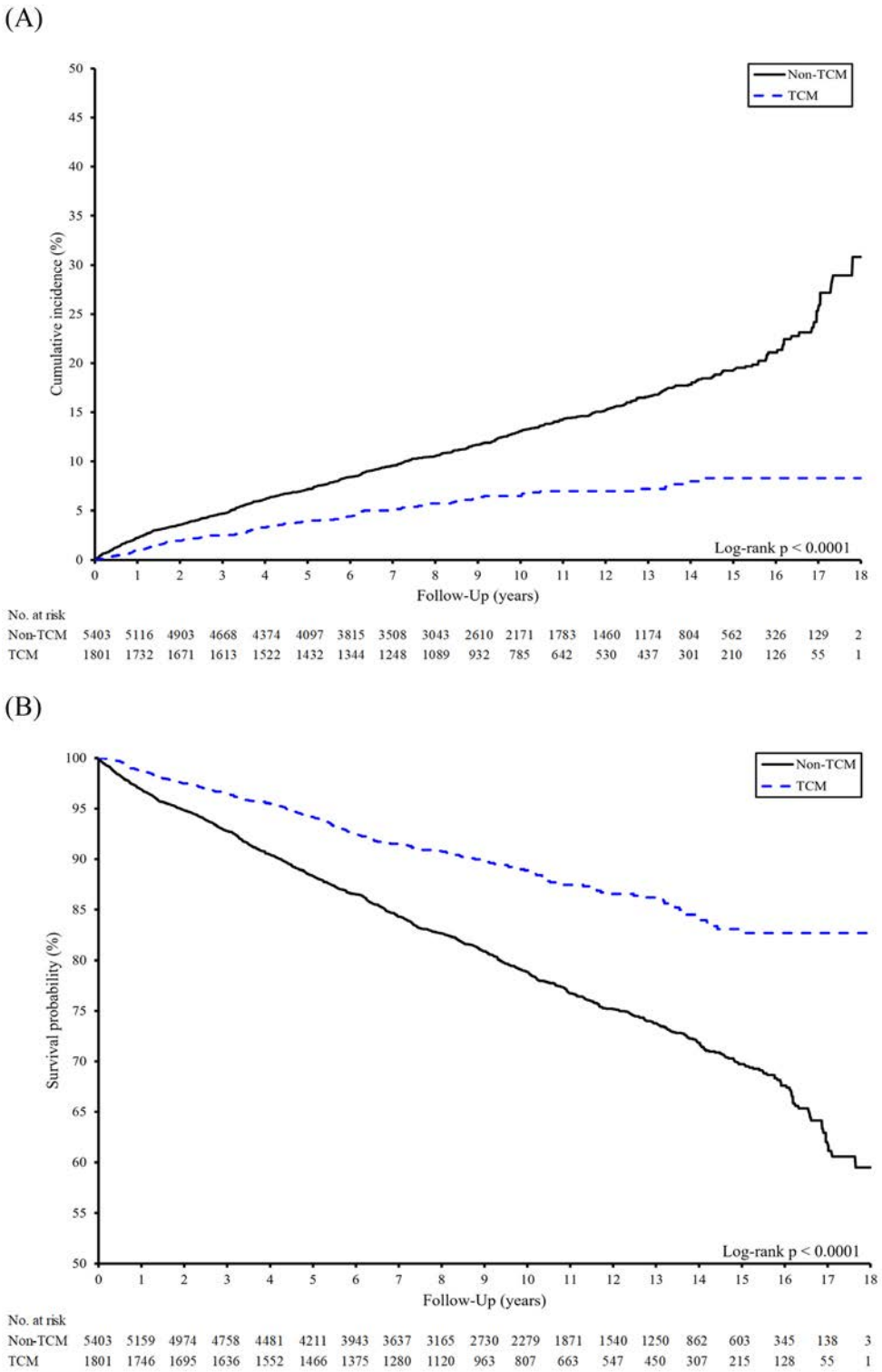

FIGURE 2 | (A) Cumulative incidence of sepsis and (B) overall survival probability among patients with systemic lupus erythematosus (SLE) stratified by Traditional Chinese Medicine (TCM) use.

3.5 | Association Between Specific Single Herbs and Herbal Formulas and the Risks of Sepsis and Mortality

The top 10 most frequently used single herbs and herbal formulas among patients with SLE are summarized in Table 4. The most commonly used single herb was "Dan Shen", prescribed to 644 patients (35.76%), followed by "Bai Shao", "Mai Men Dong", "Zhe Bei Mu", and "Huang Qi". Among herbal formulas, "Jia-Wei-Xiao-Yao-San" was the most frequently used (767 patients, 42.59%), followed by "Zhi-Bo-Di-Huang-Wan", "Gan-Lu-Yin", "Shu-Jing-Huo-Xue-Tang", and "Qi-Ju-Di-Huang-Wan". All listed single herbs and formulas were significantly associated with a lower risk of both sepsis

TABLE 3 | Association between the use of Traditional Chinese Medicine and glucocorticoid dosage.

	Non-TCM group, $n = 3702$	TCM group, $n = 1851$	p[a]
Equivalent doses (unit), mean ± SD	1056.93 ± 2293.06	973.93 ± 2122.79	0.18
Duration (day), mean ± SD	580.78 ± 1034.90	550.69 ± 1038.40	0.31
Daily equivalent dose (unit/day), mean ± SD	2.47 ± 16.75	1.74 ± 5.19	0.02

Abbreviations: SD, standard deviation; TCM, Traditional Chinese Medicine.
[a]An independent *t*-test was used for group comparisons.

TABLE 4 | Hazard ratios for sepsis and mortality associated with commonly used single herbs and formulas compared with matched non-TCM users.

			Adjusted HR[a] (95% CI)			
No.	**Herbs**	**Patients/total TCM users**	**Sepsis**	***p***	**Death**	***p***
Single herbs						
1	Dan Shen	644/1801 (35.76%)	0.44 (0.31–0.63)	< 0.001	0.55 (0.43–0.70)	< 0.001
2	Bai Shao	484/1801 (26.87%)	0.52 (0.34–0.82)	0.004	0.46 (0.33–0.64)	< 0.001
3	Mai Men Dong	478/1801 (26.54%)	0.68 (0.46–0.99)	0.05	0.40 (0.29–0.54)	< 0.001
4	Zhe Bei Mu	474/1801 (26.32%)	0.51 (0.33–0.79)	0.003	0.39 (0.27–0.55)	< 0.001
5	Huang Qi	445/1801 (24.71%)	0.49 (0.33–0.73)	< 0.001	0.47 (0.35–0.64)	< 0.001
6	Sheng Di Huang	424/1801 (23.54%)	0.51 (0.34–0.78)	0.002	0.37 (0.26–0.54)	< 0.001
7	Huang Qin	422/1801 (23.43%)	0.46 (0.28–0.75)	0.002	0.39 (0.27–0.58)	< 0.001
8	Mu Li	416/1801 (23.10%)	0.50 (0.33–0.77)	0.002	0.36 (0.25–0.52)	< 0.001
9	Xuan Shen	415/1801 (23.04%)	0.41 (0.24–0.69)	< 0.001	0.35 (0.23–0.52)	< 0.001
10	Mu Dan Pi	396/1801 (21.99%)	0.40 (0.24–0.67)	< 0.001	0.48 (0.34–0.69)	< 0.001
Herbal formulas						
1	Jia-Wei-Xiao-Yao-San	767/1801 (42.59%)	0.52 (0.38–0.72)	< 0.001	0.39 (0.29–0.51)	< 0.001
2	Zhi-Bo-Di-Huang-Wan	734/1801 (40.76%)	0.58 (0.41–0.82)	0.002	0.51 (0.39–0.67)	< 0.001
3	Gan-Lu-Yin	707/1801 (39.26%)	0.46 (0.32–0.66)	< 0.001	0.40 (0.31–0.53)	< 0.001
4	Shu-Jing-Huo-Xie-Tang	587/1801 (32.59%)	0.45 (0.31–0.67)	< 0.001	0.40 (0.30–0.54)	< 0.001
5	Qi-Ju-Di-Huang-Wan	534/1801 (29.65%)	0.35 (0.23–0.54)	< 0.001	0.38 (0.28–0.51)	< 0.001
6	Suan-Zao-Ren Tang	524/1801 (29.09%)	0.50 (0.34–0.73)	< 0.001	0.35 (0.25–0.49)	< 0.001
7	Ban-Xia-Xie-Xin-Tang	492/1801 (27.32%)	0.62 (0.43–0.91)	0.014	0.43 (0.31–0.60)	< 0.001
8	Xiang-Sha-Liu-Jun-Zi-Tang	461/1801 (25.60%)	0.52 (0.34–0.80)	0.002	0.40 (0.28–0.56)	< 0.001
9	Shao-Yao-Gan-Cao-Tang	449/1801 (24.93%)	0.58 (0.38–0.88)	0.011	0.37 (0.26–0.52)	< 0.001
10	Long-Dan-Xie-Gan-Tang	408/1801 (22.65%)	0.33 (0.20–0.55)	< 0.001	0.48 (0.34–0.69)	< 0.001

Abbreviations: CI, confidence interval; HR, hazard ratio; TCM, Traditional Chinese Medicine.
[a]Adjusted for all the parameters in Table 1.

and mortality, with aHRs ranging from 0.40 to 0.68 for sepsis and 0.35 to 0.55 for mortality among single herbs, and from 0.33 to 0.62 for sepsis and 0.35 to 0.51 for mortality among formulas. Notably, "Mu Dan Pi" and "Xuan Shen" exhibited the lowest aHRs for both sepsis (0.40 and 0.41, respectively) and mortality (0.48 and 0.35, respectively). Among formulas, "Long-Dan-Xie-Gan-Tang" was associated with the lowest aHRs: 0.33 (95% CI: 0.20–0.55) for sepsis and 0.48 (95% CI: 0.34–0.69) for mortality.

4 | Discussion

This retrospective cohort study revealed an association between the integration of TCM with conventional treatment and lower risks of sepsis and mortality among patients with SLE. Additionally, TCM usage was associated with lower dosages of GCs. The findings of our study are broadly consistent with and expand upon the existing body of real-world evidence from Taiwan's health insurance databases. Specifically, our

observation of a lower all-cause mortality risk (aHR 0.52) in TCM users aligns with the conclusions of previous large-scale cohort studies by Ma et al. [15] and Chen et al. [16] which also reported improved survival outcomes in SLE patients receiving TCM. Similarly, our primary finding—an observed association with lower sepsis risk—is in line with and extends recent studies that linked TCM use to a lower risk of pneumonia [11] and overall infectious hospitalizations [17].

While previous studies established a general protective association against mortality and infections, our study provides a novel contribution by specifically addressing the progression to sepsis—a primary driver of morbidity and mortality in patients with SLE [18, 19]. Furthermore, our study contributes methodologically by employing a stringent, duration-based definition for TCM users (≥ 28 days) and utilizing the CGRD, which may represent a population with a higher disease severity compared to the general National Health Insurance Research Database (NHIRD) population.

Infections remain to account for 30%–50% of morbidity and mortality among patients with SLE globally [20, 21]. A study from the United Kingdom has indicated an upward trend in the proportion of patients with SLE requiring hospitalization due to severe infections, with an increase exceeding 12-fold between 1996 and 2001 [22]. The reason for this escalation remains unclear. Correspondingly, a recent nationwide study in the United States also revealed that the incidence of hospitalizations due to infections among SLE patients had risen over time, with sepsis overtaking pneumonia as the predominant reason for hospital admission [23]. Additionally, the risk of death after developing sepsis is higher in patients with SLE than that in the general population. Chen et al. reported that critically ill patients with SLE had a higher sepsis-related mortality rate than those without SLE, with an incidence rate ratio of 2.8 (95% CI, 2.5–3.2) [10].

Large observational studies have indicated that GC-mediated toxicity is significantly influenced by both the dose and the duration of exposure [24–26]. Balancing efficacy against the risk of toxicity remains a challenge in the management of SLE. Long-term use of GCs is associated with several adverse effects, including a 50% increase in the risk of permanent organ damage [27] and a 2.5-fold higher risk of cardiovascular events [28]. Among all the medications used to treat SLE, GC have been identified as a major risk factor for serious infection, even when used at a moderate dose [29–31]. The dosage of GCs should be determined by the type and severity of organ involvement [2]. For the long-term prevention of flares-ups, the dose should be tapered to a maintenance level of ≤ 5 mg/day (prednisone-equivalent), with the final goal of discontinuation [2, 32]. Our study demonstrated that the daily equivalent dose was less and the duration of exposure to systemic GCs was shorter in the TCM than in the non-TCM group, suggesting a potential association with a reduction in GC-related risks. This finding aligns with a previous systematic review reporting a reduction of GC use among patients with SLE who received TCM interventions [9].

The pathogenesis of SLE and the development of sepsis are complex processes involving dysregulated inflammatory responses and immune dysfunction [33]. Previous studies have suggested that TCM may help modulate these mechanisms by downregulating proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6, and by regulating Nuclear factor kappa B (NF-κB) activity [8, 34]. Several of the frequently prescribed single herbs identified in our study have been reported to possess immunomodulatory, which may be associated with a lower sepsis risk in patients with SLE (Appendix B: Table A3). In the animal study, Danshen (*Salvia miltiorrhiza*) has been shown to suppress proinflammatory mediators, such as IL-1β and nitric oxide, while enhancing host defenses through increased monocyte and natural killer (NK) cell activity [35]. Total glucosides of peony, derived from Bai Shao (*Paeonia lactiflora*), can modulate lymphocyte proliferation and T helper (Th)/regulatory T (Treg) cell differentiation, as well as suppress proinflammatory cytokines such as interleukin-1β and TNF-α [36]. Huang Qi (Astragalus polysaccharide) can regulate immune organ function and modulate the activity of both innate and adaptive immune cells, including macrophages, dendritic cells, and lymphocytes. Together, these actions contribute to the restoration of immune homeostasis [37].

In our analysis of TCM utilization patterns within the TCM group, approximately half of the commonly used single herbs and formulas were consistent with those reported in previous studies on the prevention of lupus nephritis [38], mortality [15] and GCs dose reduction outcomes [39]. Several of the most frequently used herbal formulas, including Long-Dan-Xie-Gan-Tang, Zhi-Bo-Di-Huang-Wan, and Jia-Wei-Xiao-Yao-San, were significantly associated with a lower risk of both sepsis and mortality. Notably, Long-Dan-Xie-Gan-Tang and Zhi-Bo-Di-Huang-Wan have also been shown in human trials to reduce endothelial inflammation and lower levels of vascular endothelial growth factor and interleukin-18 [40], further supporting their potential role in modulating the inflammatory and vascular components of SLE and sepsis pathogenesis.

The present study had several strengths. Our research used a long retrospective time frame, along with more recent data, from 2005 to 2022, and leveraged the CGRD database, which offers several advantages. Compared to the NHIRD and other medical centers in Taiwan, the CGRD population exhibits a higher burden of comorbidities and a greater prevalence of specific diseases [12, 41]. Moreover, the CGRD provides more detailed clinical information, such as laboratory and pathological results, than does the NHIRD, aiding researchers in controlling confounders and obtaining accurate estimates [12]. Greater patient coverage rates were also observed in the CGRD for patients using TCM with major comorbidities [42]. Given the disease severity of SLE patients and the features of the CGRD, the findings of this study are particularly valuable. Additionally, we employed a relatively strict criterion for defining the TCM group, mandating TCM usage for a minimum of 28 days, which may have enhanced the methodological precision.

Despite the advantages of being a multicenter cohort study, our research also had some limitations that should be considered. First, the CGRD includes only TCM treatments prescribed by the CGMH. Patients with SLE who received TCM or other therapies from local clinics or different medical facilities were not identifiable. Furthermore, patients in CGRD who developed infections or were lost to follow-up may have switched to other hospitals but could not be traced, unlike in the NHIRD, which may have introduced potential bias. Third, despite rigorous PSM, residual

confounding from unmeasured variables is possible, as the CGRD lacks detailed data on socioeconomic factors (e.g., education, income) and specific lifestyle behaviors. To investigate the influence of health-seeking behaviors, we conducted a stratified analysis using vaccination status as a proxy. While the protective association of TCM remained robust in the non-vaccinated group, this specific analysis has its own limitations, including the likely under-ascertainment of vaccination events that often occur outside our hospital system. Therefore, the influence of these unmeasured confounders cannot be completely ruled out. Finally, as an observational study, our findings indicate association, not causation. Large randomized controlled trials on specific TCM herbs or formulas are needed to further confirm causal relationships.

In conclusion, our retrospective cohort study suggests a significant association between TCM use and improved outcomes in SLE therapy, including a lower risk of sepsis and a lower GC dosage. Further large randomized controlled trials on specific TCM herbs or formulas are needed in the future to identify causal relationships.

Author Contributions

Conceptualization: C.-Y.W., H.-H.Y. Formal analysis: C.-H.C., H.-H.Y., C.-Y.W. Data curation: C.-H.C., H.-H.Y., P.-C.K., P.-Y.C., C.-Y.W. Writing – original draft: C.-H.C. Writing – review and editing: C.-H.C., H.-H.Y., W.-J.C., H.-S.S., C.-Y.W. Visualization: P.-C.K., P.-Y.C. Project administration: C.-Y.W. Funding acquisition: C.-Y.W.

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Ethics Statement

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 on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Appendix A

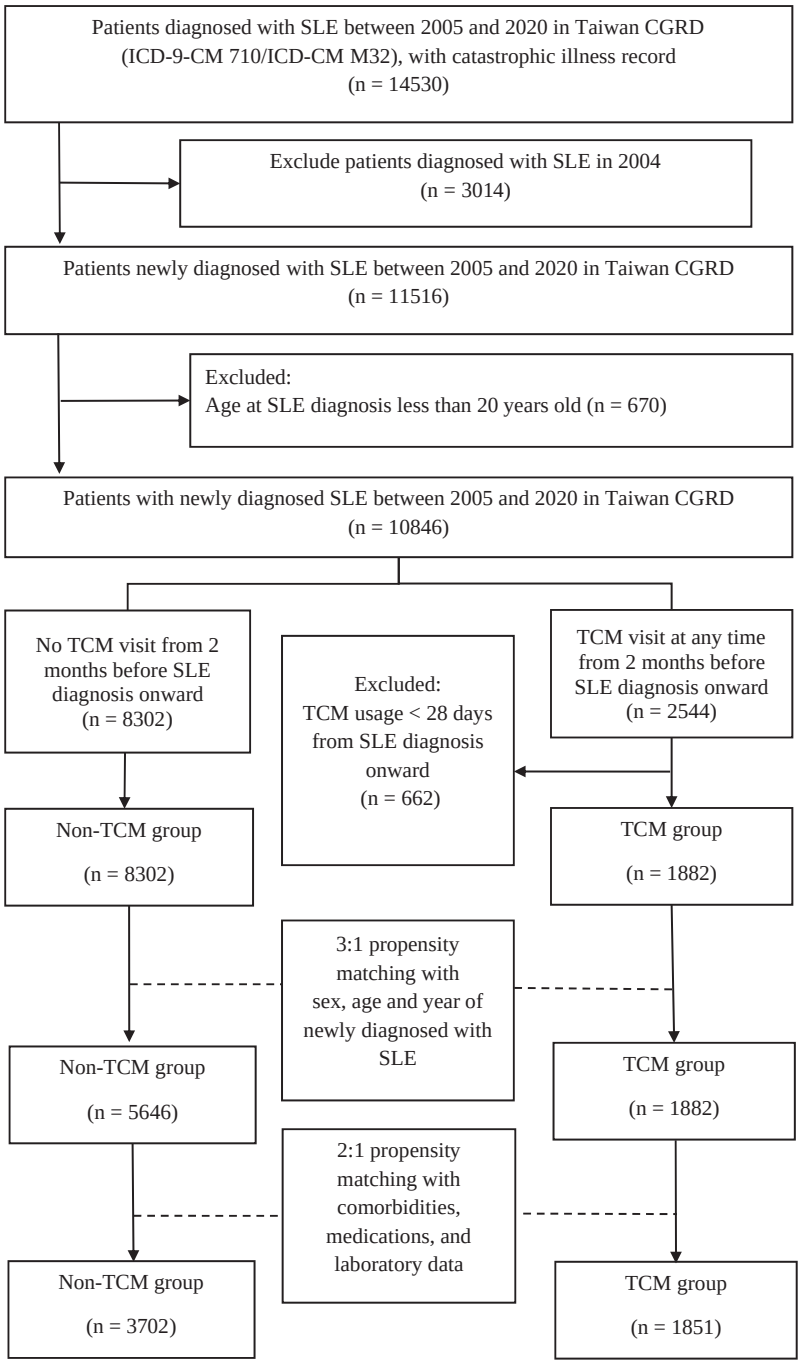

FIGURE A1 | Flow diagram of the study for identifying the effect of Traditional Chinese Medicine use on the glucocorticoid use in patients with systemic lupus erythematosus.

TABLE A1 | Potency of systemic glucocorticoid preparations.

Glucocorticoid	Equivalent doses (mg)
Hydrocortisone	20
Cortisone acetate	25
Prednisone	5
Prednisolone	5
Methylprednisolone	4
Triamcinolone	4
Dexamethasone	0.75
Betamethasone	0.6

TABLE A2 | Demographics, physiological profiles, and laboratory findings of patients with systemic lupus erythematosus using glucocorticoids: A comparison between Traditional Chinese Medicine (TCM) users and non-TCM users.

	Non-TCM group, $n = 3702$	TCM group, $n = 1851$	p[a]	Absolute standardized mean difference[b]
Age (year), median (IQR)	50 (39, 59)	50 (38, 59)	0.89	< 0.01
Male	374 (10.1%)	187 (10.1%)	> 0.99	0
Comorbidities				
Hypertension	544 (14.69%)	265 (14.32%)	0.71	0.01
Diabetes mellitus	183 (4.94%)	83 (4.48%)	0.45	0.02
Chronic kidney disease	268 (7.24%)	132 (7.13%)	0.88	< 0.01
Gout	62 (1.67%)	26 (1.4%)	0.45	0.02
Baseline medications				
NSAIDs	475 (12.83%)	239 (12.91%)	0.93	< 0.01
Glucocorticoids	918 (24.8%)	462 (24.96%)	0.90	< 0.01
DMARDS				
Hydroxychloroquine	1158 (31.28%)	578 (31.23%)	0.97	< 0.01
Azathioprine	206 (5.56%)	106 (5.73%)	0.80	0.01
Methotrexate	201 (5.43%)	107 (5.78%)	0.59	0.02
Leflunomide	17 (0.46%)	8 (0.43%)	0.89	0.01
Mycophenolate mofetil	55 (1.49%)	25 (1.35%)	0.69	0.01
Cyclosporin	45 (1.22%)	19 (1.03%)	0.53	0.02
Cyclophosphamide	15 (0.41%)	8 (0.43%)	0.88	0.01
Rituximab	0 (0%)	0 (0%)	—	—
Biochemical profiles				
Creatinine (mg/dL), median (IQR)	0.72 (0.60, 0.90)	0.70 (0.59, 0.84)	< 0.01	0.05
Proteinuria (mg), median (IQR)	413.93 (119.05, 1576.45)	250.91 (107.56, 1651.52)	< 0.001	0.29
C3 (mg/dL), median (IQR)	92.90 (75.40, 112.00)	92.20 (77.10, 109.00)	0.92	0
C4 (mg/dL), median (IQR)	18.60 (12.90, 23.60)	17.90 (12.20, 23.60)	0.38	0.03
Anti-dsDNA (U/mL), median (IQR)	71.70 (40.50, 209.40)	73.30 (40.50, 222.30)	0.68	0.05
Anti-dsDNA-positive	118 (3.19%)	51 (2.76%)	0.43	0.03

Abbreviations: dsDNA, double-stranded DNA; IQR, interquartile range; NSAIDs, nonsteroidal anti-inflammatory drugs; TCM, Traditional Chinese Medicine.
[a]Chi-squared test for categorical data, Mann–Whitney *U* test for continuous data.
[b]Calculation of effect size: mean for continuous data and percentage for categorical data.

Appendix B

TABLE A3 | Mechanisms related to immunomodulation of the top 10 frequently used single herbs.

No.	Single herbs	Latin name	Mechanisms related to immunomodulation
1	Dan Shen	*Salvia miltiorrhiza* Bge.	Suppress IL-1β and NO; increased monocyte and NK cell activity [35]
2	Bai Shao	*Paeonia lactiflora* Pall.	Modulation of lymphocyte proliferation, Th/Ts balance, and proinflammatory mediators [36]
3	Mai Men Dong	*Ophiopogon japonicus* (L.f.) Ker Gawl.	Reduction of IFN-γ/IL-4 ratio; Activation of macrophages, increase phagocytosis, NO, IL-1β [43]
4	Zhe Bei Mu	*Fritillaria thunbergii* Miq.	Not known
5	Huang Qi	*Astragalus membranaceus* (Fisch.) Bunge.	Activation of macrophages, NK cells, DCs, T/B cells, microglia [37]
6	Sheng Di Huang	*Rehmannia glutinosa* Libosch.	Stimulate T cell proliferation, IL-2/IFN-γ production, DC antigen-presenting ability [44]; active ingredient catalpol suppress Th17 via STAT3/let-7g-5p [45]
7	Huang Qin	*Scutellaria baicalensis* Georgi	Downregulation of TLRs and inhibit proinflammatory immune signaling as MAPK, NF-κB, JAK-STAT [46]; Promotes splenocyte proliferation and increases $CD4^+/CD8^+$ T cell populations during parasitic infection [47]
8	Mu Li	*Crassostrea gigas* (Thunberg)	Not known
9	Xuan Shen	*Scrophularia ningpoensis* Hemsl.	Promotes macrophage pinocytosis, increases NO and TNF-α production, upregulates TLR2 and MAPK/NF-κB signaling [48]
10	Mu Dan Pi	*Paeonia suffruticosa* Andr.	Not known

Abbreviations: CD, cluster of differentiation; DC, dendritic cell; IFN, interferon; IL, interleukin; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; NO, nitric oxide; STAT, signal transducer and activator of transcription; Th, T helper cell; TLR, toll-like receptor; Ts, T suppressor cell.

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LETTER TO THE EDITOR

A Case Report of Systemic Lupus Erythematosus in GATA2 Deficiency: Expanding the Spectrum of Rheumatological Features

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Dear Editor,
GATA2 deficiency is a rare, autosomal dominant primary immunodeficiency (PID) caused by mutations in the *GATA2* transcription factor, which is essential for the development and maintenance of hematopoietic stem cells and immune cells such as monocytes, dendritic cells, B cells, and NK cells. It presents with a broad clinical spectrum, including recurrent granulomatous infections, persistent fevers, lymphadenopathy, cytopenia, and sterile abscesses. Some rheumatological manifestations, such as erythema nodosum and arthritis, have been reported in association with GATA2 deficiency. Here, we present one such rare case of GATA2 deficiency with a coexisting and hitherto unreported rheumatological disease.

A 33-year-old woman was referred to our center with a 3-month history of intermittent low-grade fever and a 1-month history of neck swelling. Her medical history was remarkable for recurrent fevers, granulomatous lesions, and multiple courses of antitubercular therapy (ATT) over the preceding 12 years. Eleven years ago, she was diagnosed with minor organ systemic lupus erythematosus (SLE) based on positive antinuclear antibody (ANA, 1:160, nuclear homogeneous pattern), anti-Ro, and anti-dsDNA antibodies. Since then, she has been maintained on low-dose prednisolone (5 mg daily) and hydroxychloroquine.

Her symptoms began 12 years ago when she presented with fever, cough, and polyarthralgia. A chest radiograph suggested pulmonary tuberculosis (TB), for which she received a 6-month course of ATT, resulting in symptom resolution. Eleven years ago, she developed dyspnoea on exertion. High-resolution computed tomography (HRCT) of the thorax revealed tiny nodules scattered across the lung parenchyma without significant mediastinal lymphadenopathy. Blood tests showed mild leukopenia but were otherwise unremarkable. ANA was positive (nuclear homogenous, 1:160), with positive anti-Ro and anti-dsDNA antibodies (ELISA). She was diagnosed with minor organ SLE (hematological, musculoskeletal, and constitutional) and started on hydroxychloroquine.

Nine years ago, she developed a right gluteal abscess. Histopathology revealed caseating granulomas without acid-fast bacilli (AFB). She was empirically treated for TB with another 6-month ATT course, with symptomatic improvement. Seven years ago, she developed necrotizing abdominal lymphadenopathy and underwent empirical ATT for 9 months, again with radiological resolution. She remained asymptomatic until 1 year ago, when she presented with high-grade fever, chills, and pancytopenia. PET-CT revealed multiple FDG-avid necrotic and non-necrotic lymph nodes. A flare of SLE was suspected, and she was started on high-dose steroids. Lymph node biopsy showed granulomatous inflammation with beaded AFB, raising suspicion for a non-tuberculous mycobacterial (NTM) infection. Empirical NTM therapy (rifampicin, ethambutol, azithromycin, amikacin, and levofloxacin) was initiated; however, the fever persisted despite 1.5 months of therapy, and pancytopenia worsened. Bone marrow examination showed trilineage hematopoiesis without definite hemophagocytosis, and bone marrow cultures (MGIT) were sterile. NTM treatment was discontinued,

and a disease flare was again suspected. She received rituximab (500 mg weekly for 4 weeks), resulting in temporary remission for the next 3 months.

Three months ago, she again developed a low-grade fever and neck swelling. Examination revealed a fluctuant swelling over the left sternoclavicular joint (Figure 1A). No other significant findings were noted. Laboratory investigations showed pancytopenia (Hb 8.4 g/dL, WBC 2490/μL, platelets 70000/μL) with normal complement levels and normal liver and renal parameters. Twenty-four-hour urine protein quantification revealed 460 mg/24 h. Contrast-enhanced CT of the thorax and abdomen showed multiple collections in the neck, subdiaphragmatic region, right psoas muscle, and D12 vertebral body (Figure 1B,C). Aspiration of the neck swelling yielded frank pus that was AFB-negative on microscopy, and cultures were sterile. Bronchoscopy and bronchoalveolar lavage (BAL) were performed; CBNAAT and cultures for TB were negative, but multiplex PCR of the BAL fluid confirmed the presence of NTM, establishing the diagnosis of disseminated NTM infection. However, the mycobacterial culture of the BAL sample was sterile, thus denying NTM species identification. The journey of the patient over the past 12 years through various episodes of infection is shown in Table 1.

The combination of recurrent granulomatous infections and persistent fevers without other features of active SLE prompted consideration of an underlying PID, along with a review of the SLE diagnosis. Considering her history of polyarthralgia and leukopenia with ANA and anti-dsDNA positivity, the case fulfilled the 2019 ACR/EULAR classification criteria for SLE. Immunological evaluation revealed lymphopenia with absent B cells, reduced T and NK cells, and slightly low IgG levels (Figure 1D–F). Whole-exome sequencing identified a heterozygous missense variant in exon 5 of the *GATA2* gene (c.1061C>T), confirming a diagnosis of underlying GATA2 deficiency. Parental testing showed no such mutation, suggesting a de novo mutation in the patient.

She was started on broad-spectrum antimycobacterial therapy (imipenem, amikacin, linezolid, and azithromycin) for disseminated NTM infection. Given the risk of progressive infections, bone marrow failure, and hematologic malignancies, she was planned for allogeneic hematopoietic stem cell transplantation (HSCT), which remains the definitive cure for GATA2 deficiency.

GATA2 deficiency is a PID resulting from germline *GATA2* mutations. The clinical features mainly arise from bone marrow dysfunction. According to the 2019 IUIS phenotypic classification, GATA2 deficiency is categorized as a disorder of phagocytes, with monocytopenia as its hallmark feature [1]. Once marrow failure is established, B and NK cell precursors are significantly depleted, with a relative preponderance of terminally

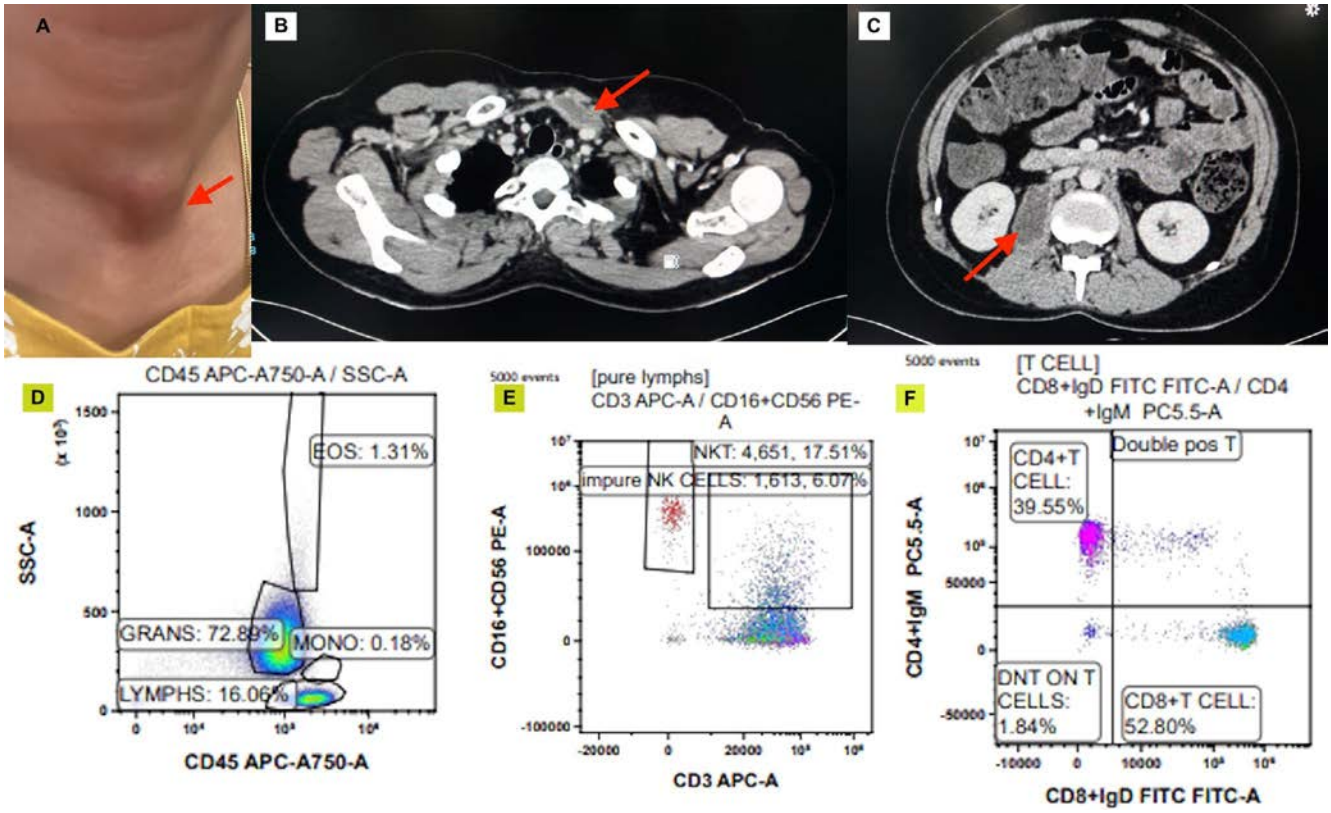

FIGURE 1 | (A) Fluctuant swelling in front of the neck. (B) Axial contrast-enhanced CT scan at the level of the lower neck in the infrathyroid region shows a well-defined peripherally enhancing collection within the strap muscles on the left side. (C) Axial contrast-enhanced CT scan of the abdomen at the level of the upper pole of both kidneys shows a well-defined peripherally enhancing collection in the right psoas muscle. (D) Flow cytometry dot plots showing decreased lymphocytes and monocytes. (E) Flow cytometry dot plots showing decreased NK cells. (F) Flow cytometry dot plots showing decreased CD4 and CD8 T cells.

TABLE 1 | The timeline showing various events, investigations and treatment that happened through the course of patient's illness.

Time	Event	Details
12 years ago	First symptoms and TB diagnosis	Fever, cough, polyarthralgia → CXR showed pulmonary TB → ATT for 6 months → resolution
11 years ago	Nodular lung lesions, SLE diagnosis	HRCT: scattered lung nodules, mild leukopenia, ANA + (nuclear, homogeneous), anti-Ro, anti-dsDNA positive by ELISA → SLE diagnosed → HCQ started
9 years ago	Gluteal abscess	Right gluteal abscess → biopsy: caseating granulomas, AFB negative → empirical ATT → improvement
7 years ago	Necrotising abdominal lymphadenopathy	Necrotic nodes → empirical ATT for 9 months → resolution
1 year ago	High-grade fever, pancytopenia	FDG-PET: multiple necrotic & non-necrotic lymph nodes → Lymph node biopsy: granulomatous, beaded AFB → empirical NTM therapy → persistent fever
1 year ago	Bone marrow evaluation	Bone Marrow: trilineage hematopoiesis, no haemophagocytosis → MGIT sterile → NTM therapy stopped
1 year ago	Rituximab therapy	Suspected SLE flare → Rituximab 500 mg weekly x4 → Temporary remission
3 months ago	New neck swelling and abscesses	Fluctuant left sternoclavicular swelling → Multiple sterile abscesses (neck, psoas, vertebra) → BAL multiplex PCR positive for NTM
Now	Immunodeficiency workup and genetic diagnosis	Immunological workup: absent B cells, reduced T & NK cells, low IgG → Whole Exome Sequencing: heterozygous *GATA2* mutation → Diagnosis: GATA2 deficiency

differentiated cell populations [2]. There is typically a reduction in naïve B cells, with an increased proportion of memory B cells. Although the T-cell population is relatively preserved, there is inversion of the CD4:CD8 ratio, a reduction in naïve cells, and an expansion of terminally differentiated effector cells [3]. It usually presents in the late teens or early twenties. Monocytopenia leads to increased susceptibility to NTM infections, often disseminated, due to disruption of the IFNγ–IL12 axis [4, 5]. The lack of dendritic cells further raises the risk of mycobacterial infections. Patients are also at risk for invasive fungal and viral infections such as HPV, VZV, and EBV [3]. Besides immunodeficiency, there is a high lifetime risk of developing myeloid neoplasms, with around 80% of patients presenting by the age of 40 years [6]. In our patient, cytopenia with absent B cells and reduced T and NK cells was consistent with marrow dysfunction, likely attributable to both underlying SLE and prior rituximab therapy, but also to the GATA2 deficiency. The patient experienced recurrent granulomatous infections, likely representing disseminated and recurrent TB infections, although these were not microbiologically confirmed until the final episode. A possibility of false-positive autoantibodies due to granulomatous infection was also considered. However, while false-positive ANA is well documented in TB, it typically shows a nuclear speckled pattern [7]. In our case, the ANA was nuclear homogeneous. Furthermore, among various autoantibodies, anti-cardiolipin IgG and anti-Scl-70 are the most commonly reported false positives in TB patients. False-positive anti-dsDNA is uncommon in TB [8]. Additionally, population-based studies suggest that NTM infections rarely cause false-positive autoantibody results [9]. Thus, the autoantibody positivity in our patient was, in all likelihood, genuine, supporting the diagnosis of SLE. Although rheumatological manifestations such as psoriasis, arthritis, and erythema nodosum have been described in GATA2 deficiency, an association with connective tissue diseases like SLE has not been reported until now [10].

Treatment for GATA2 deficiency includes supportive care, management of infections, and often prophylaxis for NTM infections. Allogeneic HSCT remains the definitive treatment. Although there is no consensus on the optimal timing of HSCT in the absence of hematological malignancy, our patient is planned for HSCT once the infection is controlled.

This case describes a rare and previously unreported rheumatological manifestation of GATA2 deficiency. It illustrates the diagnostic challenges in distinguishing PID from chronic infections and autoimmune conditions. It highlights the importance of considering an underlying immunodeficiency in patients with recurrent granulomatous infections, sterile abscesses, and cytopenia.

Author Contributions

J.S., M.K., and D.S. planned this study. J.S., M.K., D.S., J.M., and V.R.P. took part in management; J.S. and M.K. wrote the initial manuscript; D.S., J.M., and V.R.P. critically reviewed it. All checked and approved the final manuscript.

Consent

Written informed consent was obtained from the patient.

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

Cervical Facet Joint Ankylosis in Patients With Axial Spondyloarthritis Serves as a Surrogate Radiographic Indicator of Propensity for Bone Formation

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ABSTRACT

Objective: To investigate the involvement of facet joints and its clinical implications during the treatment course in patients with axial spondyloarthritis (axSpA).

Methods: This was a retrospective observational study. Radiographs of the cervical and lumbar spine were evaluated for 799 patients with axSpA using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) and the de Vlam methods. Differences in radiographic progression indices and initiation of TNF inhibitors between patients with and without facet joint fusion were investigated using propensity score matching.

Results: Facet joint involvement at the cervical spine was observed in a subset of patients with axSpA (20.6%). Patients with facet joint fusion ($n = 71$) had higher mSASSS and a higher number of syndesmophytes at both cervical and lumbar levels compared with the matched patients without facet joint fusion. Dagger signs and trolley-track signs were more commonly observed ($p < 0.001$), and trolley-track signs developed more frequently in patients with facet joint fusion during follow-up (Δ9/49 vs. Δ1/68, $p = 0.0038$). Disease activity score and CRP level at baseline were also significantly higher in patients with facet joint fusion. The risk of initiation of TNF inhibitor was significantly higher in patients with facet joint fusion (hazard ratio [95% CI] 1.909 [1.187, 3.07], $p = 0.007$).

Conclusions: Facet joint involvement in the cervical spine was identified in a subset of patients with axSpA and was closely associated with advanced radiographic structural damage and the initiation of TNF inhibitors.

1 | Introduction

Axial spondyloarthritis (axSpA), including ankylosing spondylitis (AS), is a chronic progressive disease characterized by inflammation of the entheses, leading to new bone formation and ankylosis of the joints, primarily in the axial skeleton [1, 2]. Diagnosis of axSpA is usually delayed by 5–7years due to practical reasons [3, 4] and a considerable subset of patients already had radiographic damage in their sacroiliac joints and/or spines [5, 6]. Radiographic progression of the spine after 2years was observed in approximately 20%–500% of patients with AS [7–9] and baseline structural change, particularly the presence of syndesmophytes, is a critical predictor for ongoing spinal radiographic progression in early axSpA [7, 10]. Spinal structural change is usually irreversible, and inevitablyhas an negative effect on physical activity and work productivity [11]. Tumor necrosis factor (TNF) inhibitors, one of the core therapeutic modalities, failed to show a significant protective effect on radiographic progression, and only long-term

treatment of TNF inhibitors might confer an impeding effect on spinal radiographic progression [12, 13].

Spinal radiographic change is assessed with the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), which evaluates structural changes in the anterior corners of the cervical and lumbar vertebral bodies. However, mSASSS has a limitation of not involving facet joints, a commonly affected joint [14], or the thoracic spine, the most frequently affected region [15]. Facet joints of the spine, also known as zygapophyseal joints, are diarthrodial synovial joints playing a biomechanical role in facilitating the articulation of the vertebrae [16]. Impairment of the facet joint can critically impinge upon the functional activity of the spine [17]. Facet joints are enclosed by joint capsules, which are extended and closely coupled with interspinous ligaments. The intervertebral structure of the cervical spine is much more clearly visualized on lateral radiographs, unlike thoracic and lumbar spines. de Vlam et al. proposed a measurement method for facet joint involvement [14], which has been used to evaluate facet joint involvement of the cervical spine in axSpA [18–20]. Evaluation of the facet joint using the de Vlam method has enabled the identification of more patients with AS who have spinal radiographic progression beyond cases that can be captured with conventional mSASSS or bridging syndesmophytes [18, 19]. A study found the that presence the facet joint fusion was associated with male sex, age, higher disease activity, and worse functionality [20].

In this study, we further investigated the clinical implication of cervical facet joint involvement in an observational cohort of patients with axSpA. We examined the association of cervical facet joint involvement with other ossification findings of the lumbar intervertebral structures, such as dagger sign and trolley-track sign, and initiation of TNF inhibitors through propensity score matching (PSM).

2 | Patients and Methods

2.1 | Patients

A total of 799 patients with axSpA who fulfilled the Assessment of Spondyloarthritis International Society (ASAS) classification criteria for axSpA [21] and had received care at St. Vincent's Hospital, Catholic University of Korea (Suwon, Republic of Korea) between 2005 and 2023 were identified. Clinical, laboratory data, and radiographic images were retrieved from medical records. At baseline, sex, age at diagnosis, time since diagnosis, HLA–B27 status, smoking status, and history of extra-articular manifestations (uveitis, psoriasis, inflammatory bowel disease, peripheral arthritis, and enthesitis) were recorded. Disease activity was assessed according to the ankylosing spondylitis disease activity score (ASDAS) using C-reactive protein (CRP) level [22]. The study was conducted in accordance with the Helsinki Declaration and was approved by the Institutional Review Board of St. Vincent's Hospital, The Catholic University of Korea (No. VC24RISI0291).

2.2 | Radiographs and Scoring

Radiographs of the sacroiliac joints and the cervical and lumbar spine were obtained at baseline and during follow-up. The anterior column of the cervical and lumbar spine was assessed using mSASSS [23] and cervical facet joint involvement was evaluated according to the method described by de Vlam [14, 20]. Figure 1 presents radiographic examples of the de Vlam method, the Dagger sign, and the Trolley-Track sign. All available radiographs for each patient were independently scored simultaneously by two experienced readers who were blinded to all other data except for radiograph chronology. The interobserver reliability was assessed by calculating the interclass correlation coefficient, which was 0.945 (95% confidence interval [CI] 0.939–0.952). If the difference between the scores assigned by the two readers exceeded five units (defined as a major disagreement), the same assessors re-evaluated these radiographs. In the event of continued major disagreement after reevaluation, an independent adjudicator assigned a final score.

2.3 | Propensity Score Matching

Because we expected systematic differences in baseline characteristics of subjects with or without facet joint involvement, we performed propensity score matching (PSM) to adjust for potential imbalances [24, 25]. We used logistic regression to obtain the propensity score [26], and the variables included in PS estimation were sex, age at diagnosis, and disease duration. Patients without facet joint involvement were matched based on PSs using the nearest-neighbor matching algorithm without replacement and with a 1:1 ratio within 0.05 of the estimated propensity score [26].

2.4 | Statistical Analyses

For continuously distributed data, the results are presented as means with standard deviations. Between-group comparisons were conducted using Student's *t*-test. Categorical or dichotomous variables were presented as frequencies and percentages and were compared using the chi-squared test or Fisher's exact test. Correlation analysis between two continuous variables was conducted using Pearson's method. The initiation of TNF inhibitors was assessed through a Kaplan–Meier analysis and compared using log-rank tests. A hazard ratio (HR) was used to compare the rate of an event occurring in a treatment group to the rate of the same event occurring in a control group. A two-sided p-value of <0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.4.2, R Project for Statistical Computing, www.r-project.org).

3 | Results

3.1 | Baseline Characteristics of the Study Population

In total, 799 patients with axSpA were enrolled: 364 patients (45.6%) had cervical spine involvement (cervical spine mSASSS ≥ 1), and 228 patients (28.5%) had more than one syndesmophyte at baseline. Facet joint involvement was scored by the de Vlam method [14]. The presence of facet joint fusion was defined as significant involvement according to a previous study [20],

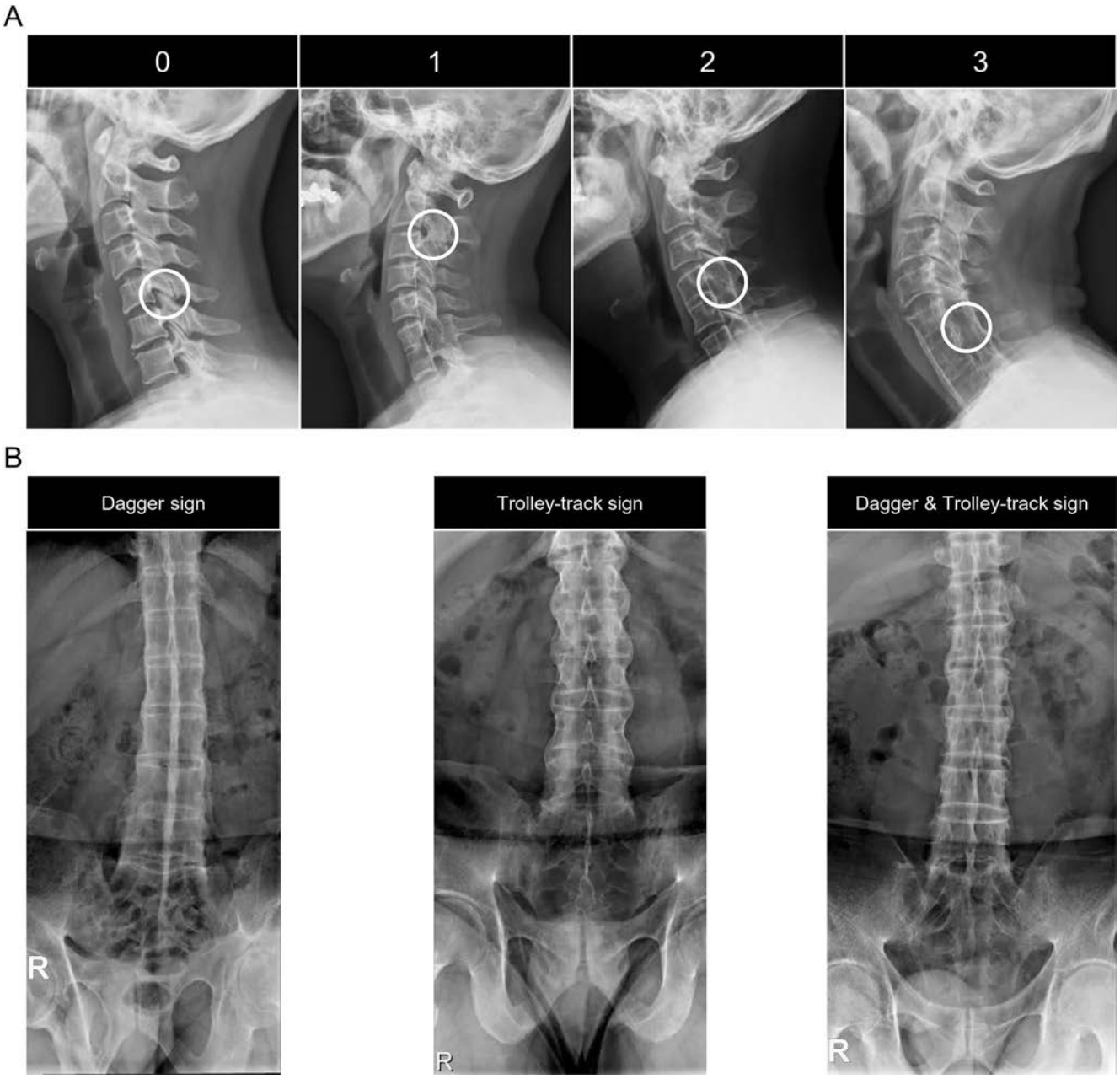

FIGURE 1 | Examples of radiographic findings. (A) Scoring examples according to De Vlam method, 0: Normal; 1: Decreased joint space; 2: Partial obliteration of the space; 3: Complete obliteration of the space or ankylosis. (B) Dagger sign and Trolley-track sign.

and patients who were identified to have facet joint fusion at their cervical spine at the last follow-up evaluation were categorized into the group with facet joint fusion. In total, 165 patients (20.6%) were identified to have facet joint involvement (facet joint score ≥ 1), and 71 patients (8.9%) had facet joint fusion at their cervical spine (Figure 2). Facet joint fusion without damage to the anterior portion of the cervical spine (cervical spine mSASSS = 0) was observed in 11 of the facet joint fusion patients (15.5%). Compared with patients without facet joint fusion ($n = 728$), patients with facet joint fusion ($n = 71$) were older at the time of diagnosis and had longer disease duration (39 [30, 50] vs. 34 [25, 45] years, $p = 0.003$, and 14 [7, 22] vs. 8 [4, 12.5] years, $p < 0.001$, respectively) (Table 1). The proportion of male patients was marginally higher in the facet joint fusion group (84.5 vs. 73.6%, $p = 0.062$). ASDAS was significantly higher in patients with facet joint fusion (3.4[2.7, 4.2] vs. 2.8[2.3, 3.5], $p = 0.003$). Mean CRP levels were more than twice as high in the facet joint fusion group than in the group without facet joint fusion group (1.5[0.4, 3.3] vs. 0.6[0.2, 1.7] mg/dL, $p < 0.001$).

Age at diagnosis, disease duration, and male sex are known to be significant but uncontrollable clinical factors for radiographic progression. In an effort to mitigate bias, we performed a 1:1 PSM for age, sex, and disease duration. Consequently, the sex ratio, age at diagnosis, disease duration, and HLA-B27 positivity were comparable between groups with facet joint fusion and without facet joint fusion (Table 1). However, ASDAS and inflammatory indices including erythrocyte sedimentation rate (ESR) and CRP levels at baseline were still significantly higher in patients with facet joint fusion (all $p < 0.05$).

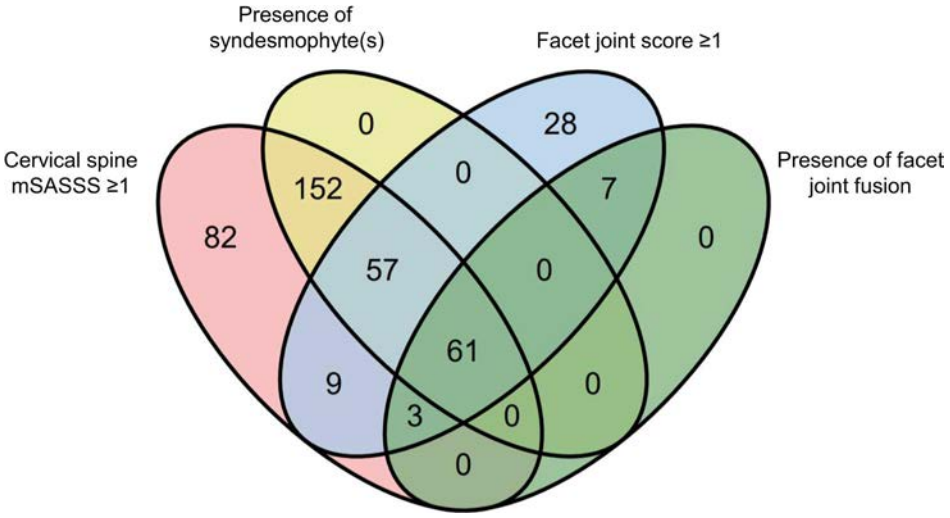

FIGURE 2 | Venn diagram for cervical radiographic damages. mSASSS ≥ 1, presence of syndesmophytes, facet joint score ≥ 1, or presence of facet joint fusion. mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score.

3.2 | Radiographic Structural Damages in Association With Facet Joint Involvement

Radiographic damage indices of the cervical spine at both baseline and last follow-up, including mSASSS, presence of syndesmophytes, number of syndesmophytes, presence of bridging syndesmophytes, and number of bridging syndesmophytes, were significantly higher in patients with facet joint fusion compared with patients without facet joint fusion (All $p < 0.01$) (Table 1). Radiographic damage indices of the lumbar spine at both baseline and last follow-up, except for the presence of syndesmophytes, were also significantly higher in patients with facet joint fusion (all $p < 0.05$) (Table 1).

Overall, facet joint score was significantly correlated with cervical mSASSS ($\gamma = 0.7196$, $p < 2.2 \times 10^{-16}$). A strong correlation between cervical mSASSS and facet joint score ($\gamma = 0.7213$, $p = 1.282 \times 10^{-12}$) was observed in the facet joint fusion group, while the correlation was moderate in the group without facet joint fusion ($\gamma = 0.4145$, $p = 0.0003$) (Figure 3).

Advanced ossification findings of lumbar intervertebral structures, for example, dagger sign and trolley-track sign, were observed in 106 (13.27%) and 60 (7.51%) patients with axSpA, respectively (Figure 4A). The frequency of dagger and trolley-track signs wase significantly higher in patients with facet joint fusion (all $p < 0.01$) (Table 1). Specifically, the frequency in the development of trolley-track sign at follow-up in patients with facet joint fusion was significantly higher than in patients without facet joint fusion (Δ9/49 vs. Δ1/68, $p = 0.0038$) (Figure 4B).

3.3 | Risk of Initiation of TNF Inhibitors

TNF inhibitors are usually recommended for patients with persistently high disease activity despite conventional treatment including non-steroidal anti-inflammatory drug (NSAID) therapy according to the endorsed treatment recommendation [27, 28]. The lack of response to conventional treatment and the initiation of TNF inhibitor is a surrogate index of high disease activity. The frequency of TNF inhibitor treatment was higher in patients with facet joint fusion compared with patients without facet joint fusion (62.0% ($n = 44$) vs. 39.4% ($n = 28$), $p = 0.012$). The cumulative incidence of TNF inhibitor initiation was compared between patients with and without facet joint fusion, and the risk of TNF inhibitor initiation was significantly higher in patients with facet joint fusion (HR [95% CI] 1.909 [1.187, 3.07], $p = 0.007$) (Figure 5).

4 | Discussion

In this study, we identified facet joint involvement at the cervical spine in a subset of patients with axSpA. Patients with facet joint fusion had more advanced radiographic structural damage, including new bone formation, on standard radiographic measurement in their spine compared with matched patients without facet joint fusion. Intervertebral ossification findings such as dagger sign and trolley-track sign were more commonly observed and tended to develop more frequently in patients with facet joint fusion during follow-up. In particular, the risk of initiation of TNF inhibitor was significantly higher in patients with facet joint fusion.

Facet joint inflammation and ankylosis are key pathologies in terms of development and spinal functionality in axSpA [17, 20, 29, 30] but have been underestimated possibly due to unclear visualization on conventional radiographs. Strong correlations between structural damage of the anterior corners and facet joint involvement of the cervical vertebrae have been reported. However, 8.1% of patients with cervical spine involvement (cervical mSASSS ≥ 1) (35/435) did not have facet joint damage, and 2.8% of the patients without cervical syndesmophytes (16/571) had facet joint ankylosis. A previous study also reported that facet joint ankylosis was present in 8% of the cervical levels without bridging syndesmophytes and in 21% of the lumbar levels without bridging syndesmophytes [14]. In

TABLE 1 | Baseline characteristics of axSpA patients with and without facet joint fusion.

Variable	Patients with facet joint fusion ($n=71$)	Patients without facet joint fusion			
		Post-PS matching ($n=71$)	p[b]	Pre-PS matching ($n=728$)	p[c]
Male, *n* (%)	60 (84.5)	64 (90.1)	0.449	536 (73.6)	0.062
Age at diagnosis, years	39 [30, 50]	43 [33, 54]	0.199	34 [25, 45]	0.003
Disease duration, years	14 [7, 22]	13 [6, 20]	0.347	8 [4, 12.5]	<0.001
BMI, kg/m^2, *n* (%)					
Underweight (<18.5)	3 (4.2)	2 (2.9)	0.800	32 (4.4)	0.960
Normal, 18.5–25	40 (56.3)	36 (51.4)		427 (59.1)	
Overweight, 25–30	22 (31.0)	27 (38.6)		202 (27.9)	
Obese, <30	6 (8.5)	5 (7.1)		62 (8.6)	
HLA-B27, *n* (%)	63 (88.8)	62 (87.3)	1.000	620 (85.2)	0.524
Smoking					
Non-smoker, *n* (%)	30 (42.3)	33 (46.5)	0.735	384 (52.7)	0.117
Smoker, *n* (%)	41 (57.7)	38 (53.5)		344 (47.3)	
Uveitis, *n* (%)	16 (22.5)	24 (33.8)	0.192	190 (26.1)	0.608
Peripheral arthritis, *n* (%)	22 (31.0)	16 (22.5)	0.343	169 (23.3)	0.187
Enthesitis, *n* (%)	1 (1.4)	11 (15.5)	0.007	74 (10.2)	0.028
Psoriasis, *n* (%)	1 (1.4)	7 (9.9)	0.069	40 (5.5)	0.227
IBD, *n* (%)	1 (1.4)	1 (1.4)	1.000	19 (2.6)	0.825
ESR, mm/h	36 [21, 54]	24 [14, 44]	0.016	27 [13, 49]	0.035
CRP, mg/dL	1.5 [0.4, 3.3]	0.6 [0.2, 1.7]	0.003	0.6 [0.2, 1.9]	0.001
ASDAS-CRP	3.4 [2.7, 4.2]	2.8 [2.3, 3.5]	0.003	2.9 [2.3, 3.6]	<0.001
Use of TNF inhibitor, *n* (%)[a]	44 (62.0)	28 (39.4)	0.012	364 (50.0)	0.072
Baseline					
Cervical spine					
mSASSS	16 [5, 27]	3 [0, 8]	<0.001	0 [0, 2]	<0.001
Facet joint score	7 [3, 11]	0 [0, 0]	<0.001	0 [0, 0]	<0.001
Presence of syndesmophyte(s)	55 (77.5)	38 (53.5)	0.005	173 (23.8)	<0.001
No. of syndesmophyte	4 [1, 10]	1 [0, 3]	<0.001	0 [0, 0]	<0.001
Presence of bridging syndesmophyte(s)	42 (59.2)	7 (9.9)	<0.001	38 (5.2)	<0.001
No. of bridging syndesmophyte(s)	2 [0, 6]	0 [0, 0]	<0.001	0 [0, 0]	<0.001
Lumbar spine					
mSASSS	14 [2, 28]	8 [0, 13]	<0.001	0 [0, 4]	<0.001
Presence of syndesmophyte(s)	46 (46.5)	45 (63.4)	1.000	191 (26.3)	<0.001

(Continues)

TABLE 1 | (Continued)

Variable	Patients with facet joint fusion ($n=71$)	Patients without facet joint fusion: Post-PS matching ($n=71$)	p[b]	Patients without facet joint fusion: Pre-PS matching ($n=728$)	p[c]
No. of syndesmophyte	4 [0, 8]	2 [0, 5]	0.103	0 [0, 1]	<0.001
Presence of bridging syndesmophyte(s)	38 (53.5)	22 (31.0)	0.011	94 (12.9)	<0.001
No. of bridging syndesmophyte(s)	2 [0, 8]	0 [0, 2]	0.003	0 [0, 0]	<0.001
Dagger sign	29 (40.8)	11 (15.5)	0.002	46 (6.3)	<0.001
Trolley-track sign	22 (31.0)	3 (4.2)	<0.001	19 (2.0)	<0.001
Last follow-up					
Time interval from baseline	88 [30, 130]	89 [27, 151]	0.887	62 [3, 101]	0.002
Cervical spine					
mSASSS	21 [8, 36]	5 [1, 11]	<0.001	0 [0, 3]	<0.001
Facet joint score	9 [5, 12]	0 [0, 1]	<0.001	0 [0, 0]	<0.001
Presence of syndesmophyte(s)	61 (85.9)	45 (63.4)	0.004	209 (28.7)	<0.001
No. of syndesmophyte	7 [3, 12]	1 [0, 4]	<0.001	0 [0, 1]	<0.001
Presence of bridging syndesmophyte(s)	53 (74.6)	13 (18.3)	<0.001	59 (8.1)	<0.001
No. of bridging syndesmophyte(s)	4 [1, 12]	0 [0, 0]	<0.001	0 [0, 0]	<0.001
Lumbar spine					
mSASSS	18 [6, 33]	10 [2, 20]	0.003	0 [0, 7]	<0.001
Presence of syndesmophyte(s)	57 (80.3)	53 (74.6)	0.547	253 (34.8)	<0.001
No. of syndesmophyte	2 [0, 10]	4 [0, 7]	0.023	0 [0, 2]	<0.001
Presence of bridging syndesmophyte(s)	45 (63.4)	31 (43.7)	0.029	132 (18.2)	<0.001
No. of bridging syndesmophyte(s)	2 [0, 10]	0 [0, 4]	0.003	0 [0, 0]	<0.001
Dagger sign	38 (53.5)	16 (22.5)	<0.001	68 (9.3)	<0.001
Trolley-track sign	31 (43.7)	4 (5.6)	<0.001	29 (4.0)	<0.001

Note: Values are presented as median [interquartile ranges] or number (percentage).
Abbreviations: ASDAS, Ankylosing Spondylitis Disease Activity Score; BMI, body mass index; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; PS, propensity score; TNF, tumor necrosis factor; ZJ, zygapophyseal joint.
[a]Counted if ever used. TNF inhibitors include etanercept, adalimumab, infliximab, and golimumab.
[b]Comparison between patients with facet joint fusion and matched patients without ZJ fusion.
[c]Comparison between patients with facet joint fusion and unmatched patients without ZJ fusion.

other words, facet joint damage can develop independent of pathologic changes in the anterior portion of the vertebrae in axSpA. A typical morphology was presented in a case report, in which total ankylosis of the facet joint of the lumbar spine without definite bridging syndesmophytes on 3-dimensional computed tomography was identified as trolley-track sign on conventional radiograph [31].

Dagger sign is a single central radiodense line on frontal radiographs running along the spinous processes, presenting as ossification of the supraspinous and interspinous ligaments secondary to enthesitis. Trolley-track sign refers to ossification of apophyseal joint capsules appearing as three vertical radiodense streaks in the radiograph [32]. In our results, dagger and trolley-track signs were observed more frequently in patients with

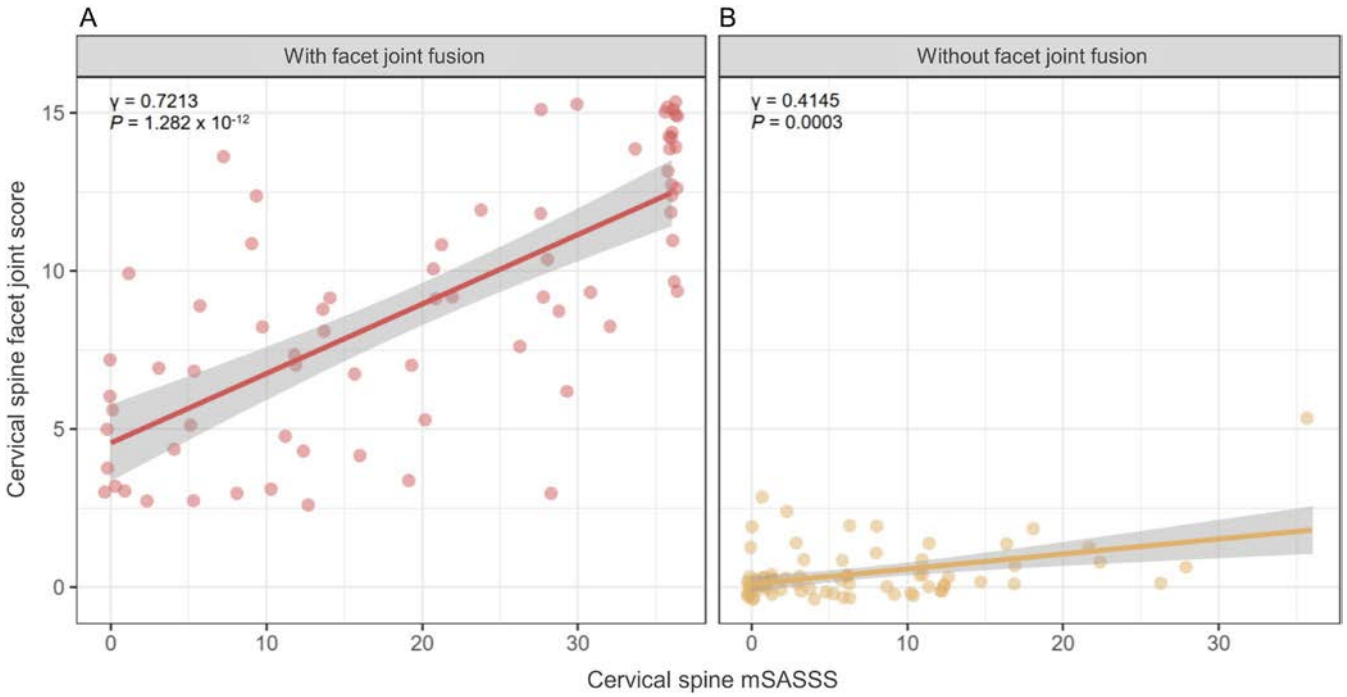

FIGURE 3 | Correlation between cervical spine mSASSS and facet joint score. (A) In a group with facet joint fusion. (B) In a group without facet joint fusion.

cervical facet joint fusion. The posterior column of the vertebrae includes the ligamentum flavum, interspinous ligament, facet capsule, and supraspinous ligament. This implies that involvement of ossification pathology could be dominant in a specific vertebral column (e.g., anterior, middle, or posterior) in a subset of patients with axSpA. In the biomechanical theory of the spine, spinal instability is explained by the three-column model [33, 34].

The facet joints are synovial joints linking the articular processes of two adjacent vertebrae, and each pair of facet joints biomechanically guides and limits movement of the spinal motion segment [16]. Damage and impairment of facet joints necessarily lead to back pain and a limited range of motion of the spine. Recent studies confirmed the close association between facet joint damage and functional impairment of the spine [17, 20]. Patients with facet joint fusion showed worse mobility and more radiographic damage [20], and facet joint ankylosis was significantly associated with a history of uveitis, ASDAS, sacroiliitis, and syndesmophyte score [35]. It is worth noting that facet joint ankylosis was most common in the thoracic spine; on examination of whole spine computed tomography (CT), the frequency was more than two-fold greater than that of facet joint ankylosis of the cervical and lumbar spines (42%–45% vs. 10%–16% and 15%–24%) [35]. Another study suggested that adding the thoracic spine to the mSASSS might improve the sensitivity of detecting changes in scoring radiographic progression [15], but another was skeptical of the contribution of thoracic vertebral corners to the mSASSS [36]. Evaluation of vertebral body damage at the thoracic spine might not play a clear contributory role in the time-consuming overall evaluation of radiographic spinal damage, but evaluation of facet joint ankylosis, especially at the thoracic spine, could help identify change in new bone formation. TNF inhibitors over the course of 2 years or 4 years failed to halt radiographic progression in patients with axSpA [12], and an IL-17 inhibitor, secukinumab, did not demonstrate superiority in suppressing radiographic progression over adalimumab in a recent head-to-head randomized controlled trial, although the change from baseline in mSASSS in the secukinumab-treated group was numerically lower than in the adalimumab-treated group [37]. The mSASSS does not have sufficient scope for assessing new bone formation. Increases in mineral density or volumetric change in the preformed syndesmophtye(s) are not counted as progression, and syndesmophyte formation at posterior or lateral portions of the vertebral corners, which are clearly identifiable on CT scan [38, 39], is not subject to assessment. Facet joint ankylosis, especially at the thoracic spine, was also excluded despite a substantial component of bone formation. If these limitations are considered for assessing the degree of bone formation, a better picture of the inhibitory effects of treatment drugs on bone formation could be obtained.

Patients with cervical facet joint fusion not only had worse radiographic progression but also had higher CRP levels, higher disease activity, and a high risk of early initiation of TNF inhibitors. These patients present complex cases with multiple poor prognostic factors for radiographic outcomes [7, 13]. Therefore, they need close observation and effective treatment against abnormal osteoproliferation and ossification. Involvement of the posterior spinal column, including facet joint damage, might take the lead in the inflammatory response in axSpA [30] because the posterior spinal column covers the extended joint and enthesis structures compared with the anterior column without synovial joint structures [29]. It would be intriguing to investigate the clinical course of patients with axSpA in terms of radiographic progression and response to biologic or targeted synthetic agents according to the involvement of different regions of the spinal columns.

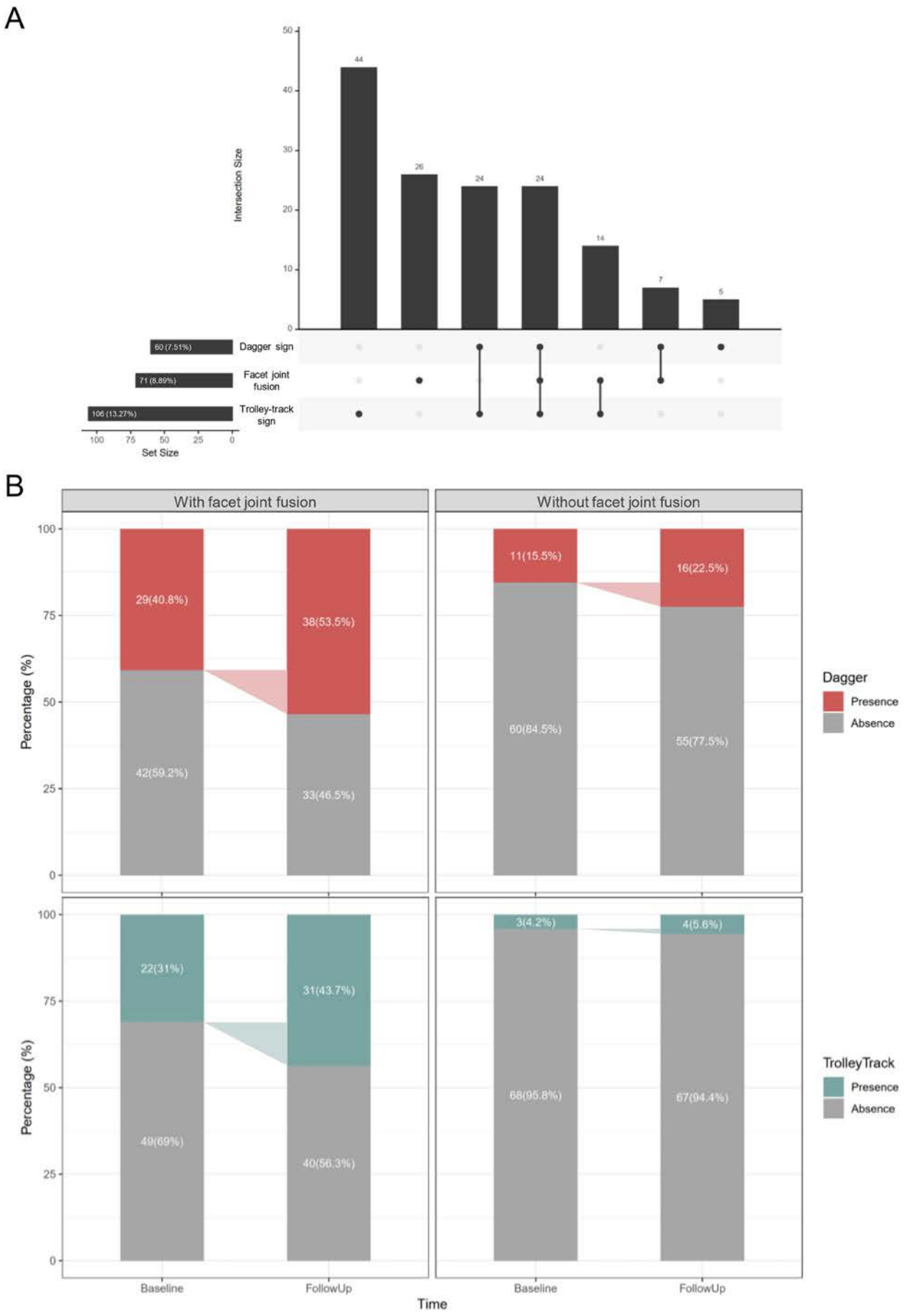

FIGURE 4 | Legend on next page.

FIGURE 4 | Frequency of ossification pathology in posterior spinal column. (A) Upset plot for facet joint fusion, dagger sign, and trolley-track sign. (B) Change in frequency of dagger and trolley-track sign between baseline and follow-up by presence of facet joint fusion.

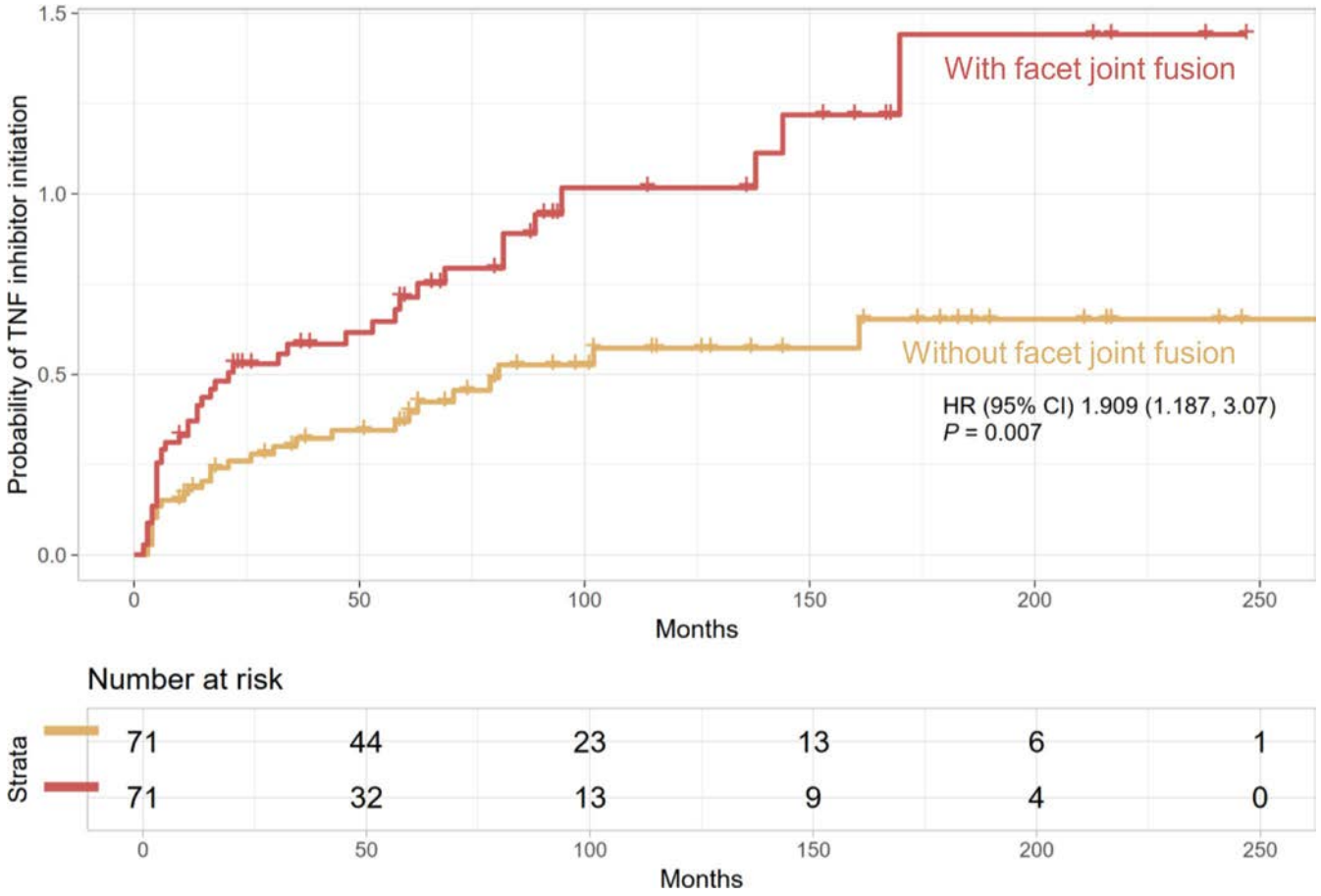

FIGURE 5 | Cumulative incidence of initiation of TNF inhibitor based on presence of cervical facet joint fusion. Red and yellow lines indicate patients with and without cervical facet joint fusion. Initiation of TNF inhibitor with hazard ratios (HRs) and corresponding 95% CIs were assessed via Kaplan–Meier analysis and were compared using log-rank tests.

This study has a few limitations. First, the data were retrospectively collected. Retrospective data collection is necessarily susceptible to misclassification and information bias. Specifically, the frequency of the recorded enthesitis was relatively low, possibly due to a lack of full history taking and physical examination regarding enthesis. Second, conventional radiograph is less powerful to discern pathologic structural deformities and discriminate them from degenerative changes than CT scan. However, the influence of confounding factors would be evenly distributed across the study subjects, and the aging factor was adjusted partially with the PSM method. Third, a key limitation of this study was the incomplete retrieval of the Bath Ankylosing Spondylitis Metrology Index (BASMI) and the Bath Ankylosing Spondylitis Functional Index (BASFI) data, as these measures were not systematically recorded in the medical records. However, previous studies indicated that physical function impairment (BASFI) in patients with axSpA significantly correlates with spinal structural damage (mSASSS), disease activity (BASDAI), and spinal mobility (BASMI) [11, 40].

In conclusion, a subset of patients with axSpA have facet joint involvement, which was closely associated with more progressed structural damage and functional impairment of their spine. It is probable that facet joint lesions play an important role in the inflammatory response and osteoproliferation in the disease progression of axSpA, given that it was closely associated with high disease activity and the initiation of TNF inhibitor. This study re-evaluates the clinical significance of facet joint involvement in patients with axSpA. Facet joint pathology in axSpA is potentially underrecognized, primarily due to suboptimal visualization on conventional plain radiography. Therefore, developing and validating more accurate imaging modalities for facet joint assessment—and integrating them into standard structural damage evaluations—is important.

Author Contributions

Ki-Jo Kim conceived and designed the study. Seung Min Jung, Yun-Jung Park, Kyung-Su Park, and Ki-Jo Kim carried out data collection. Ki-Jo Kim performed statistical analysis. Ki-Jo Kim and In-Woon Baek wrote the manuscript. Ki-Jo Kim supervised all aspects of the project. All authors participated in discussion and interpreting the results. All authors read and approved the final manuscript.

Ethics Statement

This study was approved by the Institutional Review Board of St. Vincent's Hospital, The Catholic University of Korea (No. VC24RISI0291). The requirement for informed consent was waived due to the retrospective design of the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data underlying this article cannot be shared publicly for the protection of the privacy of individuals that participated in the study.

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LETTER TO THE EDITOR

Case Report: Severe Legionnaires' Disease Complicated by Rhabdomyolysis and Acute Kidney Injury in a Patient With Rheumatoid Arthritis

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To the Editor,

The diagnosis of severe infections in patients with rheumatic diseases is challenging, as overlapping symptoms and confounding serologies can obscure the etiology [1]. We report a case of Legionnaires' disease complicated by rhabdomyolysis and acute kidney injury (AKI) in a patient with seropositive rheumatoid arthritis (RA) that highlights this dilemma.

A 53-year-old male with a history of hypertension and seropositive RA presented to the emergency department with shortness of breath and altered mental status. His RA, with a clinical history of symmetric polyarthritis since his mid-30s, was characterized by high-titer rheumatoid factor, anti-CCP antibodies, and hand X-rays confirming erosive changes. His disease was poorly controlled on methotrexate 15 mg weekly and prednisone 10 mg daily. No other medications associated with rhabdomyolysis, such as statins, were identified. He had recently completed outpatient Augmentin for presumed pneumonia without improvement.

On admission (Hospital Day [HD] 1), he was febrile to 40.5°C with hypoxemia, AKI (creatinine 1.71 mg/dL; baseline 0.62), and hyponatremia. He was started on empiric intravenous vancomycin and piperacillin-tazobactam for presumed sepsis. He was initially managed on the medical floor, but deteriorated rapidly on HD 4 and was transferred to the ICU. He progressed to acute respiratory distress syndrome requiring intubation. Bronchoscopy revealed diffuse alveolar hemorrhage (DAH). He developed severe rhabdomyolysis (peak creatine kinase 16 000 U/L) and worsening AKI (creatinine peak 9.06 mg/dL) necessitating continuous renal replacement therapy.

Given this constellation of findings and positive ANA, anti-dsDNA, and anti-SSA antibodies on admission, a catastrophic autoimmune flare, such as an overlap syndrome with developing SLE and myositis, was suspected. He was treated with high-dose intravenous methylprednisolone, plasma exchange, and one dose of rituximab (1000 mg).

Diagnosis was clarified by two findings. First, a urine *Legionella* antigen test sent on HD 3 returned positive on HD 6, prompting intravenous levofloxacin (750 mg every 48 h for a 5-day course). Second, a kidney biopsy on HD 5 demonstrated acute tubular injury (Figure 1A,B) with myoglobin casts (Figure 1D–F), no immune complex deposition (Figure 1C), and no vasculitis (Figure 1G); the biopsy tissue itself was not specifically tested for the *Legionella* organism. A myositis antibody panel and ANCA screen were negative. This confirmed pigment nephropathy from rhabdomyolysis, rather than autoimmune glomerulonephritis.

While myositis can occur in RA, rhabdomyolysis of this magnitude is uncommon in inflammatory myopathies and strongly suggests an infectious etiology [2]. After initiating levofloxacin, the patient's CK levels and oxygenation improved. His renal function fully recovered after approximately 2 months of intermittent hemodialysis. At 7-month follow-up, his RA remained clinically active, necessitating a transition to alternative biologic therapies.

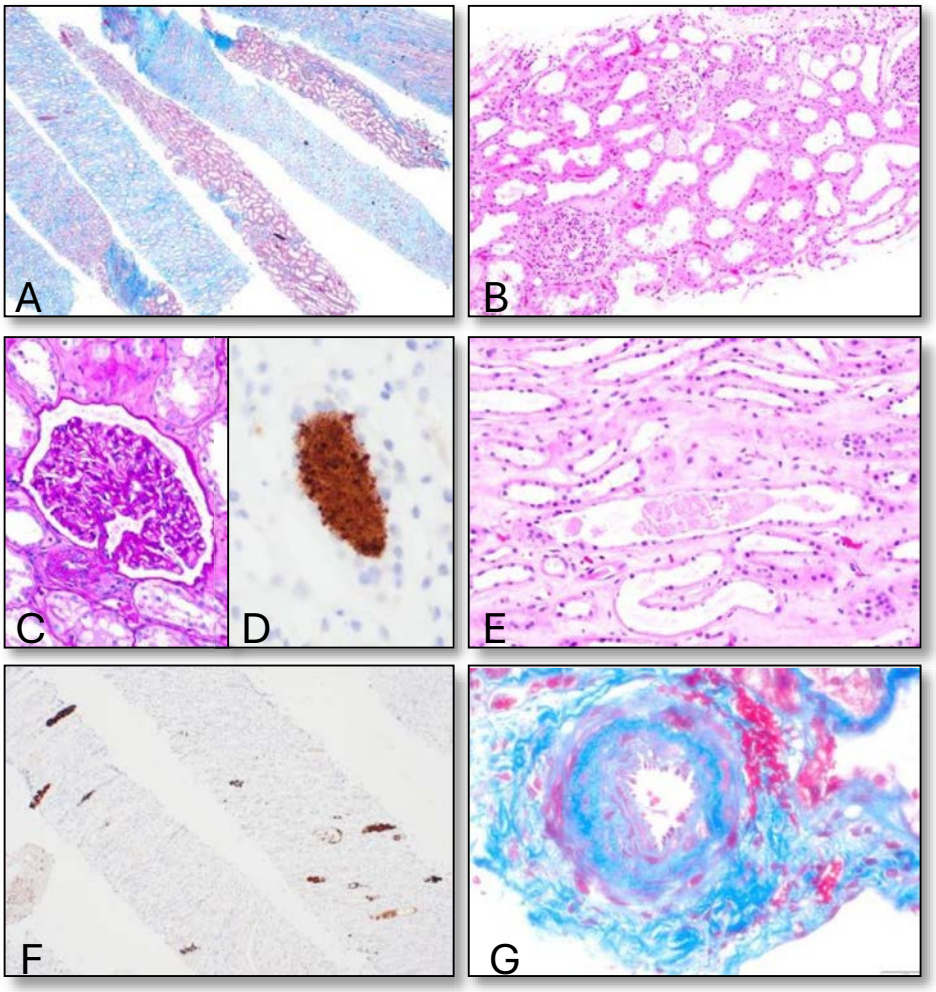

FIGURE 1 | Kidney biopsy findings. (A) Low magnification overview showing multiple renal cores with acute tubular injury and interstitial edema (Masson's trichrome stain original magnification ×100). (B) Acute tubular injury characterized by tubular epithelial flattening and debris (H&E stain, original magnification ×200). (C) Normal glomerulus without immune complex deposition (PAS stain, original magnification ×400). (D) Strong positive myoglobin immunostaining within tubular casts confirms myoglobin cast nephropathy (immunohistochemistry for myoglobin, original magnification ×200). (E) Eosinophilic tubular cast consistent with pigment nephropathy (H&E stain, original magnification ×200). (F) Strong positive myoglobin immunostaining within tubular casts confirms myoglobin cast nephropathy (immunohistochemistry for myoglobin, original magnification ×100). (G) Interlobular artery with preserved structure, showing no vasculitis (Masson's trichrome stain, original magnification ×400).

1 | Discussion

This case highlights the diagnostic challenge of acute multiorgan dysfunction in RA. The overlapping features of a potential autoimmune flare, given a positive autoantibody profile and severe infection, created a complex clinical picture requiring careful consideration [1]. Patients with RA on immunosuppressive therapy are not only at higher risk for infections but can also present with atypical symptoms, making the differentiation between an infectious process and an autoimmune catastrophe particularly difficult.

However, the diagnostic landscape shifted significantly when the urine *Legionella* antigen test returned positive, confirming Legionnaires' disease. Urinary antigen and PCR tests are highly sensitive for Legionella (96% and 92%, respectively), outperforming culture (50%) [3]. The urinary antigen test is the most used and cost-effective, while PCR helps confirm active infection. High-dose steroids may increase lung bacterial load, potentially enhancing the yield of PCR and culture. Further research is needed to assess test performance in immunosuppressed patients. Severe rhabdomyolysis with myoglobinuria was also a key diagnostic clue pointing to infection rather than autoimmune disease [2, 4]. In Legionnaires' disease, muscle injury may result from direct bacterial invasion or endotoxin-induced ischemia. Legionella is a well-known bacterial cause of rhabdomyolysis, which significantly worsens disease outcomes [4]. The kidney biopsy ruled out lupus nephritis and identified pigment nephropathy secondary to rhabdomyolysis as the cause of the AKI, firmly linking the renal failure to the *Legionella*-induced muscle injury, which is further supported by a negative myositis antibody panel.

Attributing the patient's clinical improvement solely to one intervention is difficult due to the concurrent administration of high-dose steroids, plasma exchange, rituximab, multiple

antibiotics, and renal replacement therapy. But the timing of CK decline and improved oxygenation after initiating targeted Legionella therapy, along with kidney biopsy findings, strongly implicates Legionnaires' disease as the primary cause of illness.

Rituximab was initially given for suspected immune-complex nephritis and capillaritis causing DAH, and for its potential benefit in treating his severe, active RA. While muscle involvement can occur in RA, it typically manifests with less dramatic CK elevations and non-specific inflammatory changes rather than severe rhabdomyolysis [5]. The patient's smoking history represents a relevant risk factor for *Legionella* infection [6, 7].

Key takeaways include: the need for prompt consideration of infection, particularly *Legionella*, in patients with rheumatic diseases presenting with pneumonia and rhabdomyolysis, even with positive autoantibodies; the diagnostic value of rhabdomyolysis as a potential indicator against a purely inflammatory myopathy; the importance of maintaining a broad differential; and the utility of tissue diagnosis (kidney biopsy) in differentiating complex presentations. Update immunizations before escalating immunosuppression in RA patients to reduce *Legionella* and other infection risk.

Author Contributions

The authors take full responsibility for this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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