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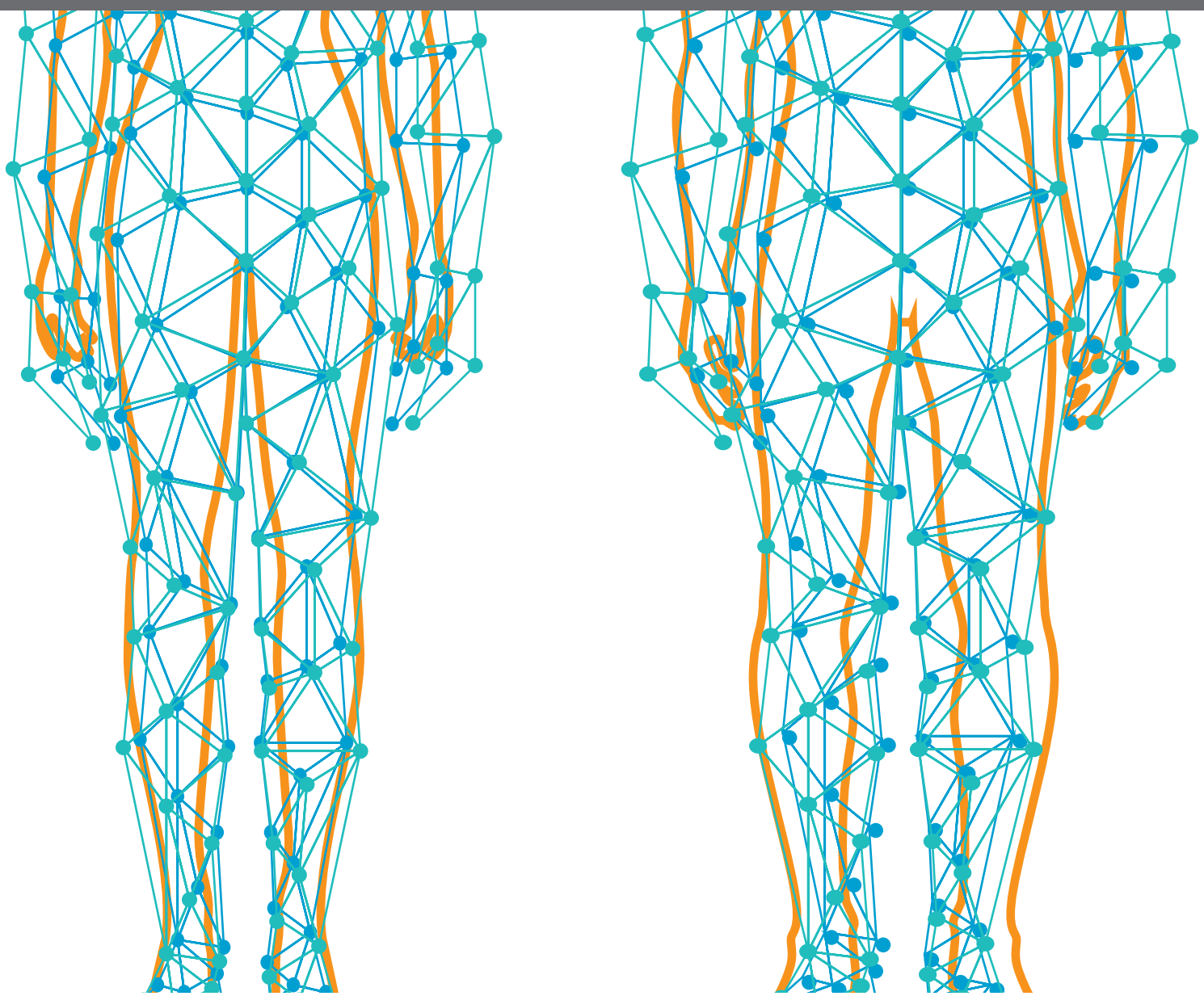

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GLOBAL EXCELLENCE IN RHEUMATOLOGY: ASIA AND AUSTRALASIA

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Dawn of Precision Medicine in Psoriatic Arthritis

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The establishment of precision medicine is considered particularly important in heterogeneous autoimmune diseases (e.g., psoriatic arthritis, systemic lupus erythematosus), which reveal clinical and molecular heterogeneity. The selection of optimal treatment strategies for individual patients may be more important and complex in autoimmune diseases than in other diseases. Two factors are important in precision medicine: patient stratification and use of targeted. When both factors work, patients are likely to have good outcomes. However, research into precision medicine and its practice in systemic autoimmune diseases is lacking. In contrast, the usefulness of peripheral immune cell phenotyping in the evaluation of immunological characteristics and stratification into subgroups of individual patients with systemic autoimmune diseases such as immunoglobulin 4-related disease, systemic lupus erythematosus, and anti-neutrophil cytoplasmic antibody-related vasculitis was reported. Furthermore, the potential of precision medicine using biological disease-modifying antirheumatic drugs based on peripheral immune cell phenotyping was recently demonstrated for psoriatic arthritis in the clinical setting. Precision medicine has not yet been sufficiently investigated in real world clinical settings. However, a dawn of precision medicine has emerged. We should shed further light on precision medicine in PsA and other autoimmune diseases. Here, we first review the usefulness of peripheral immune cell phenotyping in systemic autoimmune diseases and the potential of precision medicine in PsA based on this method.

Keywords: psoriatic arthritis, precision medicine, immune cell phenotyping, targeted therapy, treatment

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INTRODUCTION

In cancer care, genomic research has garnered attention since around 1990. Ever since the Precision Medicine Initiative for cancer care was proposed in the State of the Union address delivered by then-US President Obama in January 2015, attention has been drawn to precision medicine, wherein patients with cancer are classified based on oncogenic driver mutations, and the molecular-targeted drug that best matches each mutation is used for treatment (1).

The establishment of precision medicine is considered particularly important in heterogeneous autoimmune diseases such as psoriatic arthritis (PsA), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). These diseases show clinical and molecular heterogeneity. In these diseases, various symptoms require simultaneous improvement; however, the number of available treatment options is limited. With the elucidation of pathological conditions of these autoimmune diseases, molecules involved in the pathological processes have been clarified. It has become clearer which molecules should now be targeted for treatment. Advances in monoclonal

antibody technology, and the emergence of Janus kinase inhibitors (JAK-i) have made molecular-targeted therapy possible. However, in many autoimmune diseases, due to the heterogeneity of the disease, centrally involved molecules differ depending on individual patient. Differences in patient's molecular profiles can render molecular-targeted therapy inefficient. Therefore, patient stratification according to differences in the patient's molecular profile would allow for more efficient treatment outcomes by precision medicine as opposed to one-size-fits-all approach (2). However, although precision medicine is being developed for cancer and rare diseases, research on its development and use in systemic autoimmune and rheumatic diseases is lacking.

PsA is a rheumatic disease with high clinical heterogeneity; it is sometimes accompanied by nail psoriasis, spine, entheses, and eyes (iritis). In the pathogenesis of PsA, various cytokines such as interferon gamma, interleukin (IL)-12, IL-23, IL-17, IL-6, and tumor necrosis factor alpha (TNF-α) play important roles. Recently, biological disease-modifying antirheumatic drugs (bDMARDs) targeting TNF-α, IL-17A, IL-17A/F, IL-17 receptor, IL-12/23 (p40), and IL-23 (p19) and targeted synthetic DMARDs (tsDMARDs) targeting Janus kinase have demonstrated efficacy and are widely used in routine clinical practice (3–22). Abatacept, a selective T-cell co-stimulation modulator, has been also approved by US Food and Drug Administration (23). EULAR recommends using targeted therapies, such as TNF-inhibitors (TNF-i), IL-17-inhibitors (IL-17-i), IL-12/23-inhibitors (IL-12/23-i), JAK-i, and phosphodiesterase 4 inhibitors (PDE4-i), especially in patients with PsA who fail to adequately respond to synthetic DMARDs (24). Therefore, first of all, it is important to consider whether a patient needs to use biologics or not. Despite the availability of b/tsDMARDs, some patients are resistant to treatment. While these drugs target different molecules, clinical trials directly comparing TNF-i and IL-17-i have shown at least a comparable efficacy on musculoskeletal manifestations (25, 26). This suggests that individual patients diagnosed with PsA may possess different therapeutic targets to attain an optimal response. The diversity of pathologies in patients with such heterogeneous diseases may result in treatment resistance if key aspects or molecules of a disease in that individual are not properly targeted. However, no optimal drug selection method has been established, and some patients are resistant to these drugs and require treatment changes.

To date, precision medicine has not been achieved for any autoimmune disease. In contrast, we recently demonstrated the usefulness of peripheral immune cell phenotyping in the evaluation of immunological characteristics (phenotypic differences) and stratification of patients into subgroups in individual patients with IgG4-related disease (IgG4-RD), SLE, RA, and anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis (27–30). Moreover, we reported the potential of precision medicine based on this method in the real clinical setting of PsA (31). Here, we first review the usefulness of peripheral immune cell phenotyping in some systemic autoimmune diseases and the potential of precision medicine in PsA based on this method.

APPLICATION OF PERIPHERAL IMMUNE CELL PHENOTYPING IN STRATIFYING PATIENTS WITH SYSTEMIC AUTOIMMUNE DISEASES

The strategy of precision medicine involves patient stratification to improve diagnosis and treatment outcomes. In brief, the therapeutic target is narrowed by stratifying patients within a single disease. These two factors are important for achieving precision medicine: patient stratification and the use of targeted therapies (2). When both factors work, patients are likely to have good outcomes.

To stratify patients, some methods or strategies (e.g., genomic, proteomics, metabolomics) are considered available similar to cancer care. However, acquiring tissue biopsies from patients with autoimmune diseases is logistically more difficult than acquiring samples from patients with cancer. Peripheral immune cell phenotyping, which clarifies the differentiation stage such as naïve or memory T cells, the differences in lineage or functional differences represented by T helper (Th)1 and Th2 cells or Th17 cells, and the activation status or involvement of cellular signaling molecules in the pathological process, is useful for classifying individual patients based on immunological characteristics and can often reflect the pathological condition of involved organs or tissues themselves (32).

We performed peripheral immune cell phenotyping in 16 patients with IgG4-RD (28). Compared with healthy controls (HCs), IgG4-RD showed comparable proportions of Th1 and Th17 cells but higher proportions of Treg and follicular helper T (Tfh) cells. The proportions of class-switched memory B cells, particularly plasmablasts, were higher in IgG4-RD. A histopathological examination revealed marked Tfh cell infiltration, and the increase in Tfh cells in the peripheral blood reflected their degree of infiltration into the tissue. It indicated that peripheral immune cell phenotyping can reveal the immunological status and reflect the immunological condition of the involved lesion (tissue and organ). The abundance of Tfh cells was reported also in another cohort with 15 IgG4-RD patients (33). In this study, there was a correlation between Tfh2 cells, serum IgG4 levels and IL-4 producing plasmablasts. Various other attempts of immune phenotyping in IgG4-RD patients have been performed. In the study with 67 IgG4-RD patients, the frequency of circulating pan-innate lymphoid cells (ILCs) and ILC1s were lower than in HCs, whereas circulating ILC2s were higher in IgG4-RD. Circulating ILC2s correlated positively with $CD19^+$ B cells, serum IgG4 and IgE levels (34). In 48 patients with IgG4-RD, circulating $CD27^{low}CD28^{low}CD57^{high}$ $CD4^+$ CTLs were identified as a dominant effector subset. There were prominent infiltration of Granzyme A-expressing $CD8^+$ CTLs in involved tissue and clonal expansion of effector/memory $CD8^+$ T cells in blood (35). This abundance of $CD4^+$ CTLs in blood and tissues were observed also in another cohort with 101 IgG4-RD patients. After clinical remission by B cell-depletion therapy with rituximab, the resolution of these $CD4^+$ CTLs were

demonstrated (36). The role of immune cells in pathogenesis of IgG4-RD and treatment impact are becoming clearer by immune phenotyping (37).

We also attempted to stratify 143 patients with SLE by immune phenotyping (27). We showed that patients with SLE can be statistically stratified into three subgroups: patients who did not show characteristic features other than a high proportion of plasmablasts, those with a high percentage of Tfh cells, and those with a high percentage of activated and memory Treg cells and a low percentage of naïve Treg cells. Similar attempt in patients' stratification based on immune phenotyping was performed in 105 IgG4-RD patients. In this study, IgG4-RD patients were divided into 3 subgroups by cluster analysis: subgroup 1 with low memory B cells and normal Breg, subgroup 2 with high memory B cells and low Breg, and subgroup 3 with high plasmablasts and low naive B cells. Subgroups 2 and 3 were more likely to be resistant to treatment (38). Patients with a high percentage of Tfh cells were more resistant to treatment with immunosuppressants, in addition to high-dose of glucocorticoids (39). The proportions of $CXCR5^{+}$ $CCR7^{low}$ $PD\text{-}1^{high}$ Tfh cells or $CXCR5^{high}$ $ICOS^{high}$ $PD\text{-}1^{high}$ Tfh cells were also reported to be associated with disease activity in SLE (40, 41). Not only peripheral immune phenotyping, mass cytometry is identifying responsible cell subsets and markers characteristic of SLE heterogeneity. Transcriptome analysis is discovering molecular networks responsible for disease activity, disease subtype and future relapse. The elucidation of disease heterogeneity in SLE toward further development of precision medicine is becoming clearer by immune cells phenotyping and recent technological advances in single-cell and omics analysis (42).

Peripheral blood lymphocyte phenotyping in 108 patients with RA revealed that the proportions of Tfh, IgD^{-} $CD27^{-}$ double-negative B cells, and plasmacytoid dendritic cells (pDCs) were higher in patients with active RA than in HCs (29). Treatment with TNF-is reduced the proportion of pDCs, while tocilizumab reduced the proportion of double-negative B cells but increased proportions of naïve and activated Treg cells. Notably, the proportion of T follicular helper cells in the peripheral blood was an independent predictor of favorable responses to treatment with abatacept. In rheumatoid arthritis, increasing number of studies based on immune cell phenotyping and other procedures are performed to reveal the genetic, cellular and molecular heterogeneity for further development of precision medicine (43).

We also demonstrated that excessive B-cell differentiation, defined as the proportion of class-switched memory B cells or $IgD^{-}CD27^{-}$ B cells among all B cells that was >2 SD higher than the mean in HCs, was associated with treatment resistance in ANCA-associated vasculitis and that rituximab was more effective in patients with circulating B-cell abnormalities (30). Thus, molecular-targeted therapies induced different changes in different immune cell phenotypes, and immune phenotyping might be useful for predicting responses to treatment. As described, recently, there has been much effort toward development of precision medicine in autoimmune diseases around the world.

We next attempted to practice precision medicine for PsA in the real clinical setting as a representative heterogeneous rheumatic disease (autoimmune disease) similar to SLE. However, contrary to SLE, ANCA-associated vasculitis, and IgG4-RD, various types of available molecular-targeting therapies such as bDMARDs and JAK-i are currently under development.

PRECISION MEDICINE BASED ON PHENOTYPIC DIFFERENCES IN PERIPHERAL T HELPER CELLS IN PsA

Several predictors of treatment response in PsA have been reported (44–50). Biomarkers are also reported predictors (51). However, attempts to establish precision medicine based on these predictors are insufficient. Evidence of the use of precision medicine is lacking. We previously conducted a systematic literature review of PubMed and the Cochrane Library for our literature search from January 2000 to July 2019 (52). However, a quantitative meta-analysis could not be conducted. In PsA, there is insufficient evidence of a quantitative meta-analysis, and our report is currently the only study demonstrating the potential of precision medicine in the real clinical setting.

We reported the potential of precision medicine using bDMARDs based on lymphocyte phenotypes in PsA (31). The first group (strategic bDMARDs) included 26 patients treated with bDMARDs that were selected according to immune cells phenotyping flow cytometry. The second group (standard bDMARDs) included 38 patients who started treatment with a TNF-i that was selected according to the 2015 EULAR or GRAPPA treatment recommendations.

The results revealed that patients with PsA could be classified into four groups based on lymphocyte phenotypes: $CD3^{+}CD4^{+}CXCR3^{-}CCR6^{+}CD38^{+}HLA\text{-}DR^{+}$-activated Th 17-dominance, $CD3^{+}CD4^{+}CXCR3^{+}CCR6^{-}CD38^{+}HLA\text{-}DR^{+}$-activated Th1-dominance, activated Th1/Th17-high (hybrid pattern), and activated Th1/Th17-low (normal pattern / comparable with HCs). In the preliminary assessment prior to this study, decreases in activated Th17 cells and improvements in arthritis and skin lesions were observed in activated Th17-dominant patients treated with IL-17-i. Similarly, a decrease in activated Th1 cells and improvements in arthritis and skin lesions were observed in activated Th1-dominant patients treated with IL-12/23(p40)-i, while a decrease in activated Th1/Th17 cells and improvement in arthritis and skin lesions were observed in Th1/Th17-high patients who received TNF-i. Other combinations of treatment and a lymphocyte phenotype resulted in poor or no changes in clinical improvement and/or a lymphocyte phenotype. Therefore, the following treatments were administered to patients according to their lymphocyte phenotype: Ustekinumab (UST) for patients with activated Th1-dominance, IL-17-i for patients with activated Th17-dominance, TNF-i for patients with hybrid pattern and major joint complaints, IL-17-i for patients with a hybrid pattern and major skin complaints, and TNF-i for patients with a normal pattern (**Figure 1**). Six

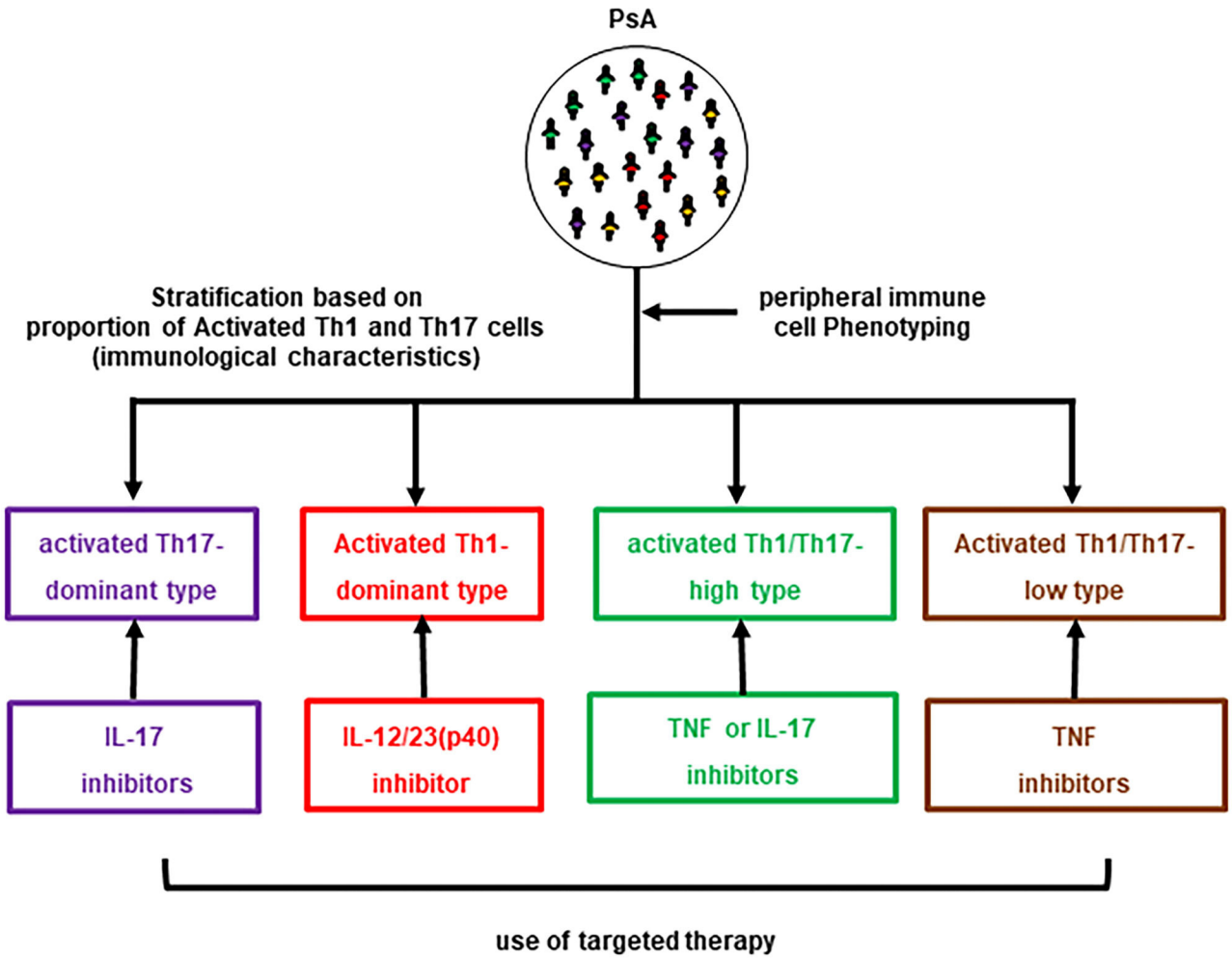

FIGURE 1 | Selective use of bDMARDs in PsA based on the lymphocyte phenotype.

months after treatment, an intergroup comparison of the efficacy showed that the proportion of patients achieving low disease activity based on Simplified Disease Activity Index (SDAI) was significantly higher in the strategic bDMARDs treatment group (92.3%) than in the standard bDMARDs treatment group (55.2%).

We also assessed the correlation of activated Th1/Th17 cells and clinical severity (SDAI and PASI) in each group, e.g., the association between activated Th17 cells and PASI/SDAI in activated Th17 dominant type. However, there was no significant correlation between activated Th1 or Th17 cells and clinical severity. In this context, it was assumed that the phenotypes of peripheral T helper cells might reflect the latent pathological condition in individual patients rather than reflecting overall PsA disease activity. This study excluded IL-23(p19) and JAK-i since they were recently approved and have just become available in the clinical setting with high efficacy. Based on their mode of action, Il-23(p19)-i is assumed to be more effective in activated Th17 cells-dominant type. Regarding JAK-i, different JAK-is have selectivity for different JAK isoforms and are unlikely to perform equally well for all PsA patients. Further assessment is required to establish precision medicine approach using JAK-i. However, this strategy has not been validated anywhere. Due to the pragmatic reason, we performed CCR6 and CXCR3 staining. It seems to be over simplistic. Other markers, such as CD161 which is enriched on Th17, allow for more accurate immunophenotype. In addition, involvement of many types of immune cells, such as tissue-resident memory T cells, mucosal-associated invariant T cells, ILCs, have recently been reported. More detailed assessment is essential to validate this strategy

or evaluate changes in these immune cells including $CD8^+$ T cells. There are still limitations, including the fact that the method used in our study, peripheral immune cell phenotyping, is complex and feasible only at limited number of facilities. Peripheral immune cell phenotyping provides a great deal of information, but it has limitations in terms of its versatility. In addition, the advantage of strategic treatment was observed only in a low disease activity achieving rate. The establishment of strategy that leads to more effective control of disease such as achievement of DAPSA-remission, PASI 90 or zero and Minimal Disease Activity is preferable. We are currently investigating whether it can be substituted by measuring serum cytokines. Conversely, single-cell multi-omics analyses of cell surface proteins and gene expression profiles of tissue-infiltrating cells, can enable more detailed and stricter patients' stratification. Such patients-stratification would enable for higher efficacy in molecular-targeted therapy. Therefore, further accumulation of cases, including the detailed assessment of novel biologics or kinase inhibitors, is necessary to establish precision medicine in real world clinical settings.

PERSPECTIVE OF FURTHER DEVELOPMENT OF PRECISION MEDICINE

There are challenges to the treatment of autoimmune disease using a precision medicine approach vs. utilizing the same approach to treat cancer. First, the development of targeted drugs for autoimmune diseases has historically been slower than that for cancer, and a limited number of treatments are currently available. However, several targeted drugs are currently under development. Second, acquiring tissue biopsies from patients with autoimmune diseases is logistically more difficult than acquiring samples from patients with cancer. In the treatment of malignant tumors, a biopsy is performed to confirm the diagnosis or pathological features and is often readily available for laboratory investigations. This makes it challenging to identify genetic and biochemical differences that contribute to disease in individual patients.

The biopsy-driven observational studies that enrolled RA patients have suggested that certain synovial tissue signatures are associated with treatment response to TNF-i, IL-6 and B-cell depletion therapy (53–57). However, autoimmune diseases such as RA are usually treated without biopsy (joint biopsy). In PsA, contrary to other systemic autoimmune diseases, it may be relatively easier to obtain tissues (skin). The integration of information from tissue biopsies and peripheral immune cell phenotypes or serum cytokine profiles and others may contribute to the further development of novel stratification and more effective treatment strategies in PsA.

Several studies suggest that liquid biopsies are useful to guide therapeutic decisions in cancer (58). In a study using RNA-sequencing data from blood samples, two distinct 23-gene transcriptional signatures to distinguish responders to TNF-i or rituximab were identified (59). In RA, differences in a chromosome conformation signature in blood have been identified as baseline predictive markers of methotrexate treatment (60). In addition to biopsy driven studies, liquid biopsy is also likely to offer important insights into disease pathogenesis and add potential value to precision medicine. If the consistent clinical benefit of treatment strategy based on biopsied-tissue (synovium) information, liquid biopsy and other methods are proven, they will be valuable methods to practice precision medicine in PsA and other systemic autoimmune disease in real world clinical settings.

As mentioned, our study showed changes in phenotypes among patients who achieved a favorable treatment response to bDMARDs. However, evaluations of the association between changes in phenotypes after treatment and treatment responses are insufficient. Moreover, we have not yet sufficiently evaluated temporal factors such as how phenotypes change over time in patients with relapse, whether there are phenotypes that predict treatment resistance already at the time of onset, or whether treatment resistance is acquired during the disease course. Elucidation of this point may provide important insights not only in which b/tsDMARDs should be selected, but also in predicting whether patients need to be treated with b/tsDMARDS or not. Unlike cancer, systemic autoimmune disease has a long (chronic) clinical course. Immunological phenotypes are expected to differ at onset and sometimes thereafter. If immune cell phenotyping can reveal temporal changes in phenotypes, in other words, the immunological natural history of disease, it may contribute to the establishment of precision medicine with the consideration of temporal factors unique to systemic autoimmune diseases with a chronic course.

CONCLUSION

Precision medicine has not been sufficiently investigated in real-world clinical settings. However, the dawn of precision medicine has emerged. Hence, we should shed further light on precision medicine in PsA and other autoimmune diseases.

AUTHOR CONTRIBUTIONS

IM and YT: substantial contributions to review conception, interpretation of reviewed literature, drafting the article, revising it critically for important intellectual content, and final approval of the version of the article to be published. Both authors have read and agreed to the published version of the manuscript.

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Relationship Between C-Reactive Protein/Serum Albumin Ratio, Neutrophil/Lymphocyte Ratio, and ANCA-Associated Vasculitis Activity: A Retrospective Single Center Cohort Study

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Objectives: To evaluate the role of C-reactive protein/albumin ratio (CAR), neutrophil/lymphocyte ratio (NLR), and mean platelet volume (MPV) in newly diagnosed AAV patients and examine their clinical significance.

Methods: Data from 79 untreated newly diagnosed AAV patients were collected and 76 health examination subjects were included in the healthy control group. All clinical characteristics of AAV patients were extracted from their medical records. The NLR, CAR, and MPV levels of AAV patients and the healthy controls were compared and the correlation between these markers and clinical characteristics was analyzed. Patients were then divided into two groups based on the 2003 Birmingham Vasculitis Activity Score (BVAS). The correlation between NLR, CAR, and MPV and disease activity was analyzed and their effects on the cumulative survival rate were analyzed.

Results: Compared with the healthy control group, elevated CAR, NLR, and MPV were observed in AAV patients. CAR ($r = 0.701$, $P < 0.0001$) and NLR ($r = 0.369$, $P < 0.05$) were positively correlated with the BVAS while MPV did not show any significant correlation ($P = 0.85$). The optimal cutoff value for disease activity evaluation using CAR was 0.80 (sensitivity: 85% and specificity: 82%, $P < 0.05$). The optimal cutoff value for disease activity evaluation using NLR was 5.15 (sensitivity: 66% and specificity: 72%, $P < 0.05$). Kaplan–Meier survival analysis revealed that the all-cause mortality rate was higher in patients with CAR ≥ 0.8 than in patients with CAR < 0.8 ($P < 0.05$). Patients with low NLR also showed a lower cumulative survival rate ($P < 0.05$).

Conclusions: NLR and CAR can reflect the inflammatory response and disease activity in AAV patients, while MPV is not significantly correlated with disease activity in AAV patients. The all-cause mortality rate was higher in patients with high CAR and NLR than in patients with low CAR and NLR.

Keywords: CAR, NLR, associated vasculitis (ANCA), MPV, disease activity

INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of rare autoimmune diseases that are characterized by small blood vessel necrosis inflammation and trace or no immune complex deposition in vascular walls. AAV often involves the lungs, ears, nose, throat, kidneys, skin, and nervous system. The pathogenesis is the occurrence of autoantibodies against myeloperoxidase (MPO-ANCA) or proteinase 3 (PR3-ANCA) in AAV patients (1–3). Cardiovascular disease secondary to AAV is the most common cause of death (4). Currently, AAV treatment mainly involves aggressive control of disease progression and activity. Disease activity affects organ function and survival in patients but there is a lack of reliable and convenient inflammatory markers to guide clinical diagnosis. Recently, many studies have reported that many inflammatory markers, such as the monocyte/lymphocyte ratio (MLR) (5), the platelet/lymphocyte ratio (PLR), the neutrophil/lymphocyte ratio (NLR) (6), and the mean plateletcrit, are associated with inflammation and disease activity in many diseases (7). Moreover, a high C-reactive protein (CRP)/albumin (ALB) ratio (CAR) was found to be associated with poor outcome of cancer patients, and this marker could be used as a predictor of poor cancer prognosis (8). To date, many extensive studies have been conducted on the effects and use of NLR, CAR, and mean platelet volume (MPV) in disease progression and prognosis, such as psoriasis, rheumatoid arthritis, systemic lupus erythematosus, Behçet's syndrome, and Sjögren syndrome (7, 9–12). However, there are very few studies on the significance of these markers in AAV. In the present study, we examined the correlation between CAR, NLR, and MPV levels and disease activity in AAV patients. In this retrospective study, we analyzed whether CAR, NLR, MPV, and the BVAS in newly diagnosed patients can reflect AAV activity. We also carried out a survival analysis to analyze the effects of initially high CAR and NLR on the cumulative survival rate. Here, we found that NLR and CAR can reflect the inflammatory response and disease activity in AAV patients, while MPV is not significantly correlated with disease activity in AAV patients. The all-cause mortality rate was higher in patients with high CAR and NLR than in patients with low CAR and NLR. Taken together, these data suggested that the CAR and NLR can serve as potential markers for the AAV disease activity and predictors of survival prognosis.

TABLE 1 | General characteristics of AAV patients and healthy controls.

	AAV patients ($n = 79$)	Healthy controls ($n = 76$)	*P*
Age, year	65 ± 12.17	62 ± 9.56	>0.05
Gender (male), %	39.49%	31.41%	>0.05
White blood cell count, 10^9/L	8.2 (5.46, 11.38)	5.59 ± 1.27	<0.0001
Red blood cell count, 10^{12}/L	3.05 ± 0.90	4.16 ± 0.47	<0.0001
Platelet count, /L	269.33 ± 124.54	202.30 ± 48.45	<0.0001
Mean platelet volume, fl	9.98±1.50	11.04± 1.57	<0.05
Plateletcrit, %	0.53 ± 0.10	0.21 (0.19, 0.24)	<0.05
Neutrophil percentage, %	75.15 ± 9.64	56.03 ± 7.88	<0.0001
Lymphocyte percentage, %	15.40 (11.00, 19.50)	32.59 ± 7.86	<0.0001
CRP, mg/L	23.90 (11.00, 61.60)	2.32 (1.73, 3.02)	<0.0001
CAR	0.80 (0.38, 2.38)	0.05 (0.04, 0.07)	<0.0001
NLR	5.01 (3.55, 7.63)	1.80 (1.40, 2.21)	<0.0001
BVAS	15.65 ± 6.35		

CAR, C-reactive protein/albumin ratio; NLR, neutrophil/lymphocyte ratio; ANCA, anti-neutrophil cytoplasmic antibody (ANCA); MPO, myeloperoxidase; PR3, proteinase 3; P, perinuclear type; C, cytoplasmic type; BVAS, Birmingham Vasculitis Activity Score. Data are expressed as mean ± standard deviation, as median (inter-quartile range), or as numbers (%).

TABLE 2 | Baseline clinical menifestation of AAV patients.

Variables	Value (%)
General	59.49%
Cutaneous	8.86%
Mucous membranes/eyes	6.33%
Ear nose throat	8.86%
Pulmonary	54.43%
Cardiovascular	20.25%
Abdominal	3.80%
Renal	77.22%
Nervous systems	12.66%
GPA	12.66%
MPA	81.01%
EGPA	1.27%
C-ANCA (or PR3 ANCA)	12.66%
P-ANCA (or MPO ANCA)	78.48%
Both ANCAs	2.53%
ANCA negative	6.33%

MATERIALS AND METHODS

Patients

We conducted a retrospective cohort study on newly diagnosed AAV patients in Jiangxi Provincial People's Hospital from January 2011 to April 2021. AAV diagnosis was based on the American College of Rheumatology (ACR) and 2012 Chapel Hill Consensus Conferences Vasculitis nomenclature (1). The 2003 Birmingham Vasculitis Activity Score (BVAS) was used to evaluate AAV activity in 79 patients (13). All patients were treatment-naive and the exclusion criteria were as follows: (1) other comorbid autoimmune diseases; (2) liver disease; (3) hematologic disease; (4) malignancy; (5) severe infection or infectious disease; and (6) absence of complete medical history. Data of 76 subjects who underwent health examinations in Jiangxi Provincial People's Hospital between June 2012 and April 2021 were used as controls. The epidemiological characteristics of healthy controls were matched to patients. The healthy controls were free from autoimmune disease, liver disease, diabetes, hypertension, hematologic disease, malignancies, and other major underlying disease and no blood test abnormality was found. AAV patients were divided into the active group and the non-active group, with BVASs on diagnosis of >15 and ≤15,

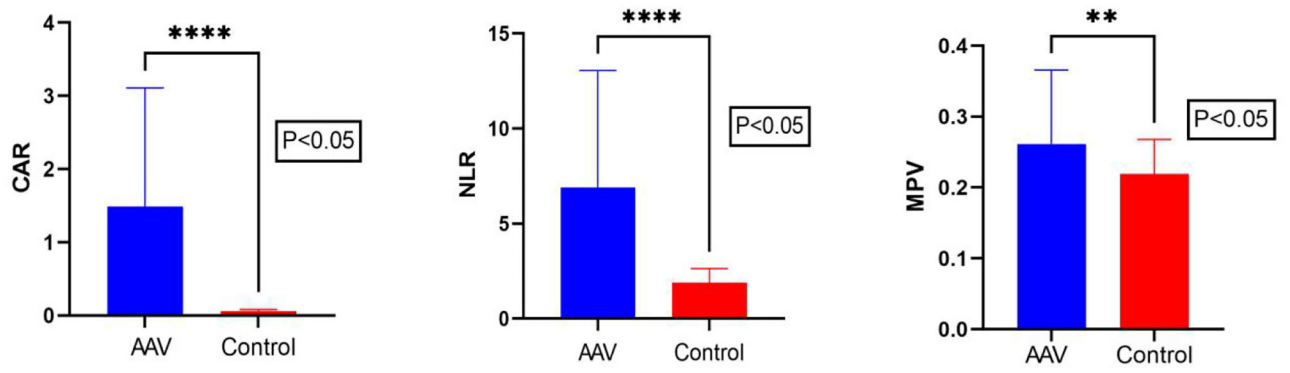

FIGURE 1 | Comparison of CAR, NLR, and MPV levels between AAV patients and the control group. The Mann–Whitney U test showed that the CAR, NLR, and MPV levels in AAV patients were significantly higher than in the control group. CAR, C-reactive protein/albumin ratio; NLR, neutrophil/lymphocyte ratio; MPV, mean platelet volume. The ****symbol indicates $p < 0.0001$ and ** symbol indicates $p < 0.01$.

TABLE 3 | Correlation between the CAR, NLR, and MPV and BVAS in AAV patients.

	r	*P*
CAR	0.701	<0.0001
NLR	0.369	<0.001
MPV	−0.065	>0.05

respectively. All AAV patients from January 2011 to April 2021 were analyzed. The date of diagnosis was taken as the starting point and the date of death as the end point. Patients who survived during this time period were considered as censored data. The patients who were lost to follow-up or did not regularly take medicine were excluded from the survival analysis. Informed consent was obtained from all recruits to this study. This study (No. 2021-06-013) was approved by the Ethics Committee of the Jiangxi Provincial People's Hospital in accordance with the World Medical Association Declaration of Helsinki.

Clinical and Laboratory Data

All clinical and laboratory data were retrospectively collected after the patients' medical records were screened. The following data were collected: age, gender, weight, blood routine analysis results, CRP levels, hepatic and renal functions, AAV subtype and antibody titer, and BVAS. The 2003 BVAS was used to determine AAV activity. The CAR was calculated by dividing the CRP level (g/dl) by the serum albumin level (g/dl). The NLR was calculated by dividing the neutrophil percentage by the lymphocyte percentage.

Statistical Analysis

All statistical analyses were conducted using SPSS software (version 21 for windows; IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to determine the normality of variable distribution. If the variable was normally distributed, the *t*-test was used to compare the variables between AAV patients and controls. If the variable was non-normally distributed, the Mann–Whitney U test was used. Continuous data that were normally distributed are expressed as the mean ± standard deviation (SD), continuous data with skewed distribution are expressed as median (inter-quartile range), and categorical data are expressed as percentages. Spearman correlation analysis was conducted to determine the correlation between variables. In addition, receiver operating characteristic (ROC) curve analysis was used to determine the sensitivity and specificity of inflammatory markers in predicting ANCA disease activity. Kaplan–Meier survival analysis was conducted to compare the cumulative survival rates between the two groups. $P < 0.05$ was considered to indicate statistically significance.

RESULTS

Basic Characteristics of Study Samples

The mean age of patients was 65 and there were 39 males (49%) and 40 females (51%). The mean age of subjects in the control group was 62 and there were 31 males (41%) and 45 females (59%). There were no statistically significant differences in age and gender between patients and controls ($P > 0.05$) and statistically significant differences were present in the other laboratory markers ($P < 0.05$) (**Table 1**). Patients with AAV exhibited a variety of systemic impairments, of which the lungs, kidneys, cardiovascular, and nervous systems are more common. More than half of patients with AAV have systemic symptoms of fever and fatigue, as well as lung and kidney damage (**Table 2**).

CAR, NLR, and MPV Levels Were Elevated in AAV Patients

The CAR of the patient group and the control group was 0.80 (0.38, 2.38) and 0.05 (0.04, 0.07), respectively. The NLR of the patient group and the control group was 5.01 (3.55, 7.63) and 1.80 (1.40, 2.21), respectively. The MPV of the patient group and the control group was 0.53 ± 0.10 and 0.21 (0.19, 0.24), respectively. There were statistically significant differences in NLR? CAR and MPV between the patient group and the control group ($P < 0.05$) (**Table 1**; **Figure 1**). We found that the lymphocyte count

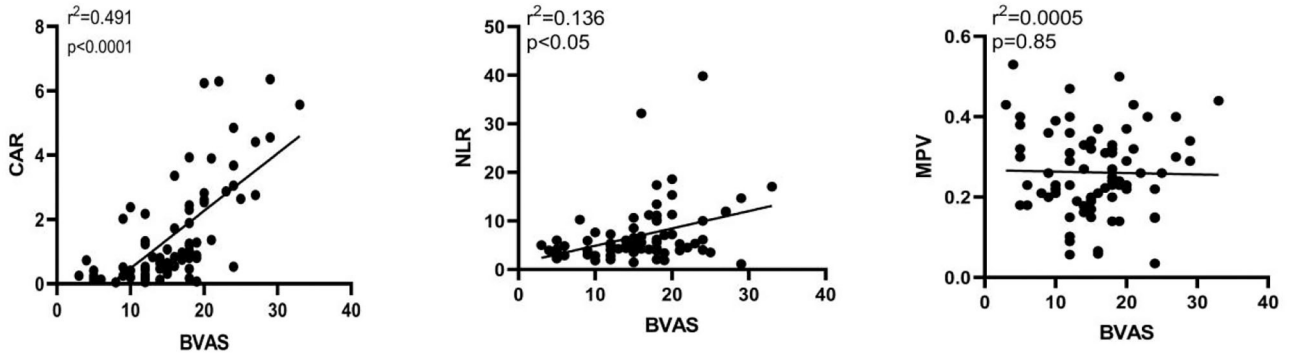

FIGURE 2 | Correlation between CAR, NLR, and MPV levels and disease activity in AAV patients. Spearman correlation analysis was conducted to test the relationship between the CAR, NLR, and MPV and disease activity. The CAR and NLR were found to be positively correlated with disease activity, while MPV did not show a significant correlation with disease activity. CAR, C-reactive protein/albumin ratio; NLR, neutrophil/lymphocyte ratio; MPV, mean platelet volume; BVAS, Birmingham Vasculitis Activity Score.

was lower in AAV patients than in healthy controls ($P < 0.0001$) (**Table 1**). The CRP level and neutrophil count were significantly elevated in AAV patients ($P < 0.0001$) (**Table 1**).

Correlation Between CAR, NLR, and MPV and Clinical Disease Activity in AAV Patients

In our study, the BVAS of AAV patients was positively correlated with CAR ($r = 0.701$, $P < 0.0001$) and NLR ($r = 0.369$, $P < 0.05$). The BVAS was not significantly correlated with MPV ($P = 0.85$) (**Table 3**; **Figure 2**). A subanalysis between CAR, NLR and MPV based on the presence of MPO or PR3 was performed, while no statistically significant was observed in these analysis, data no shown.

ROC Curve Analysis of CAR and NLR for Diagnosing Disease Activity

ROC curves of CAR and NLR relative to BVAS are shown in **Figure 3**. The optimal cutoff value for maximum specificity and sensitivity of CAR for disease activity prediction was 0.80 and the optimal cutoff value for NLR was 5.15. According to our ROC curve analysis, prediction of disease activity based on the CAR in AAV patients has a sensitivity of 85% and a specificity of 82%, and the sensitivity and specificity of the NLR were 66 and 72%, respectively.

Relationship Between CAR and NLR and the Cumulative Survival Rate of Newly Diagnosed AAV Patients

The data is current to April 2021 when the searches were last completed and the follow-up was carried out on the diagnosed AAV patients. After excluding 11 patients who were lost to follow-up and 10 patients who did not regularly take medication, 58 patients were included in the survival analysis, of whom 8 died and 50 survived. Patients were divided into the high and low CAR groups and high and low NLR groups based on the optimal cutoff values for CAR and NLR (CAR $\geq$ 0.8 and NLR $\geq$ 5.15). As shown in **Table 4**, lung injury was more common in the high CAR group than in the low CAR group. Indeed, there was markedly different cumulative survival rate between lung injury patients and non-lung injury patients ($P < 0.05$). These data shown that CAR can be used as an effective disease activity marker, and the AAV patients with high CAR might have a poor prognosis. Actually, Kaplan–Meier survival analysis revealed that the all-cause mortality rate was higher in patients with CAR $\geq$ 0.8 than in patients with CAR < 0.8 ($P < 0.05$). In addition, patients with low NLR also showed a lower cumulative survival rate ($P < 0.05$) (**Figure 4**).

DISCUSSION

This study evaluated the relationship between disease activity and CAR, NLR, and MPV in AAV patients. We found that compared with the healthy control group, the CAR, NLR, and MPV levels in newly diagnosed and untreated AAV patients were significantly elevated. With regard to how to quantify disease activity in AAV patients, we selected the BVAS scoring system for quantitative evaluation. This system includes nine sub-systems and the weighted score for new-onset/worsening or persistence of every symptom is different. Therefore, this system is considered to be the most reliable tool for evaluating AAV activity (14). We further found that the BVAS disease activity score of AAV patients was positively correlated with CRP and NLR, and patients with high CAR and high NLR had higher all-cause mortality rates.

CRP is synthesized and degraded in hepatocytes, and serum CRP levels rise drastically within 24–72 h during inflammation. CRP has a short half-life and is usually considered to be the laboratory marker of choice for acute inflammatory diseases (15). Serum ALB is also produced by the liver and severe inflammatory diseases and malnutrition will cause serum ALB levels to decrease (16). In contrast to CRP alone or serum ALB alone, the CAR includes two important inflammation markers with different presentations during inflammation and can maximally reflect

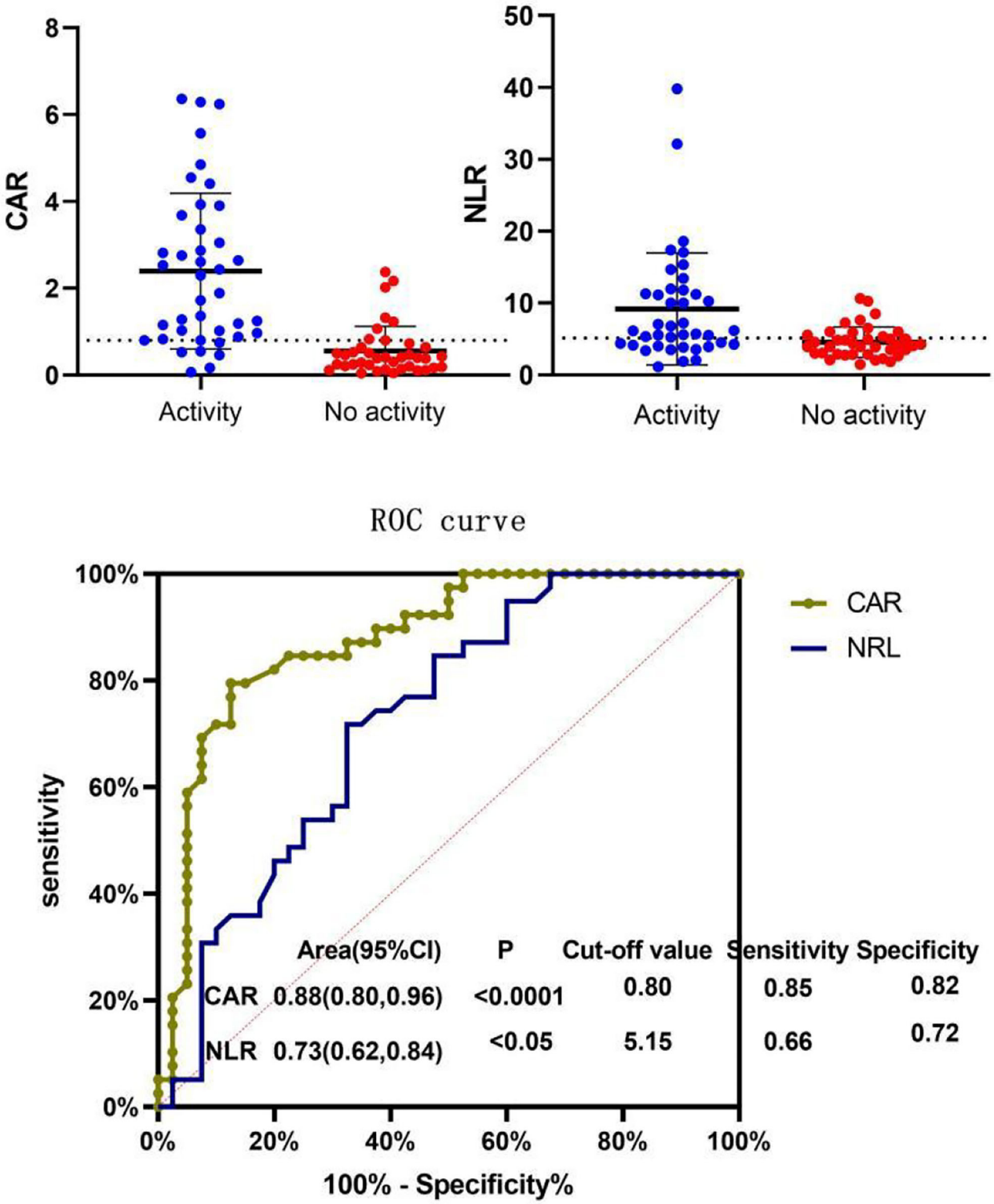

FIGURE 3 | Receiver operating characteristic (ROC) curve of NLR and CAR for BVAS. Patients were divided into the active disease group (BVAS > 15) and the non-active disease group (BVAS ≤ 15) and ROC curve analysis was conducted for the CAR and NLR. The optimal cutoff value for disease activity evaluation using the CAR was 0.80 (sensitivity: 85% and specificity: 82%, $P < 0.05$). The optimal cutoff value for disease activity evaluation using the NLR was 5.15 (sensitivity: 66% and specificity: 72%, $P < 0.05$). CAR, C-reactive protein/albumin ratio; NLR, neutrophil/lymphocyte ratio; MPV, mean platelet volume; BVAS, Birmingham Vasculitis Activity Score.

TABLE 4 | Clinical manifestation of high CAR and low CAR, high NLR and low NLR AAV patients.

Clinical manifestation	HighCAR ($n = 40$)	LowCAR ($n = 39$)	*P* value	High NLR ($n = 38$)	LowNLR ($n = 41$)	*P* value
General	27	20	0.173	23	24	1.000
Cutaneous	6	1	0.108	3	4	1.000
Mucous membranes/eyes	5	0	0.055	2	3	1.000
Ear nose throat	6	1	0.108	3	4	1.000
Pulmonary	32	11	<0.05	24	19	0.176
Cardiovascular	9	7	0.781	10	6	0.256
Abdominal	2	1	1.000	2	1	0.606
Renal	34	27	0.114	32	29	0.186
Nervous systems	6	4	0.737	5	5	1.000

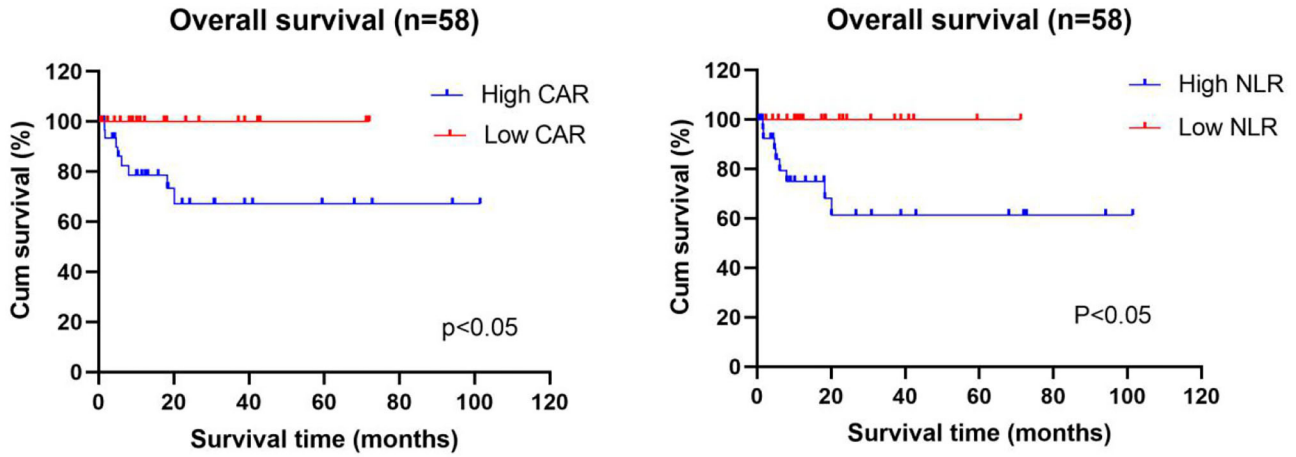

FIGURE 4 | Relationship between the CAR and NLR and the cumulative survival rate of newly diagnosed AAV patients. Kaplan–Meier survival analysis revealed that the all-cause mortality rate was higher in AAV patients with high CAR (≥0.8) and high NLR (≥5.15) than in patients with low CAR (<0.8) and low NLR (<5.15). CAR, C-reactive protein/albumin ratio; NLR, neutrophil/lymphocyte ratio.

disease burden. The assays to detect these two markers are simple and cheap (17–19). A previous study showed that the CAR is related to the all-cause mortality rate in AAV patients (20). The present study found that the CAR in AAV patients was significantly higher than in the healthy control group and showed a significant positive correlation with the BVAS disease activity score. Through plotting of ROC curves, we confirmed that the optimal cutoff value of CAR > 0.80 can be used to predict disease activity. Disease activity significantly affects patient prognosis and organ function. This study also showed that the CAR was related to the all-cause mortality rate in AAV patients.

In the human body, neutrophils account for 50–70% of all circulating leukocytes, are the most abundant circulating leukocytes, and play an important role in innate immunity (21–23). In some autoimmune diseases, neutrophils are considered to be the main source of autoantigens that trigger autoimmune diseases (24, 25). Neutrophil extracellular traps (NETs) secreted by dead neutrophils can capture and kill pathogens, damage endothelial cells, present antigens, promote platelet activation, and participate autoimmune reactions (26–28). The same ANCA can stimulate neutrophils to release NETs that include autoantigens and cause AAV patients to produce autoimmune responses to these components (29). At the same time, lymphocytopenia is considered to be related to inflammation burden, such as in rheumatoid arthritis, Crohn's disease, systemic lupus erythematosus, and vasculitis (30–33). However, some studies found that lymphocytes are negatively correlated with the recurrence rate, with lymphocytopenia associated with a lower recurrence rate (34). NLR is a combination of two independent inflammation markers. Neutrophils are mainly responsible for non-specific and early systemic inflammation while lymphocyte changes occur relatively late and participate in late immune responses. Therefore, a marker that combines two immune cells with different characteristics is more reliable than a single immune cell count and is widely used to evaluate inflammation burden and predict disease prognosis (35, 36). The NLR is related to the severity of many autoimmune diseases, such as psoriasis, rheumatoid arthritis, systemic lupus erythematosus, Behçet's syndrome, and Sjögren syndrome (7, 9–11). The results of this study showed that the NLR was significantly higher in

the AAV group than in normal subjects and that it is positively correlated with ANCA disease activity. This shows that the NLR may have clinical value in monitoring ANCA disease activity. By plotting the ROC curve, we determined that the optimal cutoff value for NLR was 5.15. Based on this result, we recommend that more frequent consultation, comprehensive laboratory tests, and evaluation of treatment results may be required in AAV patients with NLR $\geq$ 5.15.

Platelet count has always been used as a marker of inflammatory disease activity and MPV plays an important role as a deciding factor for the platelet response in many inflammatory diseases (37). MPV can be used to predict severe COVID-19 cases (38) and its elevation is an independent risk factor for coronary artery and peripheral artery diseases (39, 40). In autoimmune diseases, such as Behçet's syndrome, increased MPV can reflect Behçet's syndrome disease activity and predict ocular complications (12). However, the results of some studies also showed that MPV is not associated with mortality and recurrence rates in primary malignant bone tumors (41) and also not associated with COVID-19 severity (42). In a study on systemic lupus erythematosus, both cross-sectional and longitudinal studies found no correlation between disease activity and MPV (43). These contradictory results may be because MPV changes are affected by many factors, such as age, gender, diabetes, obesity, and hypertension (44, 45). Our study found that there is a difference in MPV between AAV patients and healthy controls, but MPV is not associated with the BVAS disease activity score.

Our study showed that the CAR and NLR can be used as two potential markers to reflect the AAV inflammation status, assess disease activity, and predict the chance of survival based on the optimal cutoff values. We recommend that frequent hospital consultation and examinations should be carried out for AAV patients with high CAR and high NLR. Neutrophil count, lymphocyte count, ALB levels, and CRP levels are easily determined and objective markers that can be obtained in almost all medical institutions and facilitate patient and physician evaluation. However, we would like to mention some study limitations. This is a single center study with small sample size and a low number of deaths. At the same time, this is a retrospective study and not all confounding factors can be controlled. For example, medication history, disease history, nutrition status, and comorbidities were not recorded in the patients' medical records. The BVAS combines the clinical characteristics of patients to cross-sectionally evaluate disease activity and has some inter-individual differences and subjectivity. Therefore, the accurate reflection of AAV disease activity is limited. We hope that a future large-sample, multicenter, prospective study can be carried out to validate our results and provide more definite optimal CAR and NLR cutoff values to predict disease activity in AAV patients and promote application in clinical practice.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the Jiangxi Provincial People's Hospital in accordance with the World Medical Association Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YT, NL, HY, and LD reviewed the medical records, analyzed the data, and wrote the first draft. YT, HY, and LD reviewed the literature and finalized the revised manuscript. All authors have read and approved the final manuscript.

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Corrigendum: Relationship between c-reactive protein/serum albumin ratio, neutrophil/lymphocyte ratio, and ANCA-associated vasculitis activity: A retrospective single center cohort study

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The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Increasing of Blood Brain Barrier Permeability and the Association With Depression and Anxiety in Systemic Lupus Erythematosus Patients

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Objective: To study changes in blood brain barrier (BBB) permeability in systemic lupus erythematosus (SLE) patients, and explore the association between the alterations in BBB permeability and depression/anxiety in SLE.

Methods: Brain dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) images were collected from 42 SLE patients and 23 healthy controls (HCs). Based on the Patlak pharmacokinetic model, the K^{trans} value of each voxel in the whole brain of each subject was calculated. BBB permeability indicator (the K^{trans} value) between SLE patients and healthy control group was compared. Hamilton Depression Scale (HAMD) and Hamilton Anxiety Scale (HAMA) were used to assess the mental health of SLE patients. The difference in BBB permeability was compared on SLE patients with depression/anxiety, SLE patients without depression/anxiety and HCs by ANOVA analysis.

Results: The K^{trans} value of the right insular region of the SLE group was significantly higher than that of the healthy control group. And the K^{trans} value of the right insular region in SLE patients with depression/anxiety was significantly increased compared with SLE patients without depression/anxiety and HCs.

Conclusions: SLE patients have increased BBB permeability, mainly in the right insular area. The increased BBB permeability in the right insular region is associated with the depression/anxiety in SLE patients.

Keywords: systemic lupus erythematosus, blood brain barrier, permeability, depression, anxiety

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease, characterized by the production of multitude of autoantibodies affecting multiple systems, which leads to multiple organ damage. Neuropsychiatric systemic lupus erythematosus (NPSLE) is one of the most serious manifestations of SLE, affecting the peripheral and central nervous systems (CNS), which can occur at any

time in the course of SLE. Depression and anxiety are the most common manifestations of diffuse CNS involvement in SLE (1). Genetic factors, immune response and blood brain barrier (BBB) dysfunction are all considered to be involved in the pathogenesis of NPSLE. Alexander et al. firstly found BBB damage in lupus mice (2). A clinical study also found that compared with healthy controls, SLE patients have increased BBB permeability, and patients with NPSLE have more severe BBB damage (3). In some diseases such as traumatic brain injury, epilepsy, and Alzheimer's disease, BBB functional damage has been proven to cause neuronal damage (4–6). Hence, we speculate that BBB damage caused by the influence of pathological changes underlying lupus makes abnormal BBB permeability, allowing inflammatory mediators in the peripheral circulation, such as cytokines, autoantibodies, to pass through the BBB and enter the CNS resulting in neuronal damage and a series of neuropsychiatric symptoms.

The traditionally accepted, reliable indicator for judging BBB damage is the albumin quotient (7). However, lumbar puncture is required to obtain the patient's cerebrospinal fluid (CSF), which is an invasive examination with related risks, so its clinical application is relatively limited. With the invention and development of medical imaging technology, a variety of imaging methods have been applied to the detection of BBB damage. This study used a non-invasive imaging technique that can quantitatively detect BBB dysfunction: dynamic contrast enhanced magnetic resonance imaging (DCE-MRI). The destruction of BBB can lead to the extravasation of low-molecular-weight magnetic resonance imaging contrast agents into the interstitial space outside blood vessels, resulting in increased signal at this site. DCE-MRI technology uses repeated brain scans to capture the changes in signal intensity caused by extravasation of intravenous low-molecular-weight contrast agents from the BBB, and uses the Patlak pharmacokinetic model to obtain the K^{trans} volume transfer constant, which can quantitatively reflect the destruction of BBB (8). The increase in the K^{trans} value represents an increase in the leakage of fluid through the BBB into the brain tissue. DCE-MRI technology has been used in the detection of BBB destruction in a variety of diseases, such as multiple sclerosis and brain tumors (9, 10).

Depression and anxiety as common emotional and mental disorders in SLE patients, present significantly higher incidence than which in the general population. Because of the lack of early symptoms and unified diagnostic criteria, depression and anxiety in SLE patients are often ignored in clinical practice. Increasing studies have shown that BBB dysfunction also plays an important role in the pathogenesis of depression. Animal experiments found that destruction of the BBB in mice produces depression-like behaviors (11). In addition, a recent study found that BBB destruction may be related to anxiety. Using DCE-MRI technology, Lyna et al. found that in patients with bipolar disorder, increased BBB permeability was associated with more severe depression and anxiety symptoms as well as the course of the disease (12).

At present, there are few studies that explored the association between BBB permeability changes and depression as well as anxiety in SLE patients. This study used DCE-MRI technology to detect changes in BBB permeability in SLE patients and evaluated depression and anxiety in SLE patients to further analyze their association with BBB dysfunction.

MATERIALS AND METHODS

Participants

This study recruited patients with SLE who were admitted to the inpatient department (IPD) of the Department of Rheumatology and Immunology of the First Affiliated Hospital of Kunming Medical University from April 2016 to December 2017. All the patients fulfilled the 1997 revised American College of Rheumatology (ACR) classification and diagnostic criteria for SLE (13). The inclusion criteria include: (1) age ranging from 15 to 50 years old, (2) right-handedness, (3) participants willing to attend the study voluntarily and sign informed consents.

The exclusion criteria for all participants were as follows: (1) participants with a history of head trauma, (2) participants with a history of drug or alcohol dependence, (3) participants suffering from other connective tissue diseases, hematologic diseases, cardiovascular and cerebrovascular diseases, malignant tumors and renal insufficiency caused by non-SLE, (4) participants with parenchymal brain disease, CNS infection, epilepsy and other neuropsychiatric diseases not caused by SLE, etc., or with family history of neurological or psychiatric disease, (5) participants who have contraindications to MRI (such as pacemaker, metal implants in the body, history of contrast agent allergy, claustrophobia, glomerular filtration rate <30 ml/min, etc.), (6) participants currently pregnant or nursing. Structured Clinical Interview for DSM-IV Non-Patient Version (SCID-NP) for the healthy control is used to assess healthy participants. Using the Edinburgh Handedness Inventory to assess participants' handedness (14).

A total of 60 patients with SLE were initially enrolled. But 17 patients who could not complete all examinations were excluded, and one patient who had a clear history of depression was also excluded. Finally, 42 patients were included in the study. There were 23 healthy controls (HCs) whose age and sex matched the SLE group were recruited. An experienced rheumatologist and a psychiatrist performed the examinations of screening.

This research has been approved by the ethics committee of the First Affiliated Hospital of Kunming Medical University, Yunnan Province, China (ClinicalTrials.gov: NCT00703742). Before the start of the trial, each participant signed a written informed consent after being informed of the trial procedures in detail.

Demographics and Psychological Assessment

We recorded the age, gender, weight, course of disease, previous history, family history, personal history of all enrolled patients and healthy volunteers. Then Hamilton Depression Scale (HAMD) and Hamilton Anxiety Scale (HAMA) were used to assess mental health of SLE patients. Scores on above mentioned scales were recorded and evaluated by two psychiatrists achieving good inter-examiner reliability after systematic training.

DCE-MRI

All enrolled patients and healthy controls accepted a cranial T1 FFE sequence scan (incoherent gradient echo sequence spoiled GE) by the same operator under the same Philips 3.0T MRI scanner in the Imaging Department of the First Affiliated Hospital of Kunming Medical University on 3rd day after admission. Five whole-brain images under two flips of 2° and 12° were collected, and then the dynamic scan was performed when the flip angle was 12°. A total of 50 periods were scanned, and from the 4th period, the Gd-DTPA contrast agent was given in a rapid intravenous injection at a rate of 3 ml/s. The DCE-MRI parameters were set as follows:

T2W: TR = 2,500 ms, TE = 80 ms, fov = 235 × 208 mm, t = 45 s, matrix = 312 × 180, slices = 18, thickness = 6 mm, slice gap = 2 mm.

T1W: TR = 1,900 ms, TE = 20 ms, TI = 800 ms, fov = 230 × 190 mm, $t = 1'14''$, matrix = 232 × 139, slices = 18, thickness = 6 mm, slice gap = 2 mm.

DCE: TR = 8.2 ms, TE = 3.1 ms, fov = 220 × 220 mm, flip angles = 2°,12°, matrix = 148 × 128, slices = 40, thickness = 3 mm, slice gap = 0 mm.

Image Processing

The original images were converted from the.dcm format to the.nii format via the dcm 2niigui software. For each subject, the first dynamic scan image was taken as the standard image, and all other images were registered to it. To correct the non-uniformity of B1 field, the 2° and 12° images were corrected by nu_correct in FSL software for N3 correction, and the mean value of the corrected uneven field was set to 1 (15). The drug concentration curve was obtained by measuring the relative value of signal enhancement E(t) at various concentrations of contrast agent C(t) (16). The K^{trans} was obtained based on the drug concentration curve and the Patlak model, and after processing, the K^{trans} image was finally produced (16).

Statistical Analysis

IBM SPSS Statistics 21 was used for data statistical analysis. Quantitative data following normal distribution were compared by t-test and quantitative data with abnormal distribution was compared by Mann-Whitney U-test to evaluate the difference. Chi-square test was used to analyze binary variable. Ranked data were analyzed by non-parametric test. When $P < 0.05$, the difference was considered to be statistically significant.

We used DPABI software to perform an independent two sample t-test (Cluster size > 100, $P \leq 0.005$ is statistically significant) to identify the area with the obvious difference of K^{trans} between SLE and HC groups. Then the three-dimensional image of that area was extracted to further calculate the K^{trans} value of each participant's whole brain and particular areas. ANOVA analysis was performed to compare the difference in the K^{trans} value of each voxel in the whole brain and particular regions between SLE patients with depression/anxiety, SLE patients without depression/anxiety, and HCs. The difference was considered to be statistically significant when the single voxel $P \leq 0.005$ and the cluster volume > 100 voxels.

TABLE 1 | Demographic of SLE and HC groups.

	SLE (n = 42)	HCs (n = 23)	P
Female (%)	33 (78.6)	18 (78.3)	0.977
Age (years) (IQR)	27.5 (23.0, 38.3)	27.0 (25.0, 33.0)	0.891

SLE, systemic lupus erythematosus; HCs, healthy controls; IQR, interquartile range.

RESULTS

Demographics of SLE and HC Groups

Totally 42 SLE patients and 23 HCs were included in this study. And there was no significant difference between the two groups on age and gender (Detailed data is shown in **Table 1**).

Clinical Data and Psychological Assessment of SLE Patients

Among 42 SLE patients, 52.4% of patients were suffering severe disease activity as well as 28.6% in moderate disease and 19% in mild disease. There were four patients who present symptoms of nervous system involvement. Scores of the psychological assessment scales showed that among 42 SLE patients, patients with depression accounted for 69.0% and patients with anxiety accounted for 47.6% of all patients. And all patients were accepting glucocorticoids treatment. Detailed data are shown in **Table 2**.

The Differences in BBB Permeability Between SLE and HC Groups

We found the K^{trans} value significantly increased in the right insular area of the SLE group. So, we extracted the three-dimensional image of the right insular area, and calculated the average K^{trans} value of the whole brain and the K^{trans} value of the right insular area of the two groups, respectively. The K^{trans} value of the right insular region of the SLE group was significantly higher than that of the HC group ($P < 0.005$), while the average K^{trans} value of the whole brain was not statistically different between the two groups (**Table 3**; **Figure 1A**).

The Difference in BBB Permeability Between SLE Patients With Depression/Anxiety, SLE Patients Without Depression/Anxiety and HCs

The results of ANOVA analysis showed that the K^{trans} value of the right insular region was significantly higher in SLE patients with depression than that of SLE patients without depression and HCs ($P < 0.005$), while there was no significant difference between SLE patients without depression and HCs. And the K^{trans} value of the right insular region was also significantly higher in SLE patients with anxiety than that of SLE patients without anxiety and HCs ($P < 0.005$), while there was no significant difference between SLE patients without anxiety and HCs (**Table 4**; **Figures 1B,C**).

TABLE 2 | Clinical features and psychological assessment of SLE patients.

	N (*n* = 42)	%
Disease activity (SLEDAI)		
Mild (≤6)	8	19.0
Moderate (7–12)	12	28.6
Severe (>12)	22	52.4
Organ involvement		
Nervous system	4	9.5
Vasculitis	2	4.8
Articular and muscular	16	38.1
Renal	21	50.0
Cutaneous and mucous	21	50.0
Serositis	20	47.6
Hematological	17	40.5
Autoantibodies (+)		
Anti-dsDNA antibody	20	47.6
Anti-Sm antibody	26	61.9
ARPA	14	33.3
Anti-nucleosome antibody	21	50.0
Anti-histone antibody	21	50.0
APLs ($n = 41$)*	12	29.3
Depression/anxiety status		
Depression (HAMD ≥ 7)	29	69.0
Anxiety (HAMA ≥ 14)	20	47.6
Treatment		
GCs	42	100.0
HCQ	36	85.7
CTX	28	66.7
MMF	7	16.7
CsA	4	9.5
MTX	5	11.9
LEF	7	16.7
Thalidomide	7	16.7
IVIG	5	11.9
Antiplatelet	11	26.2
Anticoagulation	22	52.4

*SLEDAI, systemic lupus erythematosus disease activity index; ARPA, Antiribosomal P protein antibodies; APLs, antiphospholipid antibodies; HAMD, Hamilton Depression Scale; HAMA, Hamilton Anxiety Scale; GCs, glucocorticoids; HCQ, hydroxychloroquine; CTX, cyclophosphamide; MMF, mycophenolate mofetil; CsA, cyclosporin A; MTX, methotrexate; LEF, leflunomide; IVIG, intravenous immunogloblin. *APLs was available in 41 patients.*

DISCUSSION

Compared with other invasive methods to evaluate the BBB dysfunction (17, 18), DCE-MRI not only exhibits good specificity and sensitivity but also can analyze the severity of BBB damage quantitatively. And as a non-invasive way, its' low risk will be easier to be accepted by patients.

In this study, the results showed that SLE patients have BBB dysfunction compared with HCs. And the increased BBB permeability was mainly located in the right insular area. In addition, to further explore whether the change of BBB permeability was associated with the emotional disorder of SLE patients, we divided patients into depression/anxiety groups and non-depression/non-anxiety groups to compare the degree of BBB damage in each subset. These results suggested that in SLE patients, the increased K^{trans} value in the right insula was associated with depression and anxiety.

A study using DCE-MRI technology to analyze the brain imaging of 6 SLE patients, found that the BBB permeability of SLE patients was increased compared with healthy controls (19), which was consistent with our results. And another study using DCE-MRI to assess BBB permeability found that BBB leakage was associated with gray matter loss and cognitive impairment (20), which were similar to the previous study using arterial spin labeling and diffusion-weighted brain MRI with a small sample size (21). Cagnoli et al. used magnetic resonance spectroscopy to study cell-level metabolic changes in several brain regions of SLE patients and HCs and found that compared with healthy controls and SLE patients without neuropsychiatric symptoms, neuron loss or damage was found in the right insular area of NPSLE patients (22). The abnormal brain area of NPSLE found in their study was consistent with the brain area of increased BBB permeability in SLE patients in our study.

Although there were several studies found that change of BBB permeability was associated with neuropsychiatric manifestations in SLE through other invasive methods (23–25), such as acute confusional state and cognitive dysfunction, our study was the first to explore the association between BBB permeability and depression/anxiety through the non-invasive method with relatively big sample size. This study not only provides evidence for extensive application of DCE-MRI to assess BBB damage in clinical management in SLE but also reveals that increased BBB permeability in the right insular region is associated with depression/anxiety in SLE patients.

Insula is a functional brain area involved in somatosensory and visceral sensation, regulating the conduction of pain and emotions, especially negative emotions (26). The anterior insula, as a component of ventral paralimbic regions, is an important part of the complex circuit initiating and regulating behavioral and emotional responses. In normal conditions, when the entire limbic-cortical depression network was activated, blood flow increased in the anterior insula (27). And in many neuropsychiatric diseases, abnormal damage of the insula and its network was found associated with emotional disorders. Frontotemporal dementia patients with damage of the anterior insula were found to present deficits in empathy and emotional reactivity (28). And the damage of insula networks was considered to contribute to depression in Parkinson's Disease (29). In major depression patients, bilateral amygdala and left anterior insula connectivity were found abnormally decreased in an affective network (30). These studies suggest that the destruction of structure in the insula and dysfunction of insular connectivity with other regions both can lead to depression.

Insula also plays an important role in anxiety (31). The severity of anxiety was considered to be positively correlated with central amygdala-insula functional connectivity (32), as well as somatic anxiety severity was found negatively correlated with the resting-state functional connectivity between anterior insula

TABLE 3 | The mean K^{trans} value of SLE and HC groups.

Mean K^{trans} value	SLE ($n = 42$)	HCs ($n = 23$)	*U*	*P*
Whole brain ($\times 10^{4)}$	13.331 (11.251, 21.442)	13.161 (9.722, 18.709)	421	0.395
Right insula ($\times 10^{5)}$	48.598 (35.378, 75.954)	28.017 (17.934, 43.300)	218	<0.001

SLE, systemic lupus erythematosus; HCs, healthy controls; U, U-value from Mann-Whitney U-Test; P, P-value from Mann-Whitney U-Test.

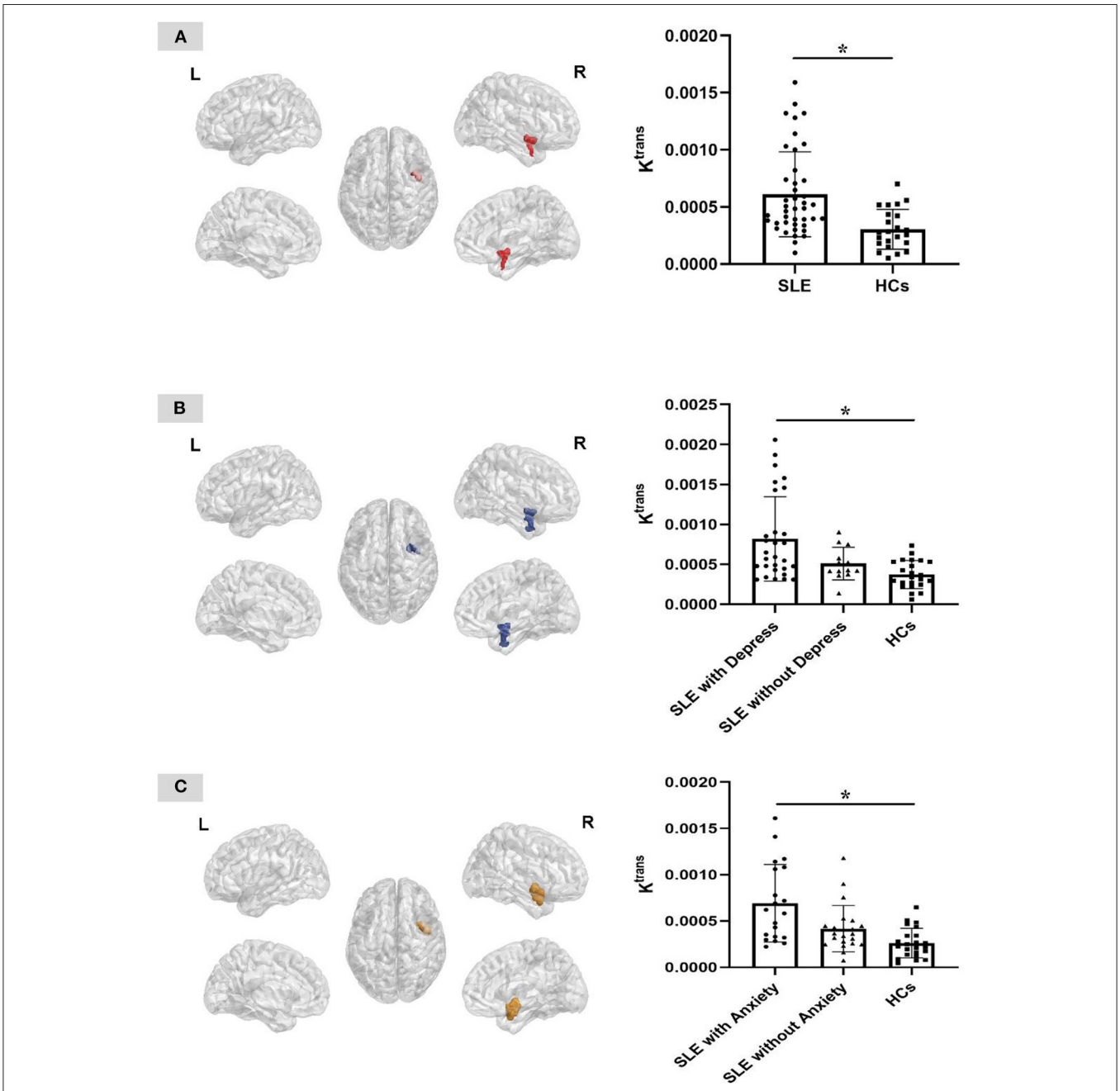

FIGURE 1 | Three-dimensional plane view of the area with different K^{trans} value between groups. **(A)** The right insular region presents significantly higher K^{trans} value in the SLE group than that of the HC group. **(B)** The right insular region presents significantly higher K^{trans} value in the SLE patients with depression group than that of the patients without depression and the HC group. **(C)** The right insular region presents significantly higher K^{trans} value in the SLE patients with anxiety group than that of the patients without anxiety and the HC group. *$P = 0.005$, cluster size > 100. L, left; R, right.

TABLE 4 | Regions with significantly different K^{trans} value in SLE patients and HCs.

Cluster location	Peak (MNI)			Number of voxels	*T*/F value
	x	*y*	*z*		
SLE patients and HCs					
Right insula	42	6	−14	265	3.6876
SLE patients with depression and HCs					
Right insula	44	0	−12	436	3.446
SLE patients with anxiety and HCs					
Right insula	44	−2	−12	196	4.2160

SLE, systemic lupus erythematosus; HCs, healthy controls.

and medial prefrontal gyrus (33). The mechanism of the insula that takes part in anxiety is complex. In rats, the insula was found to have a direct role in anxiety with a regional difference. Medial and caudal regions of the insula exhibit an anxiolytic role, while the rostral region of the insula shows an anxiogenic role (34). Robinson et al. reveal another possible mechanism that anterior insula contributes to the maintenance of anxiety through consisting of a feedback loop with the prelimbic cortex to convey the interoceptive information from visceral change to the prelimbic cortex (35).

Furthermore, besides the direct role in depression/anxiety, the insula also takes part in the brain regulation of immunity. Neurons in the insular cortex can acquire and retrieve specific immune-related information and the insular cortex activity could induce or promote inflammation (36). Hence, we speculate that the high incidence of emotional disorder in SLE patients may not merely result from neuropsychiatric pathogenesis as conventionally known, but also may be associated with its immunity dysfunction and severity of inflammation affecting insula, and the BBB damage of the insular region may be a potential way. But further research is needed to explore the underlying mechanism.

LIMITATIONS

There are also several limitations to our study. Firstly, the influence of various SLE treatment drugs could not be ruled out. For example, it has been reported that glucocorticoids have an impact on cognitive function (37). Recruiting untreated patients and comparing the DCE-MRI before and after treatment with a period of follow-up will be a reliable method to explore the effect of treatments on BBB damage. Secondly, the association of BBB damage with some clinical features could not be explored. Because of the limitation of sample size in this study, the number of patients in some subsets is insufficient to further analyze. Researches based on the larger sample size are needed.

In summary, this study found that SLE patients have increased BBB permeability, mainly in the right insular area. The increased BBB permeability in the right insular region is associated with depression/anxiety in SLE patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Kunming Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XW, LM, and YL were responsible for the management of the research and drafting the article. YY was responsible for data analysis. BU was responsible for language editing. YC was responsible for the psychiatric assessment of participants. YC, RC, and SL provided invaluable research consultation. YC and JX oversaw the entire research project and the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Prevalence of Metabolic Syndrome in Patients With Rheumatoid Arthritis: An Updated Systematic Review and Meta-Analysis

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Introduction: Rheumatoid arthritis (RA) due to systemic inflammation and insulin resistance increases the risk of cardiovascular disease and reduces life expectancy. In order to develop cardiac death prevention strategies, it is necessary to estimate the prevalence of metabolic syndrome (MetS) in these patients.

Methods: This systematic review and meta-analysis was performed to estimate the prevalence of MetS among patients with RA. International databases (i.e., Scopus, PubMed, Web of Science, and Google Scholar) were searched during the period of October 1 and October 10, 20121. Heterogeneity among the included studies was assessed through the Cochrane Q test statistics and I^2 test. Finally, a random-effects meta-analysis model was computed to estimate the pooled prevalence of MetS.

Results: Sixty-one articles with 96 groups and a sample size of 13,644 people were analyzed. The pooled prevalence of MetS was 32% (95% CI: 29.6–34.4). The highest prevalence of MetS is related to studies conducted in Asia (32.7%, 95% CI: 29–36.3) and Europe (32.7%, 95% CI: 27.5.37.9) and the lowest Prevalence was also related to studies conducted in Africa (28%, 95% CI: 28.8–32.2). The prevalence of MetS in men was 33% (95% CI: 26–39) and 34% (95% CI: 29–40) in women. Findings by diagnostic criteria showed that the highest and lowest prevalence of MetS was related to ATP III (37.5%, 95% CI: 30.9–44.2) and EGIR (14.4%, 95% CI: 10.5–18.5), respectively.

Conclusions: MetS is highly prevalent in patients with RA and identification of high-risk patients is necessary to prevent cardiovascular mortality.

Keywords: metabolic syndrome, rheumatoid arthritis, prevalence, systematic review, meta-analysis

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INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease of unknown etiology characterized by systemic symptoms, especially joint involvement and deformity (1). Patients with rheumatoid arthritis are at high risk for cardiovascular disease and premature death due to systemic inflammation, which reduces their life expectancy by 5 to 10 years (2, 3). Rheumatoid arthritis is associated with insulin resistance, dyslipidemia, and changes in adipokines profiles that are components of the metabolic syndrome (MetS) (4).

Insulin resistance is a constant risk factor for cardiovascular disease and the central mechanism in metabolic syndrome, which is present in 70% of patients with RA (5, 6).

MetS, also known as syndrome X and insulin resistance syndrome, refers to a set of cardiovascular risk factors (obesity, glucose intolerance, dyslipidemia, and high blood pressure) that can lead to cardiovascular disease (7). MetS increases cardiovascular outcomes and mortality by 2 and 1.5 times, respectively (8, 9). The increased risk of cardiovascular disease in patients with rheumatoid arthritis has been well established, so that the European League Against Rheumatism (EULAR) recommends that screening and management of cardiovascular risk in these patients be performed immediately (10, 11).

Various studies have shown that the prevalence of metabolic syndrome in these patients varies between 10 and 56% (12, 13). In this systematic review and meta-analysis, the cumulative prevalence of metabolic syndrome in patients with rheumatoid arthritis has been estimated.

METHODS

Search Strategy

The present systematic review and meta-analysis study was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (14). To access articles examining the prevalence of metabolic syndrome in patients with rheumatoid arthritis, a comprehensive search with no data limit was performed in the following databases: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search was conducted between October 1 and October 10, 2021. All article published until August 30, 2021 were included. Articles were searched with keywords ("Metabolic Syndrome"[Mesh] OR "Metabolic Syndrome*"[tiab] OR "Insulin Resistance Syndrome*"[tiab] OR "Metabolic X Syndrome*"[tiab] OR "Dysmetabolic Syndrome*"[tiab] OR "Reaven Syndrome*"[tiab] OR "Metabolic Cardiovascular Syndrome*"[tiab]) AND ("rheumatic diseases"[Mesh] OR "Arthritis, Rheumatoid"[Mesh] OR "Rheumatic disease*"[tiab] OR "Rheumatism*"[tiab] OR "Rheumatoid Arthritis"[tiab] OR "Rheumatic symptom*"[tiab]) AND ("Prevalence"[Mesh] OR "Prevalence*"[tiab] OR "Period Prevalence*"[tiab] OR "Point Prevalence*"[tiab]). The reference lists of the included articles were also reviewed to find other eligible articles.

Selection of Studies and Data Extraction

All observational studies published in English that reported the prevalence or frequency of metabolic syndrome in patients with rheumatoid arthritis were analyzed. Interventional, review, and replication studies, as well as studies investigating the prevalence of metabolic syndrome in other rheumatic diseases, were excluded. According to the inclusion and exclusion criteria, the titles and abstracts of the articles were independently reviewed by two researchers and the required information such as first author, year of publication, country of study, sample size, prevalence or frequency of metabolic syndrome in patients with rheumatoid arthritis were extracted and recorded in a pre-prepared form. To evaluate the quality of articles, the modified Newcastle-Ottawa Scale (NOS) was used, which has three main sections. The first part, rated on a scale of one to five stars, focuses on the methodological quality of each study (i.e., sample size, response rate, and sampling technique). The second section considers the comparability of the study cases or cohorts with a possibility of two stars to be gained. The last section is concerned with the outcomes and statistical analysis of the original study with a possibility of three stars to be gained. Two authors extracted the information and evaluated the methodological quality of the articles, independently. Any disagreements between the two reviewers were resolved consensus (15, 16).

Statistical Analysis

Point estimation and 95% confidence interval (CI) of metabolic syndrome due to binomial distribution formula and heterogeneity between studies was evaluated by Cochran Q test with a significance level of less than 0.1 and I^2 index. The degree of heterogeneity was assessed using the I^2 index. Heterogeneities were divided into three categories: less than 25% (low heterogeneity), 25 to 75% (moderate heterogeneity) and more than 75% (high heterogeneity). Pooled prevalence was estimated using a random-effects model. Subgroup analysis was performed based on diagnostic criteria and continent. To investigate the potential publication bias, funnel plot based on Egger's regression test was used. Univariate meta-regression was used to investigate the relationship between the prevalence of metabolic syndrome and the year of study and the mean age of patients. Data analysis was performed using Stata software version 16.

RESULTS

In the initial search, 938 potentially relevant articles were retrieved. Of these articles, 431 articles were excluded due to duplications and removing duplicate articles, 507 articles remained. The titles and abstracts of the remaining articles were reviewed and 411 irrelevant articles were removed. Of the remaining 96 articles, 34 articles were deleted for not reporting the prevalence of MetS (**Figure 1**).

Study Characteristics

In this study, 62 articles with a sample size of 13,644 people were analyzed, the characteristics of which are listed in **Table 1**. Most studies were performed in Morocco ($n = 9$) and Iran ($n = 9$). Most studies were based on NCEP/ATP III ($n = 42$) and IDF ($n = 21$) diagnostic criteria. Thirty-nine studies were conducted in Asia, 25 in Europe, 18 in the United States and 14 in Africa. All selected articles had good methodological quality.

The prevalence of MetS in patients with rheumatoid arthritis was 32% (95% CI: 29.6–34.4%). The prevalence of metabolic syndrome was 33% (95% CI: 26–39%) in men and 34% (95% CI: 29–40%)in women. The findings demonstrated that the highest prevalence of MetS was related to studies in Asia (32.7%, 95% CI: 29–36.3%) and Europe (32.7%, 95% CI: 27.5–37.9%) and the lowest prevalence was related to studies in Africa (28%, 95% CI: 22.8–33.2%) (**Figure 2**). Findings by diagnostic criteria

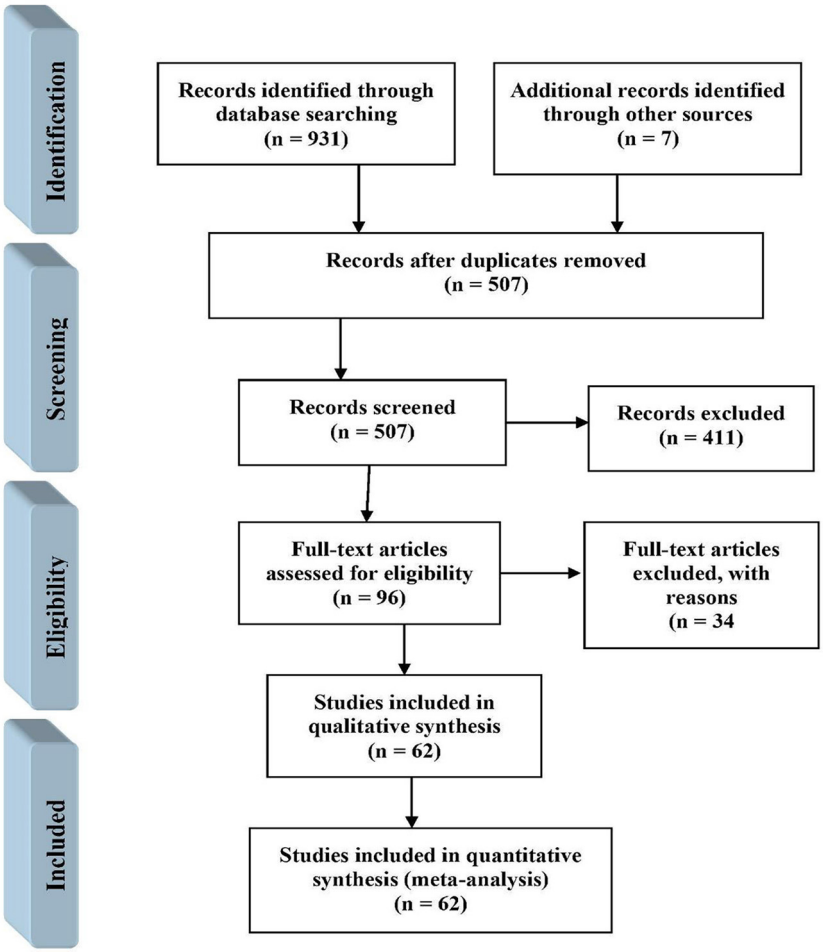

FIGURE 1 | Study selection following PRISMA guidelines.

of metabolic syndrome showed that the highest and lowest prevalence were related to ATP III (37.5%, 95% CI: 30.9–44.2%) and EGIR (14.4%, 95% CI: 10.5–18.5%) criteria, respectively (**Table 2**).

Meta-Regression

The results of meta-regression showed that the prevalence of MetShad increased significantly with increasing age (in studies in the Americas) ($p = 0.006$) (**Figure 3**). Also, the prevalence of MetS over time in studies in Asia was significantly increased ($p = 0.024$). Also, publication bias was not significant in the analyzed studies ($p = 0.569$).

DISCUSSION

The results of this study showed that one third of patients with RA have MetS. The results of a previous meta-analysis of 38 articles (with 70 groups) between 2007 and 2016 showed that the prevalence of MetS in patients with RA was 30.65%, which is almost consistent with the results of the present study (71). The reason for the high prevalence of metabolic syndrome in these patients can be attributed to traditional risk factors such as smoking, body mass index, gender, dyslipidemia and hypertension, although the role of continuous inflammation and activation of endothelial cells cannot be ignored (41). Inflammatory cytokines such as TNFα also reduce insulin function and facilitate insulin resistance (2). On the other hand, these patients use non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids to control the disease, which can cause metabolic disorders such as high blood pressure, obesity and diabetes (27). Serum levels of some biomarkers associated with metabolic syndrome, adipokines such as adiponectin, and biomarkers of endothelial cell activation and inflammation may appear to be useful in predicting cardiovascular risk in patients with RA (72).

The highest prevalence of metabolic syndrome was related to studies in Asia and Europe and the lowest prevalence was related to studies in Africa. Given that nutritional, ethnic and sociodemographic status are the determinants of the prevalence of metabolic syndrome, the reason for this finding can be attributed to these differences in these communities.

TABLE 1 | Characteristics of included articles.

First author	Year	Country	Sample size		Diagnostic criteria	Mean age	RA patients (%)		
			Total	M/F			Total	Male	Female
Turgunova et al. (17)	2021	Kazakhstan	101	31/70	IDF	-	40.5	-	-
Hee et al. (18)	2021	Singapore	561	0/561	NCEP/ATP III	-	44.9	-	-
					JC 2009	-	49.4	-	-
Giraud et al. (19)	2021	France	75	20/55	WHO	59.2	28	-	-
Kong et al. (20)	2021	China	717	152/565	CDS	61	31.2	-	-
Cioffi et al. (21)	2021	Italy	228	-	IDF	58	15	-	-
Mobini et al. (13)	2020	Iran	200	-	NCEP/ATP III	-	54.5	-	-
					IDF		56	-	-
Garcia-Chagollan et al. (4)	2020	Mexico	216	22/194	NCEP/ATP III	46	30.6	-	-
					ALAD		28.7	-	-
Xu et al. (22)	2020	Korea	247	48/199	NCEP/ATP III	58	15	-	-
Shaikh et al. (23)	2020	Pakistan	104	10/94	NCEP/ATP III	33.4	32.7	-	-
Ozkul et al. (24)	2019	Turkey	50	11/39	IDF	56.9	36	-	-
Mulumba et al. (3)	2019	Congo	75	15/60	NCEP/ATP III	51.8	25.3	-	-
Ene et al. (25)	2019	Romania	120	31/89	IDF-NCEP/ATP III	52.7	39.2	45.2	37.1
Naidu et al. (26)	2019	India	114	21/93	NCEP/ATP III	44.8	31.6	-	-
Kuriya et al. (27)	2019	USA	1543	443/1100	WHO	54	30.8	42	26
Akbal et al. (28)	2019	Turkey	53	12/41	ATP III	51	47.1	-	-
Aleksic et al. (29)	2019	Serbia	81	19/62	IDF	59.7	54.3	-	-
Mobini et al. (30)	2018	Iran	140	25/115	NCEP/ATP III	44.7	31.4	-	-
					IDF		35	-	-
Gomes et al. (7)	2018	Brazil	338	31/307	NCEP/ATP III	53.5	51.3	-	-
Burggraaf et al. (31)	2017	Netherland	212	65/147	NCEP/ATP III	54	40.1	-	-
Slimani et al. (32)	2017	Algeria	249	36/213	NCEP/ATP III	50.1	13.9	14.3	13.8
Pandey et al. (33)	2017	India	84	18/66	ATP III 2004	44.8	39.2	-	-
Ostojic et al. (34)	2016	Serbia	36	6/30	-	36	30.6	-	-
Lee et al. (35)	2016	Korea	598	110/488	AHA/NHLBI	63.6	36.4	34.5	36.9
Hugo et al. (36)	2016	France	57	15/42	IDF	57.6	24	25	24
Zafar et al. (37)	2016	Pakistan	384	97/277	NCEP/ATP III	43.8	31.3	18.5	35.5
Oliveira et al. (38)	2016	Brazil	107	0/107	NCEP/ATP III	55.5	51.4	-	51.4
					IDF		53.4	-	53.4
Muller et al. (39)	2016	Estonia	91	66/25	NCEP/ATP III	51.6	35	-	-
Dihingia et al. (40)	2016	India	72	6/66	NCEP/ATP III	41.5	16.7	-	-
Ghazaly et al. (41)	2015	Egypt	80	13/67	ATP III	40.7	50	53	49.2
Salamon et al. (42)	2015	Croatia	583	100/483	ATP III	59	43.1	40	43.7
Tantayakom et al. (43)	2015	Thailand	267	31/236	NCEP/ATP III	59	16.1	12.9	16.5
Parra-Salcedo et al. (44)	2015	Mexico	160	18/142	AHA/NHLBI	38.1	28	-	-
					IDF		18	-	-
					NCEP/ATP III		24	-	-
Craciun et al. (12)	2014	Romania	51	7/44	IDF-AHA	55.2	19	10.5	82.4
					NCEP/ATP III		23	-	-
					IDF		18	-	-
					AHA		14	-	-
Bilecik et al. (45)	2014	Turkey	100	0/100	IDF	52	33	-	33
					NCEP/ATP III		27	-	27
Ozmen et al. (46)	2014	Turkey	52	15/37	NCEP/ATP III	51	17.3	-	-
					WHO		28.8	-	-
Kumar et al. (47)	2014	India	54	6/48	IDF	46	29	-	-
					NCEP/ATP III		31	-	-

(Continued)

TABLE 1 | Continued

First author	Year	Country	Sample size		Diagnostic criteria	Mean age	RA patients (%)		
			Total	M/F			Total	Male	Female
Abourazzak et al. (48)	2014	Morocco	179	22/157	IDF	49	30.7	-	-
					NCEP/ATP III		29	-	-
					AACE 2003		24	-	-
Salinas et al. (49)	2013	Argentina	409	69/340	ATP III	55.5	30	62	23.8
					IDF		35	-	-
Abdul-Qaharr et al. (50)	2013	Iraq	203	41/162	NCEP/ATP III	46.9	51.2	12	92
Rostom et al. (51)	2013	Morocco	120	10/110	NCEP/ATP III 2004	49	30.8	10	32.7
					NCEP/ATP III 2001		24.6	-	-
					WHO		20	-	-
					IDF		48.6	-	-
					EGIR		18	-	-
					JC 2009		32.3	-	-
Lee et al. (52)	2013	Korea	84	0/84	NCEP/ATP III	50.6	19	-	19
Ormseth et al. (53)	2013	USA	162	18/144	ATP III	54	26	-	-
Karakoc et al. (1)	2012	Turkey	54	7/47	IDF	49.8	42.6	-	-
Manka et al. (54)	2012	Slovakia	87	4/83	IDF	58.8	48.3	-	-
					NCEP/ATP III		44.8	-	-
					AHA/NHLBI		47.1	-	-
Da Cunha et al. (55)	2012	Brazil	283	50/233	NCEP/ATP III	56.8	39.2	-	-
Goshayeshi et al. (56)	2012	Iran	120	14/106	NCEP/ATP III	45.5	45.2	-	-
Baker et al. (57)	2012	USA	499	83/416	IDF	49.5	10.6	-	-
Crowson et al. (58)	2011	USA	232	58/174	NCEP/ATP III	58.8	33	36	32
Sahebari et al. (59)	2011	Iran	120	14/106	IDF	45.5	30.8	28.6	41.5
					NCEP/ATP III		45.2	28.6	37.7
Karimi et al. (60)	2011	Iran	92	0/92	NCEP	48.3	27.2	-	27.2
					WHO		19.6	-	19.6
Mok et al. (61)	2011	Hong Kong	699	133/566	JS 2009	53.3	20	-	-
Dao et al. (62)	2010	Vietnam	105	0/105	IDF	56.3	40.9	-	-
					NCEP/ATP III 2004		32.4	-	-
					NCEP/ATP III 2001		24.7	-	-
					JS 2009		32.4	-	-
					WHO		19	-	-
					EGIR		16.2	-	-
Raterman et al. (63)	2010	Netherland	236	79/157	NCEP	62.1	19.9	-	-
Solomon et al. (64)	2010	South Africa	291	32/259	NCEP/ATP III	27.2	31.3	-	-
			335	65/270	NCEP/ATP III	27.2	20.3	-	-
Giles et al. (65)	2010	USA	131	51/80	NCEP/ATP III	61	36	-	-
Santos et al. (66)	2010	Portugal	98	0/98	ATP III	49.2	25.5	-	-
Toms et al. (67)	2009	UK	387	105/282	IDF	63.1	45.3	52.7	42.6
					NCEP/ATP III 2004		40.1	42.5	39.2
					NCEP/ATP III 2001		38.3	40	37.7
					WHO		19.4	25.5	17.2
					EGIR		12.1	22.6	8.2
Chung et al. (2)	2008	USA	66	18/48	WHO	59	42	-	-
Zonana-Nacach et al. (68)	2008	Mexico	107	-	NCEP/ATP III	42.9	18.7	-	-
Karvounaris et al. (69)	2007	Greece	200	53/147	ATP III	63	44	39.6	45.6
Montagna et al. (70)	2007	Italy	45	3/42	NCEP/ATP III	53.8	55.5	-	-

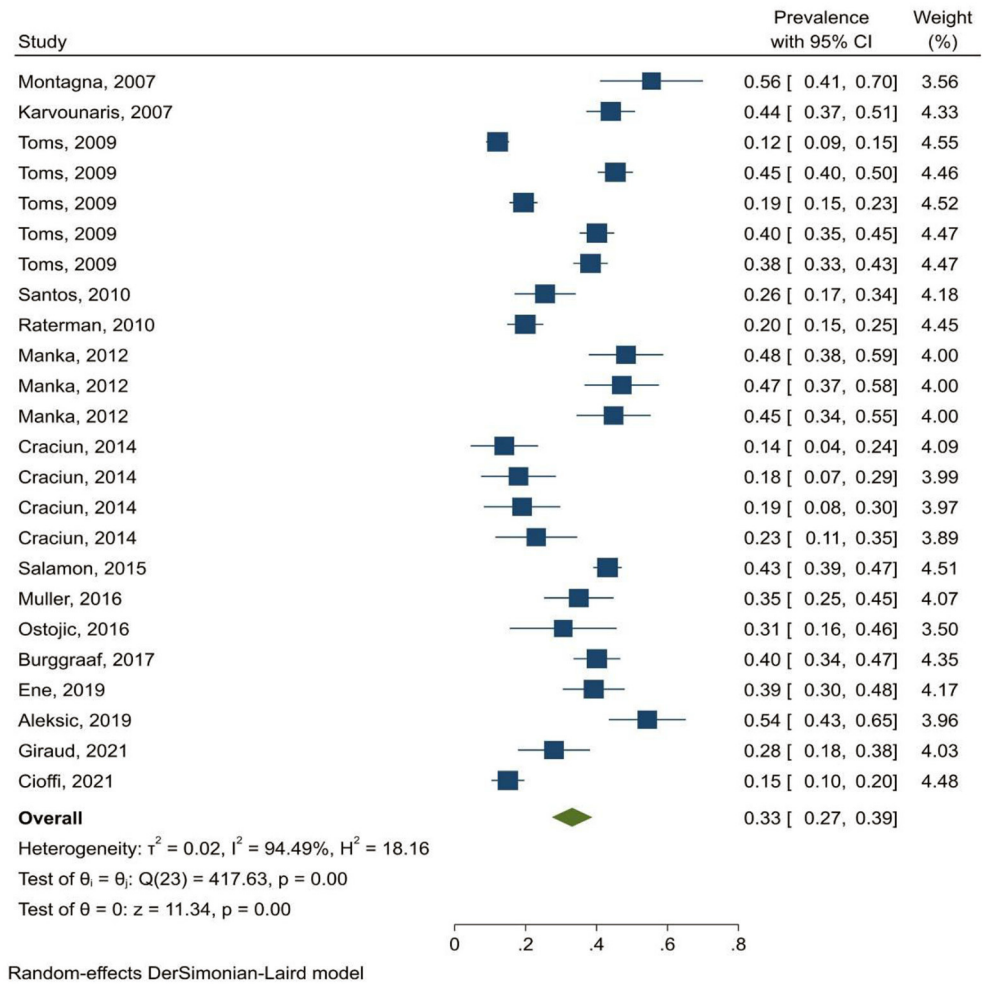

FIGURE 2 | Forest plot of the pooled prevalence of MetS in patients with RA in Europe.

TABLE 2 | Subgroup prevalence of MetS among patients with RA.

Subgroups	Number of studies	Prevalence (95% CI)	Between studies			Subgroup	
			$P_{heterogeneity}$	Q	Q	$P_{heterogeneity}$	I^2
Continent							
Asia	39	32.7 (29–36.3)	91.25%	0.001	505.13	2.39	0.495
Europe	25	32.7 (27.5–38)	93.37%	0.001	418.57		
America	18	32.3 (27–37.5)	94.66%	0.001	345.11		
Africa	14	28 (22.8–33.2)	88.24%	0.001	155.11		
Criteria							
WHO	8	25.2 (20–30.4)	81%	0.004	42.19	79.69	0.001
IDF	21	35.2 (29.4–41.1)	93.1%	0.017	482.13		
JS	4	33.5 (21–46)	95.6%	0.015	128.65		
NCEP/ATP III	42	32 (28.5–35.5)	91.2%	0.012	518.62		
ATP III	8	37.5 (31–44)	85.9%	0.007	47.09		
AACE	4	26.2 (17.3–35.2)	87.8%	0.007	25.17		
EGIR	3	14.4 (10.5–18.4)	36.75	0.001	2.92		

WHO, World Health Organization; IDF, International Diabetes Federation; EGIR, European Group against Insulin Resistance; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel; AACE, American Association of Clinical Endocrinologists; AHA/NHLBI, The American Heart Association / National Heart, Lung, and Blood Institute; JS, Joint Statement.

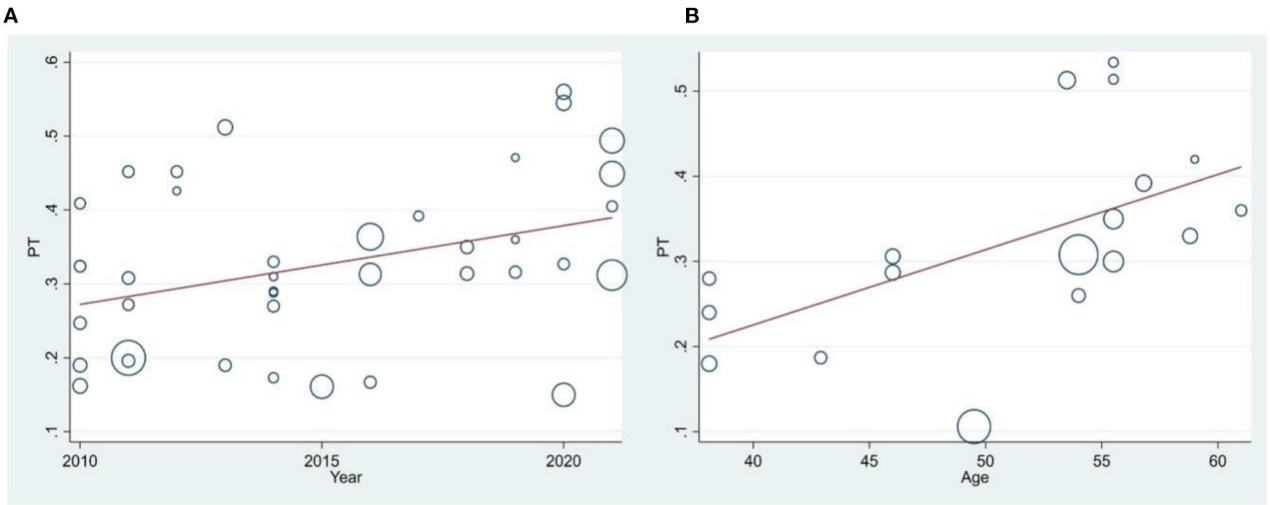

FIGURE 3 | Meta-regression of the prevalence of MetS among patients with RA based on year of study **(A)** and mean age **(B)**. Circles indicate the weight of studies.

In a study by Park et al. (73) the prevalence of metabolic syndrome in Korean and American adults was compared, and the results showed that the prevalence of metabolic syndrome and all its components (except low high density lipoprotein-cholesterol) was higher in American adults than in Korean. The two groups were not different in terms of blood pressure (73). The results of our study differ from those of Park et al. (73); in that they examined the prevalence of metabolic syndrome among patients with rheumatoid arthritis, not the general population. Therefore, further studies in this regard seem necessary.

The highest and lowest prevalence of metabolic syndrome were related to ATP III and EGIR criteria, respectively. In all diagnostic criteria, blood pressure, triglycerides, HDL cholesterol and fasting glucose are measured, and the difference between them is in the selection of the cut-off points and the measure of obesity. In WHO and EGIR criteria, the presence of hyperinsulinemia as an indicator of insulin resistance is the starting point, while in ATP III, the number of abnormalities is considered (69). These differences have led to different prevalence being reported in a group of patients (same patients) based on different criteria, so appropriate standards should be used to diagnose MetS in different regions. In a meta-analysis performed to estimate the prevalence of metabolic syndrome in postmenopausal women, the highest prevalence of metabolic syndrome was based on the ATP III screening criterion (74). The prevalence of metabolic syndrome increased significantly with age (in studies in the Americas). The prevalence of metabolic syndrome in the general population also increases with age (27), which can be due to redistribution of adipose tissue, weight gain, insulin resistance, and lipid changes (75).

Given that the prevalence of metabolic syndrome in patients with rheumatoid arthritis has not been studied in some countries and therefore has not been analyzed, the findings of this study should be generalized with caution worldwide.

CONCLUSION

Metabolic syndrome is so common in patients with RA that one-third of these patients have MetS, so identifying at-risk patients is essential to prevent cardiovascular events.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WC: concept, design, and drafting of the manuscript. WC, MP, and XT: acquisition, analysis, or interpretation of data. XT: critical revision of the manuscript for important intellectual content. MP: statistical analysis. All authors gave their final approval of this version of the manuscript.

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Resistin Expression Is Associated With Interstitial Lung Disease in Dermatomyositis

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Objective: In the current study, we aimed to assess resistin mRNA levels in the peripheral blood mononuclear cells (PBMCs) of dermatomyositis patients with interstitial lung disease (DM-ILD) and their correlation with disease activity.

Methods: We detected resistin mRNA levels in the PBMCs of 37 DM-ILD, 8 DM patients without ILD, and 19 healthy control (HC) subjects by performing quantitative reverse transcription real-time polymerase chain reaction analysis. Associations between resistin expression levels and major clinical manifestations, laboratory examinations, and disease activity were also analyzed. In addition, resistin expression in lung specimens from patients with DM-ILD was examined *via* immunohistochemistry and immunofluorescence.

Results: Resistin mRNA levels in PBMCs were significantly higher in DM-ILD than that in DM patients without ILD and HCs ($p = 0.043$, 0.014, respectively). Among these DM-ILD patients, the resistin levels were significantly elevated in those with rapidly progressive ILD than in those with chronic ILD ($p = 0.012$). The resistin mRNA levels in DM-ILD positively correlated with serum alanine aminotransferase ($r = 0.476$, $p = 0.003$), aspartate aminotransferase ($r = 0.488$, $p = 0.002$), lactate dehydrogenase ($r = 0.397$, $p = 0.014$), C-reactive protein ($r = 0.423$, $p = 0.008$), ferritin ($r = 0.468$, $p = 0.003$), carcinoembryonic antigen ($r = 0.416$, $p = 0.011$), carbohydrate antigen 125 ($r = 0.332$, $p = 0.047$), interleukin-18 ($r = 0.600$, $p < 0.001$), and lung visual analog scale values ($r = 0.326$, $p = 0.048$), but negatively correlated with the diffusing capacity of carbon monoxide (DLco)% ($r = -0.447$, $p = 0.041$). Immunohistochemical analysis of resistin showed its elevated expression in the macrophages, alveolar epithelial cells, and weak fibrotic lesions from patients with DM-ILD. Immunofluorescence staining confirmed CD68+ macrophages co-express resistin.

Conclusions: Resistin levels were increased in patients with DM-ILD and associated with disease activity and ILD severity. Therefore, resistin may participate in the pathogenesis of DM-ILD and may act as a useful biomarker.

Keywords: resistin, interstitial lung disease, dermatomyositis, disease activity, severity

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INTRODUCTION

Dermatomyositis (DM) is characterized by muscle weakness and inflammation of the skeletal muscle with progressive involvement in other organs, particularly in the lungs (1). According to previous findings, interstitial lung disease (ILD) is a major complication of DM with a high, ranging from 20 to 78% (2, 3). ILD is also a leading cause of hospitalization in patients with DM, resulting in increased mortality, and is a negative prognostic factor in patients with DM (3). Based on disease progression, two distinct subtypes of ILD have been described in DM, rapidly progressive interstitial lung disease (RP-ILD) and chronic ILD (4). In general, RP-ILD has an aggressive course with a 3-month survival rate of 51.7–72.4% (5, 6). Therefore, early diagnosis and careful monitoring are essential for the effective management of DM patients with ILD (DM-ILD).

Resistin, discovered in mice in 2001 and known as adipose tissue-specific secretory factor (ADSF) or found in inflammatory zone 3 (FIZZ3), is a 12.5 kDa cysteine-rich secreted protein that is involved in inducing insulin resistance (7, 8). Resistin is mainly expressed by peripheral blood mononuclear cells (PBMCs), macrophages, and myeloid cells (9). Resistin is associated with many inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis (10–12). Previous studies have shown that resistin can act as a pro-inflammatory cytokine. For example, resistin stimulates human macrophage-like cells and PBMCs to promote the production of pro-inflammatory cytokines (i.e., interleukin [IL]-6, IL-1β, and tumor necrosis factor [TNF]-α) (13–15). Resistin may play important roles in regulating inflammation (16). Under pathophysiological conditions, resistin has been observed in the lungs of patients with cystic fibrosis, scleroderma (SSc), and idiopathic pulmonary fibrosis (17–19). These findings suggest that the lungs are a target for resistin signaling. Resistin may be involved in the pathogenesis of ILD, which is characterized by lung injury and subsequent fibrosis (17–19). Resistin may also be a useful biomarker for ILD (11). Furthermore, serum resistin levels are elevated in patients with idiopathic inflammatory myopathy (IIM) and are associated with myositis-specific anti-Jo-1 antibodies, the overall disease activity index, and muscle damage (15). However, the clinical significance of resistin in DM-ILD has not been elucidated.

Therefore, we assessed the association between resistin mRNA levels in PBMCs and clinical variables and detected resistin expression in lung specimens from patients with DM-ILD using immunohistochemistry.

MATERIALS AND METHODS

Patients

Thirty-seven DM-ILD patients and eight DM patients without ILD, who visited the China-Japan Friendship Hospital between August 2016 and September 2020, were included in this study. All patients were diagnosed based on the criteria of Bohan and Peter (20, 21). ILD was diagnosed by high-resolution computed tomography (HRCT) (22). Patients combined with other connective tissue diseases and an age of onset of <18 years were excluded from this study. In addition, 19 sex- and age-matched healthy controls (HCs) from the Physical Examination Center of China-Japan Friendship Hospital were recruited. The patients' clinical and laboratory data (including demographics, main clinical features, laboratory data, and pulmonary function examination) were obtained from the hospital's electronic medical record. Physician global assessment (PGA) was performed to assess the disease activity of patients with DM, which was recorded on a continuous 10 cm visual analog scale (VAS). Myositis disease activity provides a comprehensive score for the whole body, skin, joints, lungs, heart, gastrointestinal system, and muscle organs or systems (23). Eight patients in this study were followed for 3–20 months. Each participant provided written informed consent before enrollment, and the study was approved by the Research Review

TABLE 1 | Characteristics of patients.

Characteristics	Patients ($n = 37$)
Female/male ratio	25/12
Onset age (years, mean ± SD)	52.89 ± 9.72
Disease duration [months, median (IQR)]	6.0 (2.5–11.5)
Clinical features, no. (%)	
Muscle weakness	14 (37.8%)
Myalgia	13 (35.1%)
Heliotrope rash	19 (51.3%)
Gottron papules/sign	18 (48.6%)
Mechanic's hands	17 (45.9%)
V sign	15 (40.5%)
Skin ulcers	3 (8.1%)
Arthritis/arthralgia	11 (29.7%)
Dysphagia	4 (10.8%)
RP-ILD	12 (32.4%)
Laboratory examinations	
Anti-MDA5, no. (%)	25 (67.5%)
Anti-ARS, no. (%)	5 (13.5%)
Other MSAs, no. (%)	4 (10.8%)
MSA negative, no. (%)	3 (8.1%)
CK, IU/l	95 (32–307)
LDH, IU/l	307.0 (231.5–447.5)
CRP, mg/dl	0.49 (0.25–0.86)
ESR, mm/H	23 (7–40.5)
Ferritin, ng/ml[a]	565.8 (140.7–1,215)
Pulmonary function test	
FVC (%)[b]	82.34 ± 22.13
FEV1 (%)[b]	75.96 ± 18.87
DLCO (%)[b]	65.23 ± 17.64
Physician VAS	5.0±2.4

ARS, aminoacyl-tRNA synthetases; CK, creatine kinase; CRP, C-reactive protein; DLCO, percent carbon monoxide diffusion capacity; DM, dermatomyositis; ESR, erythrocyte sedimentation rate; FEV1, predicted forced expiratory volume in 1 s; FVC, predicted forced vital capacity; ILD, interstitial lung disease; LDH, lactic dehydrogenase; MDA-5, melanoma differentiation-associated gene-5; MMT8, manual muscle testing of eight muscle groups; MSA, myositis-specific antibodies; VAS, visual analog scale. [a,b]*The data shown represent 36 and 34 patients, respectively.*

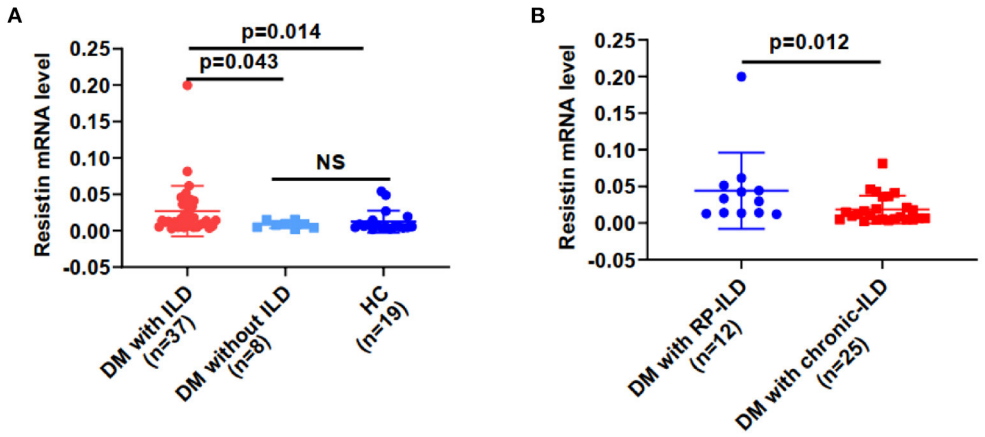

FIGURE 1 | Resistin mRNA levels were higher from PBMCs in DM patients with ILD than in HCs. **(A)** Resistin mRNA levels in DM patients with ILD, without ILD, and in HCs. **(B)** Resistin mRNA levels in patients with DM and RP-ILD or chronic ILD. DM, dermatomyositis; HC, healthy control; ILD, interstitial lung disease; RP-ILD, rapidly progressive interstitial lung disease. The relative resistin expression levels in each group were determined *via* the $2^{-\Delta Ct}$ method. The data shown are expressed as the mean ± SD.

Committee and Ethics Review Committee of the China-Japan Friendship Hospital.

Classification of ILD

Patients with DM-ILD were divided into two clinical subsets: those with RP-ILD and those with chronic ILD. RP-ILD is defined as rapidly progressing ILD with severe dyspnea symptoms and new interstitial abnormalities, upon HRCT examination, within 3 months (22). The diagnosis of chronic ILD is according to asymptomatic, slowly progressive ILD or non-RP-ILD imaging for more than 3 months (22).

Measurement of Resistin

PBMCs were separated from 6 ml peripheral blood samples from patients before immunosuppressive therapy during first hospitalization in patients with DM *via* Histopaque density-gradient centrifugation and stored in liquid nitrogen until the experiments were performed. A Rapid RNA Extraction Kit (Yishan, Shanghai, China) was used to extract total RNA from PBMCs, and NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) was used to quantify the RNA concentrations. PrimeScriptTM RT reagent (Takara Bio Inc., Japan) was performed to reverse-transcribe RNA into complementary DNA. To compare resistin mRNA levels between groups, quantitative reverse transcription real-time polymerase chain reaction analysis was performed using an ABI 7500 sequence detection system (Applied Biosystems, Foupsster City, CA) with SYBR Green Master Mix (Qiagen, Hilden, Germany) and an appropriate forward primer (5′-CTGTTGGTGTCTAGCAAGACC-3′) and reverse primer (5′-CCAATGCTGCTTATTGCCCTAAA-3′). The thermocycling conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Ribosomal protein S18 (RPS18) mRNA expression was detected as an internal reference for gene-expression analysis. Each sample was measured in triplicate. The $2^{-\Delta Ct}$ method was used to calculate the relative RNA expression levels, which were normalized to an endogenous control.

Immunohistochemistry

Lung biopsy specimens obtained by percutaneous lung biopsies were analyzed using immunohistochemistry. The tissues were fixed with 10% formalin, embedded in paraffin (6 μm thickness), deparaffinized with xylene, and preheated for 30 min in epitope-retrieval solution (Citric Acid Retrieval Solution, Aladdin, Shanghai, China). Then blocked with goat serum for 2 h. The tissue specimens were incubated with rabbit anti-resistin polyclonal antibody (1:200 dilution; Proteintech, Wuhan, China) and anti-CD68 (1:50; Abcam, Cambridge, MA, USA) overnight at 4°C. Goat anti-rabbit IgG secondary antibody (Gene Tech Shanghai Company Limited, Shanghai, China) was used to incubate the tissue specimens for 30 min at 25°C according to the manufacturer's instructions. Peroxidase activity was determined using 3,3′-diaminobenzidine (Gene Tech Shanghai Company Limited). The tissues were then counterstained with hematoxylin and observed under the microscope (Olympus, Tokyo, Japan).

Immunofluorescence Staining

Lung biopsy specimens were deparaffinized with xylene. Then, citric acid solution (Aladdin, Shanghai, China) was used for antigen retrieval at 100°C for 30 min. 0.3% Triton X-100 was used for cell membrane penetration. The samples were then blocked with goat serum for 2 h. The tissue specimens were then incubated with anti-Resistin (1:200 dilution; Proteintech, Wuhan, China), and anti-CD68 (1:50; Abcam, Cambridge, MA, USA) overnight at 4°C, and then with Alexa 488-conjugated or Alexa 555-conjugated secondary antibody (1:1,000; Cell Signaling Technology, Massachusetts, USA) for 30 min at 25°C. Next, 4′,6-diamidino-2-phenylindole (DAPI; Beyotime) was used to stain the cell nuclei, which were observed under a fluorescence

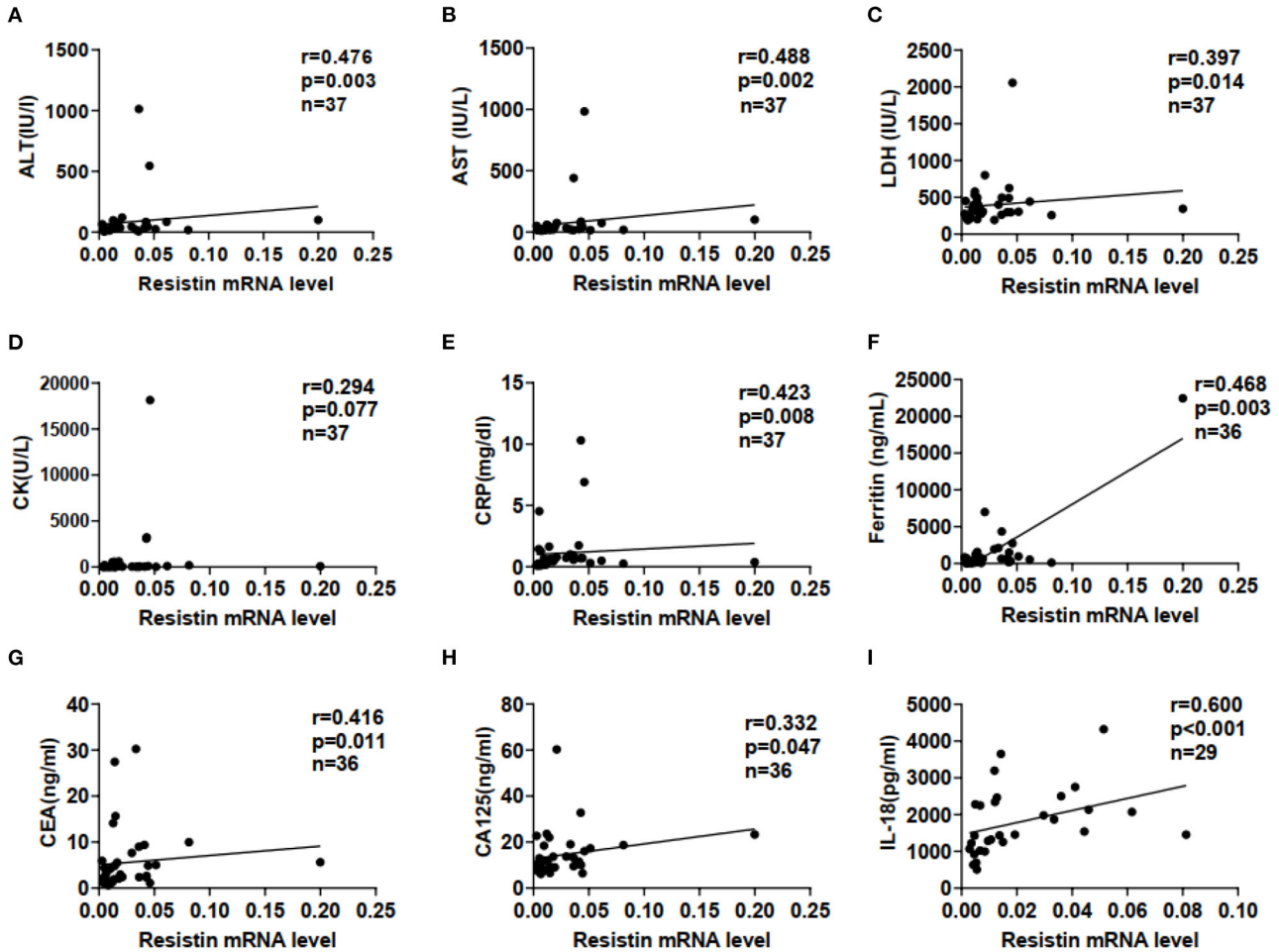

FIGURE 2 | Elevated resistin mRNA levels in PBMCs were associated with laboratory data for patients with DM-ILD. Correlation of resistin mRNA levels in PBMCs with **(A)** alanine aminotransferase (ALT), **(B)** aspartate aminotransferase (AST), **(C)** lactate dehydrogenase (LDH), **(D)** creatine kinase (CK), **(E)** C-reactive protein (CRP), **(F)** ferritin, **(G)** carcinoembryonic antigen (CEA), **(H)** carbohydrate antigen (CA)125, and **(I)** interleukin-18 (IL-18) levels. Correlations were assessed using Spearman's correlation analyses.

microscope (Olympus, Tokyo, Japan). Image J software was then used for image analysis and merging.

Measuring Serum IL-18 Levels

During the first visit, blood samples were obtained for IL-18 testing, and the data were compared with routine blood test results. The sera were stored at −80°C until they were determined. Serum IL-18 levels were measured using a Human Total IL-18 Valukine™ ELISA Kit (Novus Biologicals, USA), according to the manufacturer's instructions.

Statistical Analysis

Normally distributed data were described using the mean ± standard deviation (SD) and were compared by unpaired *t*-test. Non-normally distributed data were described using median [interquartile range (IQR)] and were compared by Mann-Whitney *U*-test. Spearman's correlation analysis was performed to assess correlations. Paired analyses between pre- and post-treatment samples were tested using the Wilcoxon's signed-rank test. Differences at $p < 0.05$ were considered statistically significant. Statistical analysis of the data was analyzed by using GraphPad Prism 8.0 and SPSS 25.0.

RESULTS

Clinical Features of the Patients Enrolled in This Study

Thirty-seven patients with DM-ILD were included in the study. Of these, 25 were women. The mean age at onset was 52.89 years, and the median disease duration was 6 months. The disease status was active at the baseline, as shown by laboratory data and physician VAS scores. Clinical manifestations, laboratory test

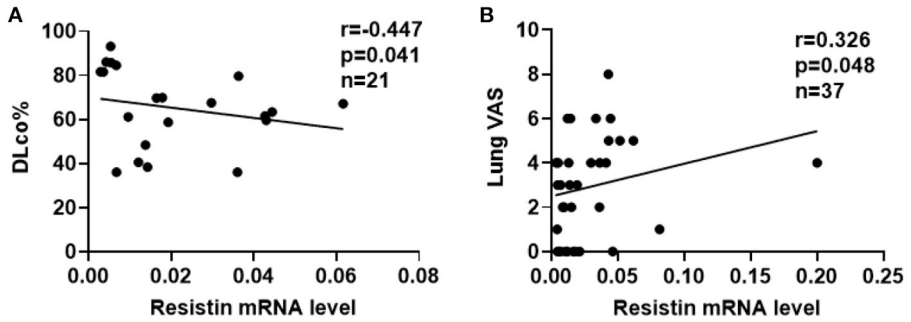

FIGURE 3 | Elevated resistin mRNA levels in PBMCs correlated with the severity of lung involvement in DM-ILD. **(A)** Resistin mRNA levels were negatively associated with the DLco% in patients with DM-ILD. **(B)** Correlation between resistin mRNA levels and lung VAS values in DM-ILD. DLco, carbon monoxide diffusion capacity; DM-ILD, dermatomyositis-related interstitial lung disease; VAS, visual analog scale.

results, and pulmonary function parameters are described in **Table 1**.

Expression of Resistin MRNA From PBMCs in Patients With DM-ILD

Resistin mRNA levels differed significantly in PBMCs from DM patients with ILD, without ILD and HCs. The median resistin mRNA levels in DM-ILD were 0.014 (0.006–0.038), which was significantly higher than that in DM patients without ILD [0.008 (0.004–0.013)] and HCs [0.005 (0.004–0.014)] ($p = 0.043$, 0.014, respectively) (**Figure 1A**). No statistical difference was observed between DM patients without ILD and HCs. The mean age of the HCs was 49.79 years, and 13 (68.4%) of the HCs were women. DM-ILD and HCs had no significant differences in age ($p = 0.31$) or sex ($p = 0.94$). DM-ILD consist of two subgroups: patients with chronic ILD and patients with RP-ILD, and their resistin mRNA levels in PBMCs were compared (**Figure 1B**). Interestingly, the resistin mRNA levels (range) in patients with RP-ILD were significantly greater than those in patients with chronic ILD (0.031, 0.013–0.049 vs. 0.010, 0.005–0.028, respectively; $p = 0.012$).

Correlations of Resistin mRNA Levels With Laboratory Data in DM-ILD

We examined correlations between resistin mRNA levels in PBMCs and laboratory data from patients with DM-ILD. Resistin mRNA levels were significantly associated with the levels of alanine aminotransferase (ALT; r = 0.476, $p = 0.003$), aspartate aminotransferase (AST; r = 0.488, $p = 0.002$), lactate dehydrogenase (LDH; r = 0.397, $p = 0.014$), C-reactive protein (CRP; r = 0.423, $p = 0.008$), ferritin (r = 0.468, $p = 0.003$), carcinoembryonic antigen (CEA; r = 0.416, $p = 0.011$), and carbohydrate antigen (CA)125 (r = 0.332, $p = 0.047$), as shown in **Figures 2A–H**. No significant correlations were found between resistin mRNA levels in PBMCs and creatinine kinase (**Figure 2D**). In addition, significant association was observed between resistin mRNA levels in PBMCs and serum IL-18 levels (r = 0.600, $p < 0.001$; **Figure 2I**).

Elevated Resistin mRNA Levels in PBMCs Correlated With the Severity of Lung Involvement in DM-ILD

To explore the associations between resistin mRNA levels in PBMCs and the severity of lung involvement in DM-ILD, we analyzed correlation's between resistin mRNA levels and pulmonary function test (PFT) parameters. We found that the diffusing capacity of carbon monoxide (DLco)% negatively correlated with resistin mRNA levels (r = −0.447, $p = 0.041$) (**Figure 3A**). No significant associations were found between resistin mRNA levels in PBMCs and the forced vital capacity (FVC) % (data not shown). Furthermore, the lung VAS scores were evaluated when the blood samples were collected. We found that resistin mRNA levels were positively associated with lung VAS scores (r = 0.326, $p = 0.048$) (**Figure 3B**). The results revealed that DM-ILD had higher resistin mRNA levels with more severe pulmonary symptoms.

Changes in Resistin mRNA Levels and Their Correlations With DM-ILD Activity

Furthermore, we compared resistin mRNA levels in PBMCs with disease activities in DM-ILD. In a cross-sectional study of 37 patients with DM-ILD, we found that resistin mRNA levels positively correlated with physician VAS scores (r = 0.343, $p = 0.037$) (**Figure 4A**). Clinical evaluations and blood tests were performed at each follow-up visit. Repeat blood samples were obtained from eight patients with DM-ILD (median pre-treatment to post-treatment interval; 12 [IQR: 3.5–16] months) before and after treatment. We tested the correlation between resistin mRNA levels and DM-ILD activities. Significantly decreased resistin mRNA levels in PBMCs were observed after immunosuppressive treatment ($p = 0.039$) (**Figure 4B**). The physician VAS scores also decreased after immunosuppressive treatment ($p = 0.007$) (**Figure 4C**).

Tissue Expression of Resistin in Patients With DM-ILD

We investigated resistin expression in lung tissues obtained from patients with DM-ILD ($n = 3$) and HCs ($n = 3$) by performing

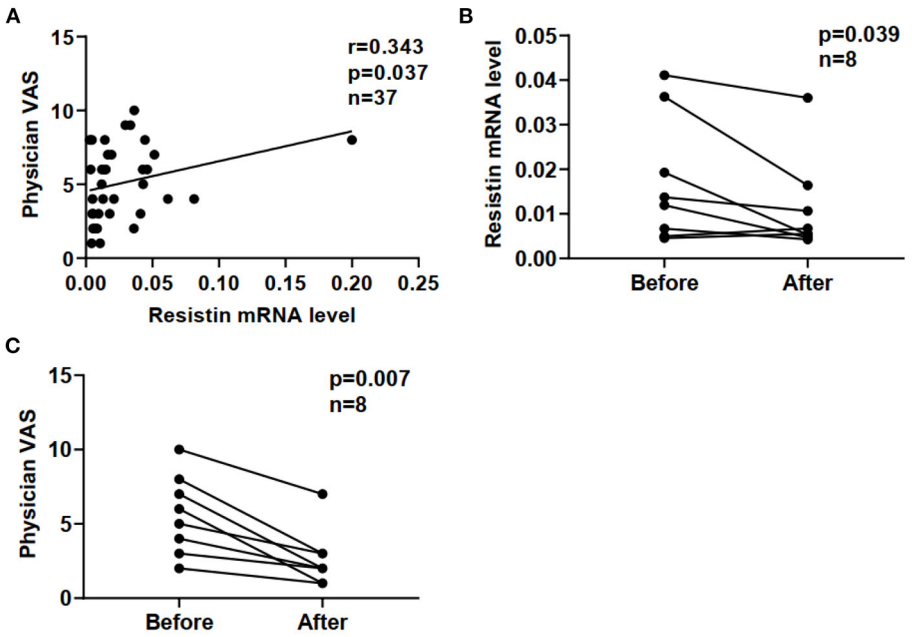

FIGURE 4 | Correlation between resistin mRNA levels and disease activities in DM-ILD. **(A)** Correlation between resistin mRNA levels and physician VAS scores. **(B)** Resistin mRNA levels before and after treatment. **(C)** Physician VAS scores before and after treatment. Paired analysis was assessed by Wilcoxon's signed-rank test. DM, dermatomyositis; ILD, interstitial lung disease.

immunohistochemical (IHC) staining. Lung sections from HCs showed a small number of resistin-positive macrophages (**Figures 5A,B**), whereas the lung sections from DM-ILD showed clear positive staining of resistin in macrophages, alveolar epithelial cells, and weak fibrotic lesions (**Figures 5C,D**). IHC staining for CD68 in DM-ILD confirmed that most resistin-expressing cells were macrophages (**Figures 5E,F**). IHC staining with an isotype-matched control antibody for resistin in DM-ILD and HC was also performed (**Figures 5G,H**). Furthermore, we performed immunofluorescence staining to confirm this as well. Two-color immunofluorescence was performed with resistin and CD68. CD68+ macrophages co-express resistin, which is rare in HC lung tissue (**Figure 5I**). However, in DM-ILD, a large number of CD68+ macrophages were seen co-expressing resistin (**Figure 5J**).

DISCUSSION

In this study, we revealed that resistin mRNA levels in PBMCs were obviously increased in patients with DM-ILD, especially in those with RP-ILD, and associated with the disease severity. We also found that resistin mRNA levels were associated with its severity in DM-ILD. Furthermore, resistin expression were more obvious in lung tissue specimens from patients with DM-ILD than in those from normal lungs. To our knowledge, this study is the first to determine the clinical significance of resistin in DM-ILD.

Previous studies have revealed that resistin levels were elevated in the synovial fluid of patients with RA and were positively associated with RA disease activity and joint damage (10). Similarly, compared with HCs or patients with osteoarthritis, serum resistin levels were increased in patients with RA and positively correlated with inflammatory markers such as ESR, CRP, and disease activity, which determined by measuring disease activity score 28 values. These studies indicated that resistin play a role in the regulation of inflammatory processes in RA (10, 24). Baker et al. revealed that serum resistin levels were increased in patients with SLE than in control subjects, and higher resistin levels were positively correlated with renal insufficiency, inflammatory markers, and disease damage (12). In another study, the serum and urine resistin levels of patients with lupus nephritis (LN) were elevated, which correlated with the relevant indicators of LN renal insufficiency and could be used as a potential marker of LN nephropathy (25). Filkova et al. observed higher serum resistin levels in patients with IIM, which strongly associated with CRP levels and the overall disease activity. In addition, resistin levels were significantly associated with overall disease activities and muscle enzyme levels in patients with DM (15). Similarly, serum resistin levels were elevated in young adult female patients with DM and correlated with age and disease activity (26). Another study showed that serum resistin levels were increased in patients with antisynthetase syndrome (26, 27). In our study, we found that resistin mRNA levels in PBMCs were higher in DM-ILD than in DM patients without ILD and normal controls. We observed correlations between resistin levels and disease activity in DM-ILD, which was consistent with the above-mentioned studies. Resistin mRNA levels varied with disease activity, and trends between resistin levels and PGA VAS scores in some

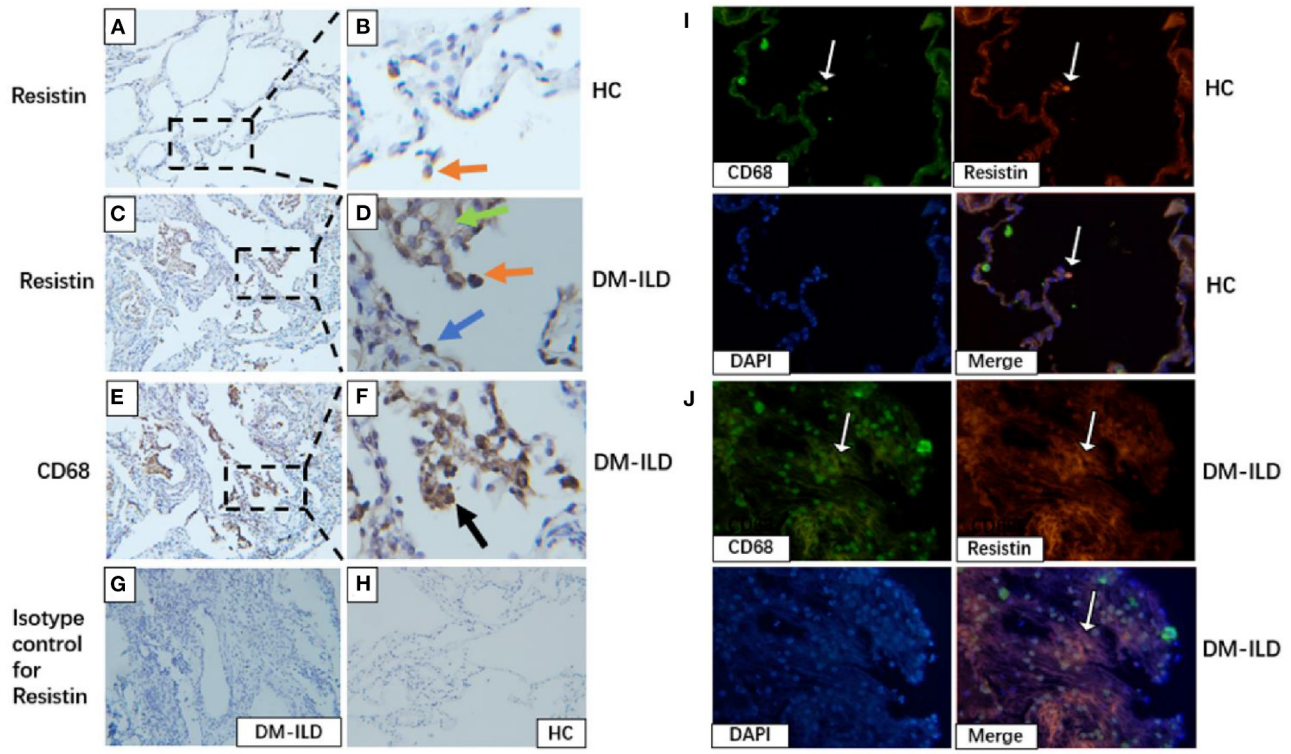

FIGURE 5 | Elevated resistin expression in the lung tissue of patients with DM-ILD. **(A,B)** Lung of healthy control: A small number of resistin–positive macrophages (red arrowhead). Scale bar = 50 μm. **(C,D)** Lung of patients with DM-ILD: Resistin was strongly expressed in macrophages (red arrowhead), alveolar epithelial cells (blue arrowhead), and weak fibrotic lesions (green arrowhead). Scale bar = 50 μm. **(E,F)** Lung of patients with DM-ILD: Immunohistochemistry analysis on serial sections using an anti-CD68 antibody confirmed that resistin-positive cells were mostly macrophages (black arrowhead). Scale bar = 50 μm. **(G,H)** Isotype-matched control antibody for resistin in DM-ILD and HC. Scale bar = 50 μm. **(I)** Lung of healthy control: Two color immunofluorescence of resistin (red) with CD68+ macrophages (green), DAPI (blue). Scale bar = 50 μm. **(J)** Lung of patients with DM-ILD: Two color immunofluorescence of resistin (red) with CD68+ macrophages (green). Scale bar = 50 μm. DM, dermatomyositis; ILD, interstitial lung disease; HC, healthy control.

patients were also shown. These results suggest that resistin levels parallel disease severity, at least in some patients with DM-ILD. Therefore, resistin levels in patients with DM-ILD may reflect overall disease activity.

Resistin is a pro-inflammatory cytokine that plays an important role in the development of inflammation by regulating the production of various cytokines and chemokines (16, 28). Previous studies have revealed that resistin mediates the secretion of IL-1, IL-6, and TNF-α in human monocytes through the nuclear factor-kappa B signaling pathway (16, 28). These pro-inflammatory cytokines can strongly induce resistin expression in monocytes/macrophages, triggering a positive feedback loop for self-injury (16). Filkova et al. observed that resistin induced the expression of several proinflammatory mediators, such as IL-1, IL-6, monocyte chemoattractant protein-1, and TNFα, in PBMCs from patients with IIM (15). These pro-inflammatory cytokines are involved in mediating the inflammatory pathogenesis of DM-ILD (29). Resistin is primarily expressed by macrophages (9). Macrophage activation also participates in the pathogenesis of DM-ILD (30). Previous results have shown that serum CRP, ferritin, IL-18, and LDH levels are related to DM-ILD, confirming this view (3, 29). During ILD progression, macrophage activation can promote hepatocyte injury, resulting in increased ALT and AST levels (31). Previous data also showed that serum CEA and CA125 levels were closely related to DM-ILD (32). In our study, resistin mRNA levels positively correlated with the above-mentioned ILD-related inflammatory markers and cytokines. Therefore, resistin may be involved in ILD pathogenesis in patients with DM.

Data from several *in vivo* and *in vitro* studies suggest that resistin is involved in the pathogenesis of fibrotic conditions. First, the murine homolog, murine resistin-like molecule alpha (mRELMα) was significantly upregulated in a rat model of bleomycin-induced pulmonary fibrosis, which stimulated alpha-smooth muscle actin and collagen I production in fibroblasts (33). mRELMα exhibits a crucial profibrotic effect in experiments in mRELMα knockout mice and adenoviral mRELMα overexpressing rats (34). Pulmonary fibrosis due to accumulation of asbestos and silica is correlated with high serum resistin levels (35, 36). These data revealed that resistin is involved in the immune response to fiber- or particle-induced fibrotic disease and may serve as a biomarker (35, 36). In a recent study, plasma and sputum resistin levels were elevated in patients with cystic fibrosis-related lung

disease and were associated with impaired lung function (17). Elevated serum resistin levels were also associated with organ involvement in SSc, including ILD (11). Our current findings also showed that resistin was associated with ILD. First, we found that resistin was elevated in PBMCs and lung tissues from patients with DM-ILD. Immunostaining of ILD specimens showed that resistin was expressed in both inflammatory cells and interstitial fibrosis tissue, suggesting that resistin may participates in inflammation and fibrosis in DM-ILD. Immunofluorescence staining confirmed CD68+ macrophages co-express resistin. Second, significantly higher resistin mRNA levels were detected in the patients with DM and RP-ILD. Furthermore, resistin mRNA levels positively correlated with ILD-related inflammatory markers and cytokines. Therefore, resistin may be involved in ILD pathogenesis in DM, and higher inflammation and fibrosis in RP-ILD may lead to higher resistin mRNA levels than in chronic ILD. Third, we found that resistin mRNA levels in DM-ILD correlated with DLco% and lung VAS scores. These metrics help to quantify the severity of ILD. Therefore, resistin mRNA levels are indicative of DM-ILD severity and disease activity (37).

This study had some limitations. First, the small sample size and single-center study design may have led to selection bias, and larger population-based multicenter studies are needed to confirm our preliminary results. Second, due to the retrospective design, some patients did not undergo laboratory tests for ferritin and lung function, so statistical bias could not be avoided. Third, the limited number of available lung-biopsy samples affected the statistics of the immunohistochemical results.

In conclusion, the current findings preliminarily show that resistin levels were elevated in PBMCs from patients with DM-ILD, especially those with RP-ILD. Furthermore, we demonstrated that resistin mRNA levels correlated with various clinical variables, including the DLco %. Therefore, resistin mRNA levels may serve as a marker of disease activity in DM-ILD. A prospective multicenter study is needed to elucidate the role of resistin in DM-ILD pathogenesis.

DATA AVAILABILITY STATEMENT

The data presented in the study are available from the corresponding authors upon request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of China–Japan Friendship Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LY, YX, PZ, HY, and SL participated in collecting the data and samples. YZ, FC, and QP participated in experimental design. GW and XS supervised the manuscript. LY wrote the manuscript. All authors contributed to the article and approved the submitted manuscript.

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The Efficacy and Safety of Pirfenidone Combined With Immunosuppressant Therapy in Connective Tissue Disease-Associated Interstitial Lung Disease: A 24-Week Prospective Controlled Cohort Study

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Objective: Interstitial lung disease (ILD) is a common manifestation of connective tissue disease (CTD) that manifests as several subtypes with significant differences in prognosis. It is necessary to evaluate the efficacy and safety of pirfenidone (PFD) combined with immunosuppressant (IS) in the treatment of CTD-ILD.

Methods: A total of 111 patients with CTD-ILD were enrolled, including those with systemic sclerosis (SSc), inflammatory myopathy (IIM), rheumatoid arthritis (RA), and other CTDs (such as systemic lupus erythematosus, primary Sjogren's syndrome, and undifferentiated CTD). After evaluation of the high-resolution computed tomography (HRCT), pulmonary function (PF), and basic disease activity, patients either were or were not prescribed PFD and were followed up regularly for 24 weeks.

Results: After 24 weeks of treatment, predicted forced vital capacity (FVC%) in the SSc-PFD group had improved by 6.60%, whereas this value was 0.55% in patients with SSc-no-PFD. The elevation in FVC% was also significant in IIM-PFD over the IIM-no-PFD controls (7.50 vs. 1.00%). The predicted diffusing capacity for carbon monoxide (DLCo%) of RA-PFD was enhanced by 7.40%, whereas that of RA-no-PFD decreased by 5.50%. When performing a subtype analysis of HRCT images, the change in FVC% among patients with SSc with a tendency toward usual interstitial pneumonia (UIP) was higher in those given PFD (SSc-PFD-UIP) than the no-PFD group (8.05 vs. −3.20%). However, in IIM patients with a non-UIP tendency, PFD displayed better therapeutic effects than the control (10.50 vs. 1.00%). DLCo% improved significantly in patients with the PFD-treated RA-non-UIP subtype compared with the patients with no-PFD (10.40 vs. −4.45%). Dichotomizing the patients around a baseline FVC% or DLCo% value of 70%, the PFD arm had a more improved FVC% than the no-PFD arm within

the high-baseline-FVC% subgroups of patients with SSc and IIM (6.60 vs. 0.10%, 6.30 vs. 1.10%). In patients with RA-PFD, DLCo% showed a significant increase in the subgroup with low baseline DLCo% compared to that in patients with RA-no-PFD (7.40 vs. −6.60%).

Conclusion: The response of PF to PFD varied between CTD-ILD subsets. Patients with SSc and IIM showed obvious improvements in FVC%, especially patients with SSc-UIP and IIM-non-UIP. In RA, the subsets of patients with non-UIP and a lower baseline DLCo% most benefited from PFD.

Keywords: connective tissue disease, interstitial lung disease, pirfenidone (PFD), systemic sclerosis, inflammatory myopathy, rheumatoid arthritis

INTRODUCTION

Connective tissue disease (CTD), including systemic sclerosis (SSc), inflammatory myopathy (IIM), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and primary Sjogren's syndrome (pSS), is a cluster of autoimmune diseases with multiorgan involvement. The lung is one of the most commonly affected organs. There are different subtypes of pathology and imaging manifestations of CTD-associated interstitial lung disease (ILD), and these patients may present with subclinical features following a slow or acute progressive course, the latter showing clinically significant rapid progression and mortality.

The incidence of CTD-ILD is reported to range from 12.4 to 34% (1). The etiology and pathogenesis of CTD-ILD are still unclear, but immune-mediated pulmonary inflammation and subsequent fibrosis are key elements in the development of the condition (2).

Different CTDs can manifest as different or same types of ILD. Thus far, pathological examination is the gold standard for typing, and the high-resolution computed tomography (HRCT) findings correspond well with the pathological changes. The common types of CTD-ILD are non-specific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), organizing pneumonia (OP), and lymphocytic interstitial pneumonia (LIP) (3–5).

In terms of CTD-ILD therapy, the Chinese guideline of 2018 emphasizes treatment of both CTD activity and ILD progression. Glucocorticoids (GCs) combined with immunosuppressive agents (IS) are used as the first-line treatment for different CTD-ILD, with no recommendations on how to use GCs for different imaging types, such as NSIP and UIP. Unfortunately, some patients have a poor response to such therapy due to fibrosis progression. Pirfenidone (PFD) is a pyridone-derived drug with extensive antifibrotic, anti-inflammatory, and antioxidant effects that modulates a number of cytokines, such as transforming growth factor-β1, interleukin (IL)-1, IL-4, IL-6, IL-8, IL-13, and tumor necrosis factor-α (6–9). It is currently approved worldwide for the treatment of idiopathic pulmonary fibrosis (IPF) based on its ability to slow the pulmonary function (PF) decline and disease progression, as shown in a number of phase III clinical trials (10–12). Based on these findings, PFD has been applied for some kinds of CTD-ILD, but few randomized clinical trials or strictly controlled studies have been reported (13, 14).

To verify whether PFD is effective for CTD-ILDs, our study was conducted to observe the efficacy and safety of PFD combined with GC and IS for the treatment of CTD-ILD.

MATERIALS AND METHODS

Patients

A total of 177 patients who met the diagnostic criteria of CTD-ILD were treated from August 2019 to May 2021 at the Department of Rheumatology of Qilu Hospital, Shandong University. All participants fulfilled the following criteria, namely, (1) age $\geq$ 18 years and (2) meeting the international classification standard of a CTD, including SSc, IIM, RA, SLE, pSS, and undifferentiated CTD (UCTD) (15–19). The ILD diagnosis conformed to the criteria of HRCT and PF formulated by the American Thoracic Association and the European Respiratory Association in 2002 (20). Detailed descriptions of the exclusion criteria are provided in **Supplementary Appendix S1**.

Study Design

In this prospective, controlled, single-center study, patients with CTD-ILD had all received a stable dosage of GC and/or IS as background therapy since 4 weeks before baseline and were followed up regularly for 24 weeks. The dosage of combined GC and the kind of IS was determined from the clinical characteristics of the different CTDs and was maintained throughout the study, regardless of antifibrosis. After the evaluation of PF (FVC% and DLCo%), HRCT, and basic disease activity, physicians recommended whether to add PFD and solicited the opinions of patients according to the inclusion criteria. PFD was initially prescribed at a 300 mg/day dosage, and the dosage was increased to the maximum tolerable dosage or to a maximum of 1,800 mg/day. The primary end point of our study was the change in PF after 24 weeks of treatment.

The prescribing principles of PFD in patients with CTD-ILD were (1) the patient fulfilled the inclusion and exclusion criteria of CTD-ILD; (2) the patient had a low FVC% and/or DLCo% value (usually <80%) or no PF improvement with GC and IS treatment in the past several months; and (3) the patient had symptoms of cough or dyspnea after activities, or the imaging area of fibrosis was large or tended to expand.

The reasons for non-use of PFD were (1) no significant deteriorations in respiratory symptoms, HRCT scan, or PF in the

screening state; and (2) patients' will. In this real-world study, most patients with CTD-ILD were reluctant to use PFD unless they felt the disease was serious, mainly due to the price of PFD and their insurance status.

All participants were forewarned of potential photosensitivity manifesting as a skin rash and were advised to use sunscreen during exposure to direct sunlight. This investigation was reviewed by the Ethics Committee of Qilu Hospital, Shandong University, and conducted in compliance with the Declaration of Helsinki (KYLL-202008-014). The study was registered in the Clinical Trial Registry (NCT04928586).

Study Assessments

Baseline data regarding the demographics, PF, HRCT, laboratory characteristics, disease activity (IIM, manual muscle test 8, myositis disease activity assessment visual analog scale, RA, disease activity score in 28 joints, clinical disease activity index, simplified disease activity index, and health assessment questionnaire) (21, 22), and previous therapy history of patients with CTD-ILD were collected. After 24 weeks of treatment, the above follow-up data and adverse events (AEs) were recorded in both the Qilu Hospital database and at the Chinese Rheumatism Data Center (CRDC) with the Chinese Rheumatology Information Platform (CRIP). The HRCT imaging characteristics were assessed independently by two experienced radiologists who were blinded to the final diagnosis of the lesions.

Statistical Analysis

Continuous variables that conformed to a normal distribution are expressed as mean ± SD, whereas continuous variables that did not conform to a normal distribution are expressed as median and interquartile range. The change between the 24-week value and the baseline value was further calculated (value of change = value at 24 weeks – value at baseline). The differences in continuous variables between two groups were analyzed by the independent-sample *t*-test and the Wilcoxon rank-sum test, whereas those within groups were evaluated using the paired *t*-test or paired Wilcoxon test. The chi-squared test and Fisher's exact probability test were used to compare the rates. Multiple linear regression analysis was used to evaluate the factors influencing the changes in FVC% and DLCo%. A $p < 0.05$ was considered statistically significant. All analyses were performed using the GraphPad Prism 8 and SPSS 24 software (IBM, Armonk, NY, USA).

RESULTS

Baseline Characteristics of Patients

The screening process of 177 patients is illustrated in **Figure 1**. A total of 136 patients were eligible after the evaluation of PF, HRCT, and basic disease. Then, 64 patients were prescribed PFD, and 72 patients were not (control group), all of whom were followed up regularly. Eventually, 111 patients completed the 24-week observation and were included in the analysis (56 in the PFD group, 55 in the control group).

We categorized the patients with CTD-ILD into 4 disease groups, namely, SSc, IIM, RA, and other CTDs (including SLE, pSS, and UCTD). The demographic and clinical characteristics, PF, HRCT imaging, and therapeutic regimen at baseline are shown in **Table 1**.

Among the four groups, patients with RA-ILD were older, had a longer disease duration, had higher ESR and CRP levels, and had lower serum albumin than the other groups (all $p < 0.05$). The SSc-ILD group was the youngest, and the patients with IIM-ILD had the shortest disease duration between the 4 groups (all $p < 0.05$). As shown in PF, the DLCo% in the other CTDs was lower than that of the SSc, IIM, and RA groups (54.58% ± 15.25% vs. 65.55% ± 18.34%, 68.71% ± 14.4%, 66.89% ± 12.17%, $p = 0.036$). In the field of HRCT imaging, a definite UIP pattern was observed in 7 (44%) patients with RA-ILD, which was more prevalent than that in the other three groups ($p = 0.010$). There were no significant differences in baseline FVC%, activity-related dyspnea, or unusual physical signs between the 4 groups.

In terms of the background treatment at baseline, the individuals with IIM-ILD were more likely to take high-dosage GC ($p < 0.001$). With regard to IS, patients with SSc-ILD were more likely to receive mycophenolate mofetil (MMF) and JAK inhibitor (JAKi). Tacrolimus (TAC) and MMF were frequently used in patients with IIM-ILD, whereas patients with RA-ILD preferred TAC and JAKi. The patients in the other CTDs group were more likely to receive MMF. There were no differences in UIP tendency, previous use of GC, hydroxychloroquine (HCQ), or IS between the 4 CTD-ILD groups.

Both baseline FVC% and DLCo% in the PFD group were lower than those in the control group ($p < 0.001$ and $p = 0.005$). Simultaneously, the individuals in the PFD group were more likely to have a high UIP tendency ($p = 0.002$). The baseline FVC% of patients with SSc-PFD and IIM-PFD was lower than that of patients with SSc-no-PFD and IIM-no-PFD, respectively ($p = 0.014$ and $p = 0.010$). Patients in the IIM group who received PFD generally had a low baseline DLCo% and high UIP tendency on HRCT ($p = 0.034$ and 0.008, respectively). There were no differences in the GC dosage or IS between the PFD and control groups in any of the 4 diseases. In addition, no differences were observed in the PF or HRCT scan type across the PFD groups of the 4 diseases (**Tables 2A,B**).

All patients with CTD-ILD were relatively stable in extrapulmonary performance throughout the study. The classification and disease activity of these patients are detailed in **Supplementary Tables 1–3**.

Changes in PF

The changes in FVC% (**Figures 2A–D,I**) and DLCo% (**Figures 2E–H,J**) from baseline to 24 weeks were compared in the PFD group and control group. We found that after 24 weeks of treatment FVC% in the SSc-PFD group was improved by 6.60% (3.10–8.46%), while this value was 0.55% (−6.80 to 5.35%) in the SSc-no-PFD group ($p = 0.042$). The elevation in FVC% was also different between the PFD and control groups of patients with IIM: 7.50% (0.55–14.45%) vs. 1.00% (−4.65

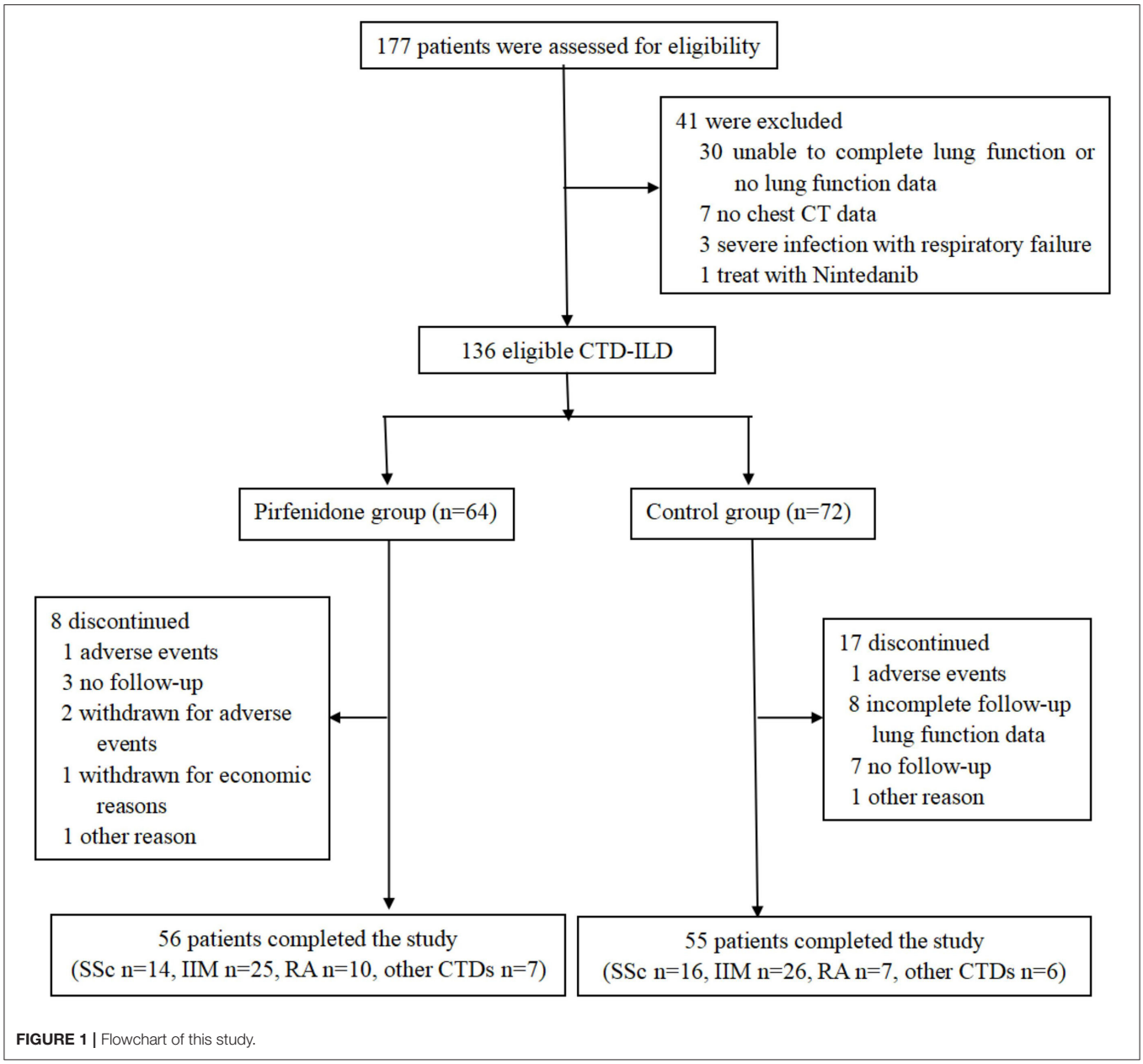

FIGURE 1 | Flowchart of this study.

to 7.43%) ($p = 0.016$). In contrast, the DLCo% of RA-PFD was enhanced by 7.40% (2.18–14.00%) compared with the RA-no-PFD decrease of 5.50% (−7.70 to −1.00%) from baseline ($p = 0.002$). No significant improvement in either the FVC% or DLCo% of the PFD group was found in the other CTD group.

Analysis of HRCT Subtype

When performing subtype analysis based on manifestations in HRCT, definite UIP and possible UIP patterns characterized by reticulation and/or honeycombing were grouped together as the UIP tendency in this study (23). Regarding the baseline FVC% of the SSc-UIP tendency subtype, the PFD-treated group had a lower value than the control ($p = 0.030$) (**Figure 3A**). A non-UIP tendency in patients with IIM was more likely to yield a poor DLCo% in the PFD group than in the control group ($p = 0.036$) (**Figure 3E**).

In addition, we found a significant improvement in FVC% from baseline to 24 weeks in the PFD group of SSc ($p = 0.021$, **Figure 3A**) and patients with IIM with UIP tendency ($p = 0.027$, **Figure 3B**). The same improvement in the FVC% of patients with IIM-non-UIP tendency was also detected after 24 weeks of PFD ($p = 0.009$, **Figure 3B**). DLCo% improved in patients with RA-non-UIP tendency after PFD treatment ($p = 0.047$, **Figure 3F**). There were no differences in the FVC% of patients with RA (**Figure 3C**) and DLCo% of patients with SSc (**Figure 3D**) in the PFD and control groups at either baseline or 24 weeks regardless of the HRCT subtypes.

TABLE 1 | The baseline clinical characteristics, PF, HRCT imaging features, and therapeutic regimen of the 4 CTD-ILD groups.

	SSc ($n = 30$)	IIM ($n = 51$)	RA ($n = 17$)	Other CTDs ($n = 13$)	p value
Age-years	45.17 ± 12.96	50.75 ± 10.57	56.12 ± 11.87	53.23 ± 10.73	**0.013**
Females (%)	29 (97.0)	39 (76.0)	13 (76.0)	12 (92.0)	**0.050**
BMI (kg/m²)	23.16 ± 3.77	23.93 ± 2.92	25.36 ± 3.10	23.23 ± 3.34	0.143
Former smoker (%)	4 (13.0)	4 (8.0)	3 (18.0)	1 (8.0)	0.611
Disease course (months)	24.00 (11.25–55.75)	7.50 (1.00–17.00)	60.00 (4.50–95.50)	31.00 (1.75–111.00)	**0.003**
FVC%	90.97 ± 21.17	84.8 ± 18.19	87.43 ± 16.16	87.08 ± 20.00	0.574
DLCo%	65.55 ± 18.34	68.71 ± 14.4	66.89 ± 12.17	54.58 ± 15.25	**0.036**
FVC%<70%	4 (13.3)	15 (29.4)	2 (11.8)	2 (15.4)	0.269
DLCo%<70%	16 (57.1)	28 (56.0)	10 (58.8)	12 (92.3)	0.064
Activity-related dyspnea (%)	21 (70.0)	33 (65.0)	9 (53.0)	6 (46.0)	0.401
Unusual physical signs (%)	8 (27.0)	14 (27.0)	4 (24.0)	3 (23.0)	1.000
Thoracic HRCT scan (%)					**0.01**
UIP	3 (10.0)	6 (12.0)	7 (44.0)	1 (8.0)	
NSIP	27 (90.0)	42 (82.0)	7 (44.0)	12 (92.0)	
OP	0 (0.0)	3 (6.0)	1 (6.0)	0 (0.0)	
LIP	0 (0.0)	0 (0.0)	1 (6.0)	0 (0.0)	
UIP tendency on HRCT (%)	11 (36.7)	17 (33.3)	9 (52.9)	4 (30.8)	0.501
ESR (mm/h)	35.50 (17.75–55.75)	18.00 (8.25–35.75)	50.00 (29.50–86.50)	28.50 (9.25–48.25)	**0.005**
CRP (mg/L)	0.80 (0.37–2.72)	0.62 (0.22–5.10)	6.49 (3.40–18.00)	1.46 (0.54–2.72)	**0.002**
Hemoglobin (g/L)	127.50 (116.50–137.75)	135.50 (127.30–146.00)	134.00 (117.00–142.00)	132.50 (117.00–143.50)	0.149
Albumin (g/L)	46.05 (42.48–48.10)	43.30 (38.08–45.98)	41.60 (37.60–44.85)	44.45 (42.43–48.18)	**0.011**
Globulin (g/L)	30.85 (28.75–33.63)	26.00 (22.75–30.85)	30.30 (25.75–33.20)	29.70 (25.20–37.35)	0.059
Baseline treatment					
GC use (%)	27 (90.0)	51 (100.0)	16 (94.1)	12 (92.3)	0.607
GC dosage (mg/d prednisone)	10.00 (5.00–15.00)	20.0 (12.5–45.00)	12.50 (7.50–20.00)	7.50 (2.80–20.00)	< 0.001
HCQ use (%)	25 (83.3)	36 (70.6)	12 (70.6)	10 (76.9)	0.608
Present DMARDs (%)					< 0.001
None	3 (10.0)	6 (11.8)	1 (5.9)	2 (15.4)	
MMF	16 (53.3)	12 (23.5)	0 (0.0)	7 (53.8)	
TAC	1 (3.3)	24 (47.1)	8 (41.7)	1 (7.7)	
JAKi	9 (30.0)	6 (11.8)	4 (23.5)	0 (0.0)	
Others	1 (3.3)	3 (5.9)	4 (23.5)	3 (23.1)	
Previous treatment					
GC use (%)	27 (90.0)	51 (100.0)	15 (88.2)	12 (92.3)	**0.037**
HCQ use (%)	23 (76.7)	36 (70.6)	11 (64.7)	11 (84.6)	0.628
DMARDs use (%)	25 (86.2)	45 (88.2)	15 (88.2)	12 (92.3)	1.000

PF, pulmonary function; other CTDs: included SLE, pSS, and UCTD; unusual physical signs: included cyanosis, velcro rale, and clubfoot; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; LIP, lymphocytic interstitial pneumonia; OP, organizing pneumonia; UIP tendency on HRCT: includes a definite UIP pattern and probable UIP pattern expressed by reticulation and honeycombing; GC, glucocorticoids; HCQ, hydroxychloroquine; DMARDs, disease-modifying antirheumatic drugs; MMF, mycophenolate mofetil; TAC, tacrolimus; JAKi, JAK inhibitor; others: other immunosuppressive drugs including iguratimod, cyclophosphamide, and cyclosporine.

Age, BMI, and baseline PF data are presented as means and standard deviations and tested by the t-test. The other data are presented as medians and ranges and were tested by the Mann-Whitney U-test. Bold numbers denote statistical significance.

After 24 weeks of treatment, the change in FVC% (**Figures 4A,C,E**) and DLCo% (**Figures 4B,D,F**) in different HRCT subtypes were compared between the PFD and control groups. We found the change in FVC% in patients with SSc-UIP given PFD was higher than in those not given PFD, 8.05% (6.15–19.43%) vs. −3.20% (−6.80 to 1.55%), $p = 0.014$ (**Figure 4A**). However, the non-UIP tendency subtype of patients with IIM given PFD showed a better change in FVC% than the patients with no-PFD: 10.50% (6.30–15.60%) vs. 1.00% (−4.65% to 7.43%), $p = 0.005$ (**Figure 4C**). DLCo% improved significantly in the PFD-treated RA-non-UIP subtype of patients than the patients with no-PFD: 10.40% (4.58–22.13%) vs. −4.45% (−8.90% to 2.1%), $p = 0.017$ (**Figure 4F**). There were no significant differences in GC or IS dosage at either baseline or follow-up between the different subgroups.

Analysis of Baseline PF Subsets

We also classified patients in both the PFD intervention and control groups according to whether the baseline FVC% and

TABLE 2A | The baseline PF and HRCT imaging in PFD-treated and control groups of patients with CTD-ILD.

	Total		SSc-ILD		IIM-ILD		RA-ILD		Other CTDs	
	Pirfenidone (*n* = 56)	**Control (*n* = 55)**	**Pirfenidone (*n* = 14)**	**Control (*n* = 16)**	**Pirfenidone (*n* = 25)**	**Control (*n* = 26)**	**Pirfenidone (*n* = 10)**	**Control (*n* = 7)**	**Pirfenidone (*n* = 7)**	**Control (*n* = 6)**
FVC%	80.58 ± 17.19***	93.81 ± 18.32	81.06 ± 18.81*	99.63 ± 19.69	78.23 ± 17.91*	91.12 ± 16.41	84.28 ± 15.60	91.93 ± 17.07	82.71 ± 15.60	92.17 ± 24.71
DLCo%	61.68 ± 13.40**	70.04 ± 16.88	60.65 ± 14.47	69.53 ± 20.57	64.25 ± 12.91*	72.82 ± 14.71	65.07 ± 9.57	69.49 ± 15.62	49.90 ± 13.47	60.03 ± 16.55
FVC%<70%	16 (28.6)	7 (12.7)	3 (21.4)	1 (6.3)	10 (40.0)	5 (19.2)	2 (20.0)	0 (0.0)	1 (14.3)	1 (16.7)
DLCo%<70	38 (70.4)**	28 (50.9)	10 (76.9)	6 (37.5)	15 (62.5)	13 (50.0)	6 (60.0)	4 (57.1)	7 (100.0)	5 (83.3)
Activity-related dyspnea (%)	36 (64.3)	33 (60.0)	11 (78.6)	10 (62.5)	18 (72.0)	15 (57.7)	5 (50.0)	4 (57.1)	2 (28.6)	4 (66.7)
Unusual physical signs (%)	16 (28.6)	13 (23.6)	3 (26.7)	5 (31.3)	10 (40.0)	4 (15.4)	1 (10.0)	3 (42.9)	2 (28.6)	1 (16.7)
Thoracic HRCT scan (%)										
UIP	12 (21.8)	5 (9.1)	3 (21.4)	0 (0.0)	5 (20.0)	1 (3.8)	3 (33.3)	4 (57.1)	1 (14.3)	0 (0.0)
NSIP	41 (74.5)	47 (85.5)	11 (78.6)	16 (100.0)	19 (76.0)	23 (88.5)	5 (55.6)	2 (28.6)	6 (85.7)	6 (100.0)
OP	2 (3.6)	2 (3.6)	0 (0.0)	0 (0.0)	1 (4.0)	2 (7.7)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
LIP	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)
UIP tendency on HRCT (%)	29 (51.8)**	12 (21.8)	8 (57.1)	3 (18.8)	13 (52.0)**	4 (15.4)	4 (40.0)	5 (71.4)	4 (57.1)	0 (0.0)

TABLE 2B | The baseline therapeutic regimen in PFD-treated and control groups of patients with CTD-ILD.

	Total		SSc-ILD		IIM-ILD		RA-ILD		Other CTDs	
	Pirfenidone (*n* = 56)	**Control (*n* = 55)**	**Pirfenidone (*n* = 14)**	**Control (*n* = 16)**	**Pirfenidone (*n* = 25)**	**Control (*n* = 26)**	**Pirfenidone (*n* = 10)**	**Control (*n* = 7)**	**Pirfenidone (*n* = 7)**	**Control (*n* = 6)**
GC use (%)	32 (94.6)	53 (96.4)	13 (92.9)	14 (87.5)	25 (100.0)	26 (100.0)	9 (90.0)	7 (100.0)	6 (85.7)	6 (100.0)
GC (mg/d prednisone)	15.00 (5.00–32.50)	15.00 (7.50–20.00)	6.25 (3.75–22.50)	10.00 (6.88–15.00)	25.00 (15.00–42.50)	16.25 (12.50–50.00)	15.00 (8.75–25.00)	10.00 (7.50–20.00)	5.00 (3.44–13.75)	17.50 (2.23–30.00)
HCQ use (%)	37 (66.1)*	46 (83.6)	10 (71.4)	15 (93.8)	16 (64.0)	20 (76.9)	6 (60.0)	6 (85.7)	5 (71.4)	5 (83.3)
DMARDs (%)										
None	8 (14.3)	4 (7.3)	2 (14.3)	1 (6.3)	4 (16.0)	2 (7.7)	1 (10.0)	0 (0.0)	1 (14.3)	1 (16.7)
MMF	18 (32.1)	17 (30.9)	5 (35.7)	11 (68.8)	8 (32.0)	4 (15.4)	0 (0.0)	0 (0.0)	5 (71.4)	2 (33.3)
TAC	12 (21.4)	22 (40)	0 (0.0)	1 (6.3)	7 (28.0)	17 (65.4)	5 (50.0)	3 (42.9)	0 (0.0)	1 (16.7)
JAKi	12 (21.4)	7 (12.7)	6 (42.9)	3 (18.8)	4 (16.0)	2 (7.7)	2 (20.0)	2 (28.6)	0 (0.0)	0 (0.0)
Others	6 (10.7)	5 (9.1)	1 (7.1)	0 (0.0)	2 (8.0)	1 (3.8)	2 (20.0)	2 (28.6)	1 (14.3)	2 (33.3)

PF, pulmonary function; other CTDs: included SLE, pSS, and UCTD; unusual physical signs: included cyanosis, velcro rale, and clubfoot; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia; LIP, lymphocytic interstitial pneumonia; OP, organizing pneumonia; UIP tendency on HRCT: includes definite UIP pattern and probable UIP pattern expressed by reticulation and honeycombing. GC, glucocorticoids; HCQ, hydroxychloroquine; DMARDs, disease-modifying antirheumatic drugs; MMF, mycophenolate mofetil; TAC, tacrolimus; JAKi, JAK inhibitor; others: other immunosuppressive drugs include iguratimod, cyclophosphamide, and cyclosporine.

*Baseline pulmonary function (PF) data are presented as means and standard deviations and were tested by Student's t-test. The others are presented as medians and ranges and tested by Mann-Whitney U-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the control group.*

DLCo% values were <70%. The results illustrated that the change in FVC% of the PFD group was higher than that of the control group in all patients with CTD-ILD regardless of whether the baseline FVC% was <70%. Particularly in the subset with baseline FVC% ≥ 70%, the PFD group exhibited an FVC% that was increased by 4.10% (−1.40 to 7.85%) compared with an increase of 0.30% (−3.10 to 4.68%) in the control group ($p = 0.050$) (**Table 3**).

Regarding the specific diseases, the improvement in FVC% was significantly higher in the SSc-PFD and IIM-PFD group with high baseline FVC% than patients with no-PFD: 6.60% (−1.23 to 11.50%) vs. 0.10% (−6.80 to 4.60%) ($p = 0.047$) and 6.30% (0.50 to 10.50%) vs. 1.10% (−3.30 to 6.80%) ($p = 0.089$), respectively. Among patients with RA, DLCo% showed a significant increase in the <70%-baseline-DLCo% subset given PFD compared to those not given PFD: 7.40% (2.18 to 14.03%) vs. −6.60% (−8.60 to 2.13%), $p = 0.011$. There was no significant improvement in DLCo% among patients with SSc after PFD treatment (**Table 3**).

Analysis of PFD Dosage

In this study, a total of 56 patients in the PFD group received PFD for 24 weeks, and the dosage of PFD was adjusted according to the protocol. Four patients increased the dosage of PFD in the first 4 weeks, which then decreased to 400 mg/day due to their intolerance at 12 weeks and maintained up to 24 weeks. The others gradually increased the dosage up to the maximum tolerable dosage or to a maximum of 1,800 mg/day, including one patient taking a maximum tolerable dosage of 400 mg/day from the beginning. The mean daily dosage of PFD was 786.63 (682.87–896.5) mg/day, at the range of 400–1,800 mg/day (**Supplementary Tables 4**, **5**).

When the PFD dosage was adjusted to 800 mg/day at 24 weeks, we found no differences in the change in PF or the reduction dosage of GC between high- and low-dosage-PFD subsets (**Supplementary Table 6**). There were no differences in the dosage of PFD across the PFD groups of the 4 diseases.

Multiple Linear Regression Model for the Change in PF

Multiple linear regression analysis was used to identify the influencing factors of the change in FVC% and DLCo%. The basic clinical characteristics of patients with CTD-ILD, such as age, sex, body mass index (BMI), smoking history, disease duration, baseline FVC% < 70%, baseline DLCo% < 70%, dyspnea after activity, laboratory results, and UIP tendency on imaging, were first included. It was found that the baseline FVC% < 70% significantly affected the change in FVC%, and the baseline DLCo% < 70% affected the change in DLCo%. The therapeutic regimen was tested in another model, which included the average dosages of GC, HCQ, IS, and PFD as variables. The data indicated that PFD and high-dosage GC were positive factors for increased FVC%, and only PFD was significant in terms of DLCo% improvement (**Supplementary Tables 7**–**10**). In the pooled analysis, all factors affecting the changes in FVC% and DLCo% with $p < 0.100$ and the basic clinical index were included in the regression model, and baseline FVC% < 70% and PFD were found as positive factors influencing the changes in FVC% and DLCo% in CTD-ILD (**Tables 4A,B**).

AEs in the Study

The AEs that occurred during the study period are summarized in **Table 5.** A total of 30 AEs occurred in 21 patients in the PFD group compared with 22 AEs in 15 patients in the control group (32.80 vs. 20.83%, $p = 0.124$). The AEs that were more often found in the PFD group than the control group were gastrointestinal events, including abdominal distension, gastroesophageal reflux, and diarrhea ($p = 0.067$). All these AEs were mild to moderate, reversible, and without clinical sequelae. Respiratory infections were equal in both PFD and control patients (10.64 vs. 9.72%). There were no significant differences in the occurrence of other AEs, such as skin infections, urinary infections, and rash.

Treatment-emergent serious AEs occurred in 3 patients (4.69%) in the PFD group and 6 patients (8.33%) in the control group. In the PFD group, a 57-year-old patient with melanoma differentiation-related gene 5 (MDA5)-positive dermatomyositis died of respiratory failure, mediastinal emphysema, and respiratory infections; this individual was receiving 15 mg/day of GC, 10 mg/day of tofacitinib, and 900 mg/day of PFD at that time. Two patients were hospitalized with infections (mumps and respiratory infections). In the control group, 1 patient experienced cerebral thrombosis, 3 patients were hospitalized for rash, and 2 subjects were hospitalized for respiratory infections.

DISCUSSION

The efficacy of PFD in the treatment of IPF has been confirmed by several clinical trials, which have shown that PFD may delay the decline in FVC and increase the progression-free survival rate (10–12, 24, 25). However, the clinical indication of PFD is still limited to patients with IPF, and no large cohort of PFD-treated patients with CTD-ILD has been reported. Therefore, our prospective study aimed to observe the efficacy of PFD in CTD-ILD and identify the best-responding HRCT subtype and baseline PF index among PFD-treated patients with CTD-ILD.

As this was a real-world study initiated by an investigator, we could not obtain free PFD for a randomized study on CTD-ILD. In contrast, the inclusion criteria of the PF threshold changed gradually in several previous studies on PFD. In a randomized controlled trial published in 2020 assessing the efficacy and safety of PFD in SSc-ILD (26), patients were included who met the disease criteria and had an FVC% value <80%. The inclusion criteria of the RELIEF study (27), a phase 2b trial on PFD, included patients with progressive fibrotic ILD, not limited to IPF, with FVC% and DLCo% values <90%. In a retrospective study on interstitial pneumonia with autoimmune features (IPAF), patients using PFD were enrolled without an upper limit for FVC% or DLCo% (28), and the therapeutic effect was also good. Although the criteria for the use of antifibrosis agents have been updated often in recent years, the benefits of PFD against CTD-ILD seem more pronounced. Meanwhile, the respiratory symptoms of patients with CTD-ILD were not completely consistent with their manifestations on HRCT scans

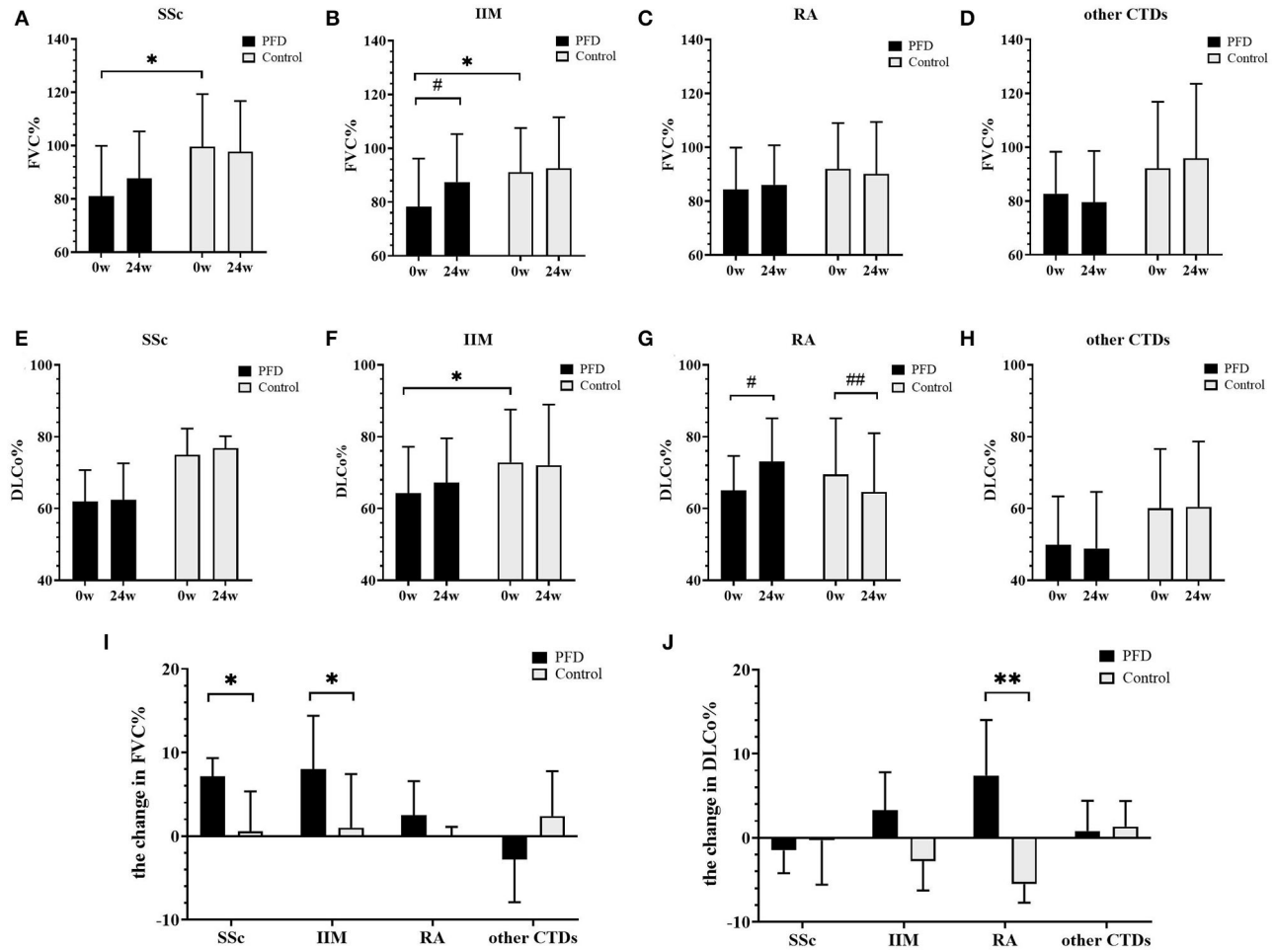

FIGURE 2 | Changes in FVC% and DLCo% in the PFD-treated and control groups of patients with CTD-ILD over 24 weeks. FVC% changes in patients with **(A)** SSc-ILD, **(B)** IIM-ILD, **(C)** RA-ILD, and **(D)** other patients with CTD from baseline to 24 weeks. DLCo% changes in patients with **(E)** SSc-ILD, **(F)** IIM-ILD, **(G)** RA-ILD, and **(H)** other patients with CTD from baseline to 24 weeks. The changed value of **(I)** FVC% and **(J)** DLCo% in patients with SSc, IIM, RA, and other patients with CTD. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the no-PFD control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the baseline value. Baseline and 24-week pulmonary function data are presented as the means and standard deviations and were tested by the unpaired *t*-test. The other results are presented as medians and ranges and were tested by the Mann-Whitney *U*-test.

and the PF. Therefore, we prescribed PFD for some patients with obvious imaging progress or UIP subtypes regardless of their PF to observe whether the rapid PF decline could be held off by PFD.

For these reasons, the patients in the PFD group had a poorer PF than the controls at baseline, which caused the imbalance and deviation in PF and HRCT. The patients needed to be grouped by their specific diseases, HRCT subtypes, and PF levels to reduce confounding effects, which made the subgroups too small for analysis. However, when we combined similar cases into a general cluster, the amount of GC and type of IS agent were not different between the HRCT and baseline PF subgroups, with or without PFD, but varied widely between different CTD-ILDs. Therefore, we described the results in terms of different diseases to minimize bias.

Recent studies have reported that a definite UIP pattern or a possible UIP pattern characterized by honeycomb and/or grid shadows in the ILD classification often demonstrates a poor prognosis (23). Therefore, we classified patients with a definite UIP pattern or a possible UIP pattern on HRCT as having a UIP tendency and found the IIM-non-UIP and RA-non-UIP tendency subtypes showed superior therapeutic effects of PFD. Interestingly, the change in FVC% in the SSc-UIP tendency subtype with PFD was higher than that in the control, a finding that needs to be supported by more data in a large number of patients with SSc-UIP tendency. Based on these results, we speculate that the PFD response could be influenced by both the imaging subtype and background disease.

To exclude the effect of differences in baseline PF between the PFD and control groups, we stratified the patients around a 70% baseline FVC% or DLCo% based on the phase III trial conducted in Japan (29). In general, the improvement in FVC% of the PFD group was higher than that of the control group

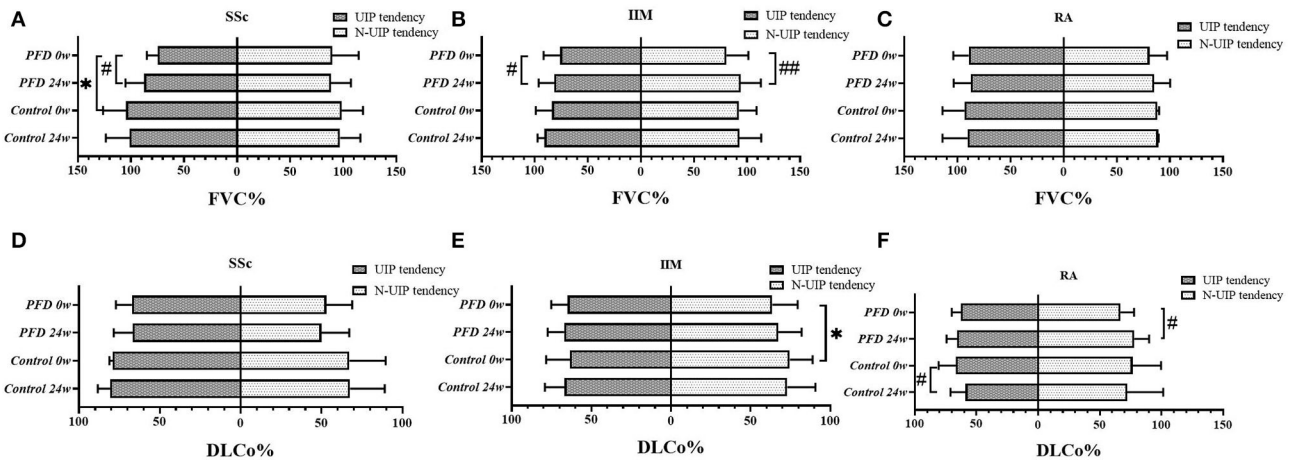

FIGURE 3 | The PF value in different HRCT subtypes of the 3 CTD-ILD groups at baseline and 24 weeks comparing PFD treatment with the no-PFD control treatment. The value of FVC% in patients with **(A)** SSc-ILD, **(B)** IIM-ILD, and **(C)** RA-ILD and the value of DLCo% in patients with **(D)** SSc-ILD, **(E)** IIM-ILD, and **(F)** RA-ILD at baseline and 24 weeks comparing the efficacy of PFD in UIP and non-UIP tendency subtypes with that of the no-PFD control. $^{*}p < 0.05$ compared to the no-PFD control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to baseline value.

regardless of the baseline value, but it was significantly greater in high-baseline-FVC% PFD-treated patients with SSc and IIM and low-baseline-DLCo% patients with RA.

We were delighted to find that the patients with CTD-ILD responded to PFD very well. The improvement in FVC% or DLCo% of the PFD group was more significant than that of the control after 24 weeks of treatment even though the PFD-treated patients were more likely to have a poorer PF and/or to have a UIP tendency at baseline. Multiple linear regression analysis also showed that baseline FVC% < 70% and PFD acted as positive factors for the changes in FVC% and DLCo% in CTD-ILD. As a result, patients with IIM and RA, with a non-UIP tendency and a lower PF at baseline, would most likely benefit from PFD.

In recent years, it has been noted that patients with IIM positive for anti MDA5 antibody usually have rapidly progressive interstitial lung disease (RP-ILD) and a poor prognosis. In this study, we enrolled 51 patients with IIM, including 20 MDA5-positive patients (10 in the PFD group and 10 in the control group) and 13 ARS-positive patients (6 in the PFD group and 7 in the control group) (**Supplementary Table 2**), and found that PFD improved the FVC% of the patients with IIM, especially the MDA5-positive patients. In the clinic, some of the MDA5-positive patients with IIM-ILD progressed too quickly to take antifibrosis agents, even with the help of a ventilator. Thus, the MDA5-positive patients in this study had an average baseline FVC% of 76.36 ± 19.95%, which was not as poor as expected, and showed a better response to PFD than other patients. In contrast, most of the patients had relatively stable disease activity during the observation, and there were no significant differences in rash, limb weakness, and hoarseness or dysphagia (**Supplementary Table 11**).

In 2015, the first multicenter study on PFD treatment in Chinese patients with IPF confirmed that PFD improved PF after 24 weeks (−0.08 ± 0.20 L vs.−0.22 ± 0.29 L) (30) and opened the door to PFD for IPF treatment in our country. A number of studies (13, 14, 26, 31) have found a beneficial effect of PFD in CTD-ILD, but the sample sizes have been too small. At present, PFD has been tested in randomized-controlled clinical studies in patients with different CTDs (SLSIII, NCT03221257; Trial 1, NCT02808871) (32), but the results have not yet been released.

Recently, a retrospective study at Tongji University (28) reported the effect of PFD on 184 patients with IPAF. The volume of FVC in the PFD group ($n = 81$) in this study was increased by 0.0390 L/year compared with a decrease of 0.0769 L/year in the control group ($n = 103$). The researchers concluded that PFD (600–1,800 mg/day) can improve FVC% and can help to reduce the GC dosage in patients with IPAF. In addition, the median dosage of PFD in that work was 1,492 mg/day.

In this study, a large proportion of patients took 800–1,200 mg/day PFD at 24 weeks, and the mean daily dosage of PFD during the 24-week period was 786.63 (682.87–896.5) mg/day, which was also lower than the standard dosage (**Supplementary Tables 4**, **5**). Despite the inability to reach the full dosage of 1,800 mg/day due to individual tolerance, PFD was still effective in this study. Therefore, we may conclude that the tolerated dosage of PFD in Chinese patients with CTD-ILD is 800–1,200 mg/day, and the use of GC and IS may be associated with the low dosage of PFD. As there were no differences between the high- and low-dosage PFD groups (**Supplementary Table 6**), it will be necessary to monitor the blood concentration of PFD to determine its appropriate dosage in view of the interaction effect between GC, IS, and PFD.

As a prospective study, this study revealed the superiority of PF improvement by PFD. This benefit may be related to (1) the characteristic of the CTD background: patients with CTD-ILD have more inflammatory exudative lesions on HRCT scans, indicating conditions such as NSIP and

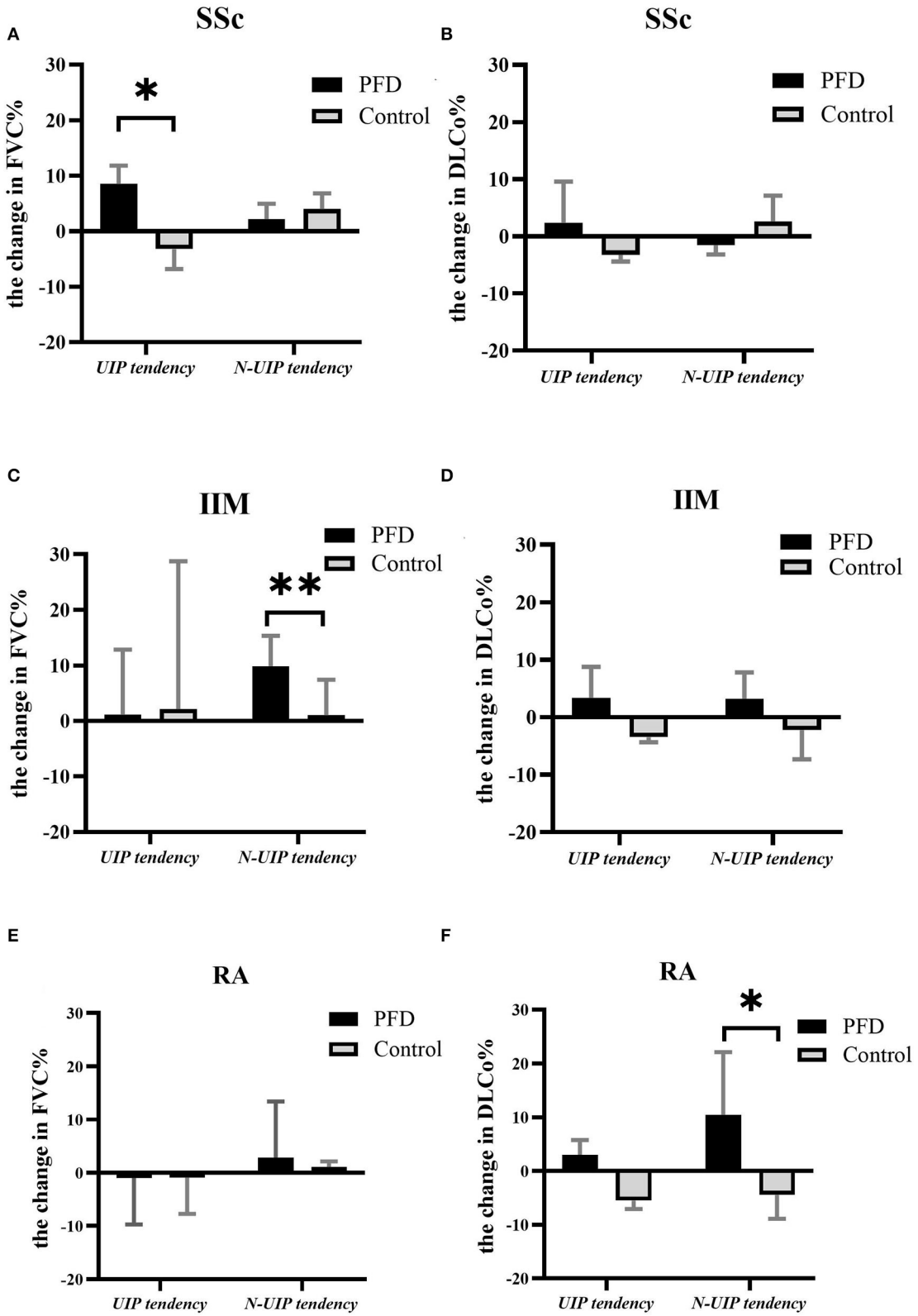

FIGURE 4 | The change in PF in different HRCT subtypes of the 3 CTD-ILD groups with vs. without PFD. The change in FVC% in UIP and non-UIP tendency subtypes of patients with **(A)** SSc-ILD, **(C)** IIM-ILD, and **(E)** RA-ILD and the change in DLCo% in patients with **(B)** SSc-ILD, **(D)** IIM-ILD, and **(F)** RA-ILD either treated or not treated with PFD. *$p < 0.05$, **$p < 0.01$ compared to the no-PFD control.

TABLE 3 | The change in FVC% and DLCo% between different baseline PF subsets of the three CTD-ILD groups, with vs. without PFD.

Item	Category		Total			SSc-ILD			IIM-ILD			RA-ILD		
	Baseline PFT		Pirfenidone	Control	*p*	Pirfenidone	Control	*p*	Pirfenidone	Control	*p*	Pirfenidone	Control	*p*
Change in FVC%	FVC% ≥ 70%	4.10 (−1.40 to 7.85)	0.30 (−3.10 to 4.68)	**0.050***	6.60 (1.23 to 11.50)	0.10 (−6.80 to 4.60)	**0.047***	6.30 (0.50 to 10.50)	1.10 (−3.30 to 6.80)	0.089	0.00 (−5.98 to 4.85)	0.00 (−2.80 to 1.10)	0.643
	(*n*)	40	48		11	15		15	21		8	7	
	FVC% ≥ 70%	10.88 (0.80 to 17.30)	1.00 (−7.20 to 14.20)	0.300	8.46 (3.80 to 8.54)	14.2 (–)	0.180	15.10 (0.35 to 19.10)	0.90 (−10.70 to 22.60)	0.221	9.85 (1.00 to 18.70)	–	–
	(*n*)	16	7		3	1		10	5		2	0	
Change in DLCo%	DLCo% ≥ 70%	−3.50 (−7.00 to 4.60)	−3.80 (−6.40 to 3.30)	0.969	−4.20 (−5.00 to 3.00)	−3.80 (−5.45 to 8.73)	0.519	−4.20 (−11.30 to 2.30)	−3.80 (−10.30 to 1.30)	0.815	6.25 (−3.75 to 18.95)	−5.00 (−6.40 to −2.50)	0.157
	(*n*)	16	27		3	10		9	13		4	3	
	DLCo% ≥ 70%	4.40 (−1.80 to 8.45)	−0.50 (−5.50 to 5.53)	0.057	−0.40 (−3.25 to 8.45)	3.65 (−6.28 to 5.60)	0.723	6.40 (−2.80 to 12.50)	0.70 (−4.70 to 10.20)	0.333	7.40 (2.18 to 14.03)	−6.60 (−8.60 to 2.13)	**0.011***
	(*n*)	38	28		10	6		15	13		6	4	

*$p < 0.05$ compared to the no-PFD control. Bold numbers denote statistical significance.

TABLE 4 | Multiple linear regression analysis of the change in PF in patients with CTD-ILD: multiple linear regression analysis of the change (A) in FVC% and (B) in DLCo%.

Variables	Hazard Ratio	95% CI		*p* value
		Lower	Upper	
(A) Multiple linear regression analysis of the change in FVC%				
Diseases				
SSc	Ref	Ref	Ref	Ref
IIM	0.85	−4.52	6.23	0.753
RA	−2.68	−9.28	3.91	0.422
Other CTDs	−2.67	−9.82	4.48	0.461
Baseline FVC<70%	5.88	0.40	11.37	**0.036**
Glucocorticoid average dosage	0.11	−0.09	0.32	0.272
Pirfenidone	4.56	0.38	8.75	**0.033**
(B) Multiple linear regression analysis of the change in DLCo%				
Diseases				
SSc	Ref	Ref	Ref	Ref
IIM	2.35	−2.85	7.56	0.372
RA	−1.32	−8.19	5.54	0.703
Other CTDs	−2.09	−9.56	5.39	0.581
BMI	−0.27	−0.93	0.40	0.427
Baseline FVC < 70%	6.81	1.28	12.33	**0.016**
Baseline DLCo < 70%	−0.44	−5.11	4.22	0.850
Pirfenidone	4.37	0.02	8.72	**0.049**

Bold numbers denote statistical significance.

TABLE 5 | Comparison of adverse events in the PFD and control groups over 24 weeks.

Adverse event	Pirfenidone (*n* = 64)	Control (*n* = 72)
Subjects (%)	21 (32.80)	15 (20.83)
Respiratory infections (%)	7 (10.94)	7 (9.72)
Skin infections (%)	1 (1.56)	1 (1.39)
Urinary infections (%)	2 (3.13)	2 (2.78)
Other infections (%)	1 (1.56)	1 (1.39)
Abdominal distension (%)	3 (4.69)	2 (2.78)
Gastroesophageal reflux (%)	4 (6.25)	1 (1.39)
Diarrhea (%)	2 (3.13)	0 (0.00)
AST and/or ALT increase (%)	1 (1.56)	2 (2.78)
Creatinine increase (%)	1 (1.56)	0 (0.00)
Rash (%)	3 (4.69)	4 (5.56)
Oral ulcer (%)	1 (1.56)	0 (0.00)
Respiratory failure (%)	1 (1.56)	0 (0.00)
Mediastinal emphysema (%)	1 (1.56)	1 (1.39)
Dizziness (%)	1 (1.56)	0 (0.00)
Palpitations (%)	1 (1.56)	0 (0.00)
Cerebral thrombosis (%)	0 (0.00)	1 (1.39)

OP, which could be absorbed in the short term; and (2) GC and IS background: PFD was used in combination with GC and IS, which helped in the recovery from ILD, especially the NSIP subtype. Although there were no

significant differences in GC dosage and no IS changes in this study (**Supplementary Tables 12**–**14**), and all the patients demonstrated stabilization of the underlying disease (**Supplementary Tables 2**, **3**), strictly paired clinical studies with large samples are needed.

Our study had some limitations. First, it was a single-center study with a limited sample size of each distinct CTD-ILD group. Due to the small numbers, there were a few differences in baseline characteristics, such as PF and HRCT features, between the study groups, and some of these factors cannot be ruled out as confounding factors. Simultaneously, masking of the differential effects of PFD due to treatment bias (GC and IS) of the underlying diseases cannot be excluded. Another shortcoming of this study was the lack of randomized control arms as the grouping in the study was partly determined by the patients based on their finances, insurance, and the self-assessment of their illness. Due to the high price of PFD, many real-world patients decide to add PFD when their disease becomes serious. Therefore, the patients in the PFD group in this study tended to have a poorer PF, and we could not exclude this baseline PF difference as a confounder. Moreover, the duration of follow-up was short, so we plan to extend this observation to 96 weeks.

CONCLUSION

The response of PF to PFD differs between several kinds of CTD-ILD. PFD has a favorable benefit/risk profile and represents a suitable treatment option for patients with SSc, IIM, and RA who have a non-UIP tendency and lower PF.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Qilu Hospital, Cheeloo College of Medicine, Shandong University (KYLL-202008-014). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JW and QS collected tissue samples, drafted the manuscript, and designed the tables and figures. XW and XQ participated in manuscript preparation. TZ contributed to the data analysis and read the manuscript critically. YC participated in the HRCT imaging assessment and read the manuscript critically. ZS read the manuscript critically. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.871861/full#supplementary-material

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The BASDAI Cut-Off for Disease Activity Corresponding to the ASDAS Scores in a Taiwanese Cohort of Ankylosing Spondylitis

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Objectives: The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) has been widely utilized to evaluate disease activity in patients with ankylosing spondylitis (AS) by an arbitrary cut-off of ≥4 to indicate high disease activity and initiate biological therapy. The Ankylosing Spondylitis Disease Activity Score (ASDAS) is a new composite index to assess AS disease activity states that have been defined and validated. ASDAS ≥2.1 was selected as a criterion to start biological therapy. The purpose of this study was to estimate the corresponding BASDAI and ASDAS cut-off in a Taiwanese AS cohort.

Methods: From November 2016 to October 2018, we assessed the ASDAS and the BASDAI regularly and recorded demographic data for 489 AS patients in Taichung Veterans General hospital (TCVGH) using an electronic patient-reported data system linked to electronic medical records. We used receiver operating characteristic curves with Youden's J statistic to determine the BASDAI values that correspond to ASDAS disease activity cut-offs (i.e., 1.3, 2.1, and 3.5).

Results: In our population, the best trade-off BASDAI values corresponding to ASDAS -C-reactive protein (CRP) 1.3, 2.1, and 3.5 were 2.1, 3.1, and 3.7, respectively. The optimal BASDAI values corresponding to ASDAS-erythrocyte sedimentation rates 1.3, 2.1, and 3.5 were 2.0, 2.6, and 4.8, respectively.

Conclusion: We propose a revised BASDAI cut-off based on our data, as BASDAI scores are commonly used globally. A more reasonable, lower BASDAI cut-off to initiate or change biological therapy will bring us closer to better decisions to treat AS patients.

Keywords: ankylosing spondylitis, biological therapy, patient-reported outcome measures, electronic medical records, BASDAI score, ASDAS

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INTRODUCTION

To define disease activity in patients with ankylosing spondylitis (AS), the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was introduced into practice in 1994 as a score calculated from a patient self-administered questionnaire (1). AS patients answer six questions, and their responses are scaled from 0 to 10. Patients are asked the degree of fatigue/tiredness experienced; AS-related pain in the neck, back, or hip; pain or swelling in other joints; discomfort from any areas tender to touch, pressure and discomfort from the time they wake up; and lastly, how long their morning stiffness lasts from the time they wake up. On a scale of 0 to 10, a BASDAI score $\geq$4 implies active disease, a consideration for initiating biologic therapy (2). Although the BASDAI is easy to administer and has been widely utilized to evaluate AS disease activity, the cut-off of BASDAI $\geq$4 used to indicate high disease activity in the ASAS EULAR guidelines was decided arbitrarily as a criterion to initiate biological therapy for AS patients.

The Ankylosing Spondylitis Disease Activity Score (ASDAS) computes the values of acute-phase reactants C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in addition to the patient-reported outcomes. ASDAS, like the Disease Activity Score in rheumatoid arthritis, is a composite index that includes five items (i.e., BASDAI question 2, BASDAI question 3, BASDAI question 6, patient global assessment and laboratory data [ESR or CRP]), resulting from a formula in which each item has a different weight for the final calculation. The ASDAS, on the other hand, has well-defined cut-off values for disease activity states and has been validated. ASDAS $\geq$2.1 was selected as a criterion for starting biological therapy (3). In a Norwegian study, when ASDAS $\geq$ 2.1 and BASDAI $>$ 4 were chosen as eligibility criteria to initiate TNF inhibitor (TNFi) treatment in AS, more patients were found eligible for TNFi using the ASDAS than the BASDAI eligibility criteria. Patients who fulfilled both criteria had the greatest likelihood of improvement, but those who fulfilled only the ASDAS criterion also improved. ASDAS could also be well applied to patient subgroups without elevated CRP and without peripheral joint swelling (4).

The construct validity of ASDAS-CRP to discriminate low and high disease activity and the cut-off values are similar in male and female patients with axSpA; however, cut-offs for ASDAS-ESR need to be defined (5). In a Turkish population study, patients with nr-axSpA and AS were assigned to low and high disease activity. The discriminatory ability of ASDAS-CRP, ASDAS-ESR, and BASDAI were found similar according to the ASAS partial remission, patient's, and physician's global assessment scores (6). A French study of 200 AS patients introduced the Patient Acceptable Symptom State (PASS) and found a significant association between disease activity and depression severity and good agreement between BASDAI and ASDAS. They found a 2.3 cut-off for both the patient-reported absence of disease activity and PASS (7). ASDAS and BASDAI showed similarly good performance in a cross-sectional setting in a local Chinese AS cohort, with ASDAS performing better in the subgroup with raised inflammatory markers (8). Several studies have used both ASDAS and BASDAI to monitor treatment responses after starting conventional synthetic disease-modifying antirheumatic drugs biological therapy. Both scores are useful to help guide clinicians in making treatment decisions in daily practice (9, 10). All of these studies are important because they correlated the ASDAS and BASDAI with physician-measured disease activity. Of note, the items of both ASDAS and BASDAI, such as fatigue, overall assessment and back pain, are nonspecific and can be found in other diseases, including fibromyalgia and degenerative joint disease. Therefore, the application of ASDAS and BASDAI in AS patients with comorbidities that shared the items of both indexes is of concern.

Determination of the BASDAI cut-off values responding to the validated ASDAS cut-off values for disease activity states is of value for clinicians to apply BASDAI to therapeutic adjustment. However, to date, only two studies have estimated the BASDAI cut-offs for disease activity states based on the validated ASDAS cut-offs (11, 12). In November 2016, we developed an electronic medical records management system for AS patients to regularly assess BASDAI, ASDAS with ESR (ASDAS-ESR) and ASDAS with CRP (ASDAS-CRP), the degree of functional impairment and in-depth evaluation of the degree of functional limitation measured by the Bath AS Functional Index, and radiographic damage measured by the modified stoke AS spinal score (mSASSS), a validated measure to assess radiographic progression (13, 14). Our electronic medical records have facilitated the study of the correlation between BASDAI and ASDAS. Therefore, the aim of the study was to determine the cut-off values of BASDAI for disease activity states corresponding to the validated ASDAS cut-offs using a retrospective analysis of a Taiwanese AS cohort from our electronic medical records.

METHODS

Ethics Approval

This study was approved by the Institutional Review Board I & II of Taichung Veterans General Hospital (TCVGH) (Approval number: CE18321A). Any patient personal information tracked was anonymized before data analysis. Therefore, informed patient consent was not required.

Study Design

This was a single-center retrospective, cross-sectional study.

Data Source

To assist Rheumatologists at TCVGH, in November 2016, we had started assessing the ASDAS, the BASDAI, medications used, and clinical outcomes of all AS patients in our electronic medical record system. Our database's reliability and validity have been examined in our previous publication on the gender difference in ASAS HI among patients with AS (15). In addition to these disease-specific data, routine demographic data, comorbidities, personal history, and family history are also part of the patient records.

Using the 1984 modified New York criteria for AS, one of our 14 rheumatologists confirmed the AS diagnosis (14), and patients were registered after confirmation as TCVGH-AS cohort. Thereafter, a trained nurse used a standard questionnaire

TABLE 1 | Demographic data and clinical characteristics at presentation of patients with AS.

	Total	Female	Male	
	***n* = 489 (100%)**	***n* = 114 (23.3%)**	***n* = 375 (76.7%)**	***P*-value**
Age, years (Mean± SD)	44.2 ± 13.8	42.9 ± 13.8	44.6 ± 13.8	0.253
Age at AS diagnosis, years (mean ± SD)	25.9 ± 10.8	29.2 ± 12.2	25.0 ± 10.2	0.001
Symptom duration, year (mean ± SD)	18.3 ± 11.9	13.7 ± 10.4	19.6 ± 12.1	<0.001
HLA-B27	441 (90.4)	93 (81.6)	348 (93)	<0.001
Smoke	164 (33.5)	7 (6.1)	157 (41.9)	<0.001
Comorbidities				
Hypertension	99 (20.2)	12 (10.5)	87 (23.2)	0.003
Diabetes mellitus	35 (7.2)	5 (4.4)	30 (8.0)	0.190
Hyperlipidemia	71 (14.5)	9 (8.0)	62 (16.5)	0.024
Hepatitis B	59 (12.1)	11 (9.6)	48 (12.8)	0.366
Hepatitis C	12 (2.5)	3 (2.6)	9 (2.4)	0.889
Chronic renal failure	15 (3.1)	2 (1.8)	13 (3.5)	0.353
Gout	23 (4.7)	2 (1.8)	21 (5.6)	0.089
Coronary artery disease	17 (3.5)	1 (0.9)	16 (4.3)	0.139*
Stroke	2 (0.4)	0 (0.0)	2 (0.5)	1.000
Periodontal disease	112 (22.9)	12 (10.5)	100 (26.7)	<0.001
Osteoporosis	35 (7.2)	10 (8.8)	25 (6.7)	0.430
Extra-spinal manifestation				
Uveitis	135 (27.6)	32 (28.1)	103 (27.5)	0.900
Psoriasis	37 (7.6)	6 (5.3)	31 (8.3)	0.285
Crohn's disease	0 (0.0)	0 (0.0)	0 (0.0)	-
Ulcerative colitis	2 (0.4)	1 (0.9)	1 (0.3)	0.371*
Peripheral arthritis	114 (23.4)	29 (25.4)	85 (22.8)	0.559
Enthesitis	75 (15.3)	18 (15.8)	57 (15.2)	0.878
Dactylitis	12 (2.5)	0 (0.0)	12 (3.2)	0.077*
Past history				
Total hip replacement	20 (4.1)	1 (0.9)	19 (5.1)	0.057*
Total knee replacement	2 (0.4)	1 (0.9)	1 (0.3)	0.412*
Fracture	49 (10.0)	8 (7.0)	41 (10.9)	0.223
Tuberculosis	16 (3.3)	2 (1.8)	14 (3.7)	0.382*
Palindromic rheumatism	5 (1.0)	0 (0.0)	5 (1.3)	0.595*
Family history of AS (first or second-degree relatives)	193 (39.5)	50 (43.9)	143 (38.1)	0.273
First degree relatives	93 (19.9)	22 (20.2)	71 (19.8)	0.926
Second-degree relatives	137 (28.8)	37 (33.0)	100 (27.5)	0.262
Current medications				
Biologics	152 (31.1)	40 (35.1)	112 (29.9)	0.292
Methotrexate	33 (6.7)	9 (7.9)	24 (6.4)	0.577
Sulfasalazine	199 (40.7)	41 (36.0)	158 (42.1)	0.240
NSAID	434 (88.8)	102 (89.5)	332 (88.5)	0.781
Tramadol/acetaminophen	127 (26.0)	28 (24.6)	99 (26.4)	0.695
Corticosteroid	58 (11.9)	14 (12.3)	44 (11.7)	0.849

Data were shown as number (percentage) unless specified otherwise.

**Fisher's exact test.*

AS, ankylosing spondylitis; HLA, human leukocyte antigen; NSAID, non-steroidal anti-inflammatory drug; SD, standard deviation.

to collect information, including clinical characteristics, age at symptom onset, smoking status, history of tuberculosis, family histories, comorbidities, and extra-spinal manifestations. These questionnaires and worksheets are maintained in a standardized format to ensure reproducibility and good laboratory practices. Screening for comorbidities at presentation included hypertension, diabetes mellitus, hyperlipidemia, hepatitis B, hepatitis C, gout, coronary artery disease, stroke, periodontal disease, and osteoporosis. The extra-spinal manifestations noted at presentation were: 1. the extra-articular manifestations (EAMs) such as uveitis, psoriasis, inflammatory bowel disease; and 2. peripheral arthritis, enthesitis, and dactylitis. The rheumatologist-in-charge then confirmed the clinical characteristics. Our trained nurses assisted the AS patients with the assessments of BASDAI, ASDAS-ESR, and ASDAS-CRP. The BASFI was generated automatically into the system. An MSK-specialist radiologist reported the radiographic images of the C-spine and L-spine to calculate mSASSS. We used the data of the first assessment to compare the correlation between BASDAI and ASDAS.

Study Subjects

From November 2016 to October 2018, a total of 489 AS patients with a complete baseline demographic and assessment data from the TCVGH electronic data system were selected. If the assessment questionnaires were incomplete for any of the four computations, BASDAI, ASDAS, BASFI, or ASAS HI, we excluded those patients from our TCVGH-AS cohort.

In general, time constraint was the only issue that prevented the completion of the questionnaire by patients. We did not collect data of patients who did not complete the questionnaires or the precise reasons for not completing the questionnaires for this study.

Statistical Analyses

Continuous variables were reported as a mean ± standard deviation (SD), and categorical variables were reported as a percentage of patients. Differences in continuous variables were examined using the Student's *t*-test, while categorical variables were analyzed using Pearson's χ^2 test. Spearman correlation coefficients were calculated to estimate the correlation between BASDAI and ASDAS-ESR/ASDAS-CRP because the distributions of BASDSI, ASDAS-ESR/ASDAS-CRP were not Gaussian distributions. We used receiver operating characteristic (ROC) curves with Youden's J statistic to determine the cut-off values of BASDAI that correspond to ASDAS disease activity cut-offs (i.e., 1.3, 2.1, and 3.5). We performed a statistical analysis of the ROC curve for the sensitivity of the BASDAI cut-off values in evaluating the disease states. The ROC curve analysis was the major method used to assess the correlation between two disease activities measured, as both were continuous variables. Data analysis was done using the SAS software (SAS Institute, Inc., Cary, NC, USA).

TABLE 2 | Baseline disease activity measures of the study population.

Disease activity measures	*n* = 489
BASDAI Q1 (fatigue), median (IQR)	3.0 (2.0–5.0)
BASDAI Q2 (neck/back/hip pain), median (IQR)	3.0 (2.0–5.0)
BASDAI Q3 (peripheral joint pain/swelling), median (IQR)	1.0 (0.0–3.0)
BASDAI Q4 (enthesitis), median (IQR)	1.0 (0.0–3.0)
BASDAI Q5 (severity of morning stiffness), median (IQR)	3.0 (1.0–4.0)
BASDAI Q6 (duration of morning stiffness), median (IQR)	2.0 (1.0–3.0)
PGA, median (IQR)	3.0 (1.0–4.0)
CRP, median (IQR)	0.3 (0.1–0.6)
ESR, median (IQR)	8.0 (3.0–15.0)
BASDAI, median (IQR)	2.4 (1.4–3.7)
ASDAS-CRP, median (IQR)	1.8 (1.2–2.3)
Inactive disease, *n* (%)	130 (26.6)
Moderate disease activity, *n* (%)	180 (36.8)
High disease activity, *n* (%)	154 (31.5)
Very high disease activity, *n* (%)	25 (5.1)
ASDAS-ESR, median (IQR)	1.7 (1.2–2.3)
Inactive disease, *n* (%)	134 (27.4)
Moderate disease activity, *n* (%)	184 (37.6)
High disease activity, *n* (%)	149 (30.5)
Very high disease activity, *n* (%)	22 (4.5)

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; Q, question; IQR, interquartile range; PGA, patient global assessment; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ASDAS, Ankylosing Spondylitis Disease Activity Score.

Subgroup Analyses Based on Disease Duration

To examine whether the correlation between BASDAI and ASDAS cut-offs is consistent regardless of disease duration, we conducted a stratified analysis based on the median of disease duration (≤16 years, >16 years).

RESULTS

As shown in **Table 1**, in our patient cohort of 489 Taiwanese patients, the male to female patients' ratio was 3:1. The mean age at presentation was 44.2 years, with an SD of 13.8 years. On average, patients had AS-related symptoms for 18 years. The average age at which AS was diagnosed was 29 years for women and 25 years for men. While one-third of the men were smokers, only 7% of the women reported a smoking history. The presence of comorbidities such as hypertension, diabetes, hyperlipidemia, hepatitis B, hepatitis C, chronic renal failure, gout, coronary artery disease, stroke, periodontal disease, and osteoporosis in the male and female cohorts is shown in **Table 1**. As shown in **Table 1**, almost one-third of the patient population (31.1%) used biologics. The non-biologic therapies used included methotrexate (6.7%), sulfasalazine (40.7%), NSAIDs (88.8%), tramadol (26%) and corticosteroids (11.9%). **Table 2** revealed the baseline disease activity measures of the study population. Of the 489 AS patients, 179 (36.6%) had an ASDAS-CRP ≥2.1, and 25 (5.1%) of the study population had very high disease activity.

TABLE 3 | Optimal BASDAI cut-off values corresponding to ASDAS cut-offs using ROC curve with Youden's J statistic in AS patients.

	BASDAI cut-off	AUC (95% CI)	Specificity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ASDAS-CRP 1.3	2.1	0.76 (0.72–0.80)	0.70 (0.65–0.75)	0.82 (0.75–0.88)	0.92 (0.88–0.95)	0.50 (0.43–0.57)
ASDAS-CRP 2.1	3.1	0.78 (0.74–0.82)	0.69 (0.62–0.76)	0.87 (0.83–0.91)	0.76 (0.68–0.82)	0.83 (0.79–0.87)
ASDAS-CRP 3.5	3.7	0.82 (0.75–0.89)	0.84 (0.66–0.95)	0.81 (0.77–0.84)	0.23 (0.15–0.31)	0.99 (0.97–1.00)
ASDAS-ESR 1.3	2.0	0.80 (0.77–0.84)	0.74 (0.69–0.78)	0.87 (0.80–0.92)	0.94 (0.90–0.96)	0.56 (0.48–0.62)
ASDAS-ESR 2.1	2.6	0.79 (0.76–0.83)	0.81 (0.74–0.86)	0.78 (0.73–0.82)	0.66 (0.59–0.73)	0.88 (0.84–0.92)
ASDAS-ESR 3.5	4.8	0.79 (0.70–0.89)	0.67 (0.46–0.83)	0.92 (0.89–0.94)	0.33 (0.21–0.47)	0.98 (0.96–0.99)

ASADS, ankylosing spondylitis disease activity score; AS, ankylosing spondylitis; AUC, area under the curve; BASDAI, bath ankylosing spondylitis disease activity index; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ROC, receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value.

BASDAI was significantly correlated with ASDAS-CRP ($r = 0.7203$, $p < 0.0001$) and ASDAS-ESR ($r = 0.7590$, $p < 0.0001$). As shown in **Table 3**, the best cut-off BASDAI values corresponding to ASDAS-CRP 1.3, 2.1, and 3.5 were 2.1, 3.1, and 3.7, respectively. The inactive disease was found in 42.9% of patients, who had an ASDAS- CRP value <1.3, corresponding with a BASDAI cut-off value of 2.1. Low disease activity was found in 35% of patients, marked by an ASDAS-CRP value of 1.3 to 2.1, corresponding to a BASDAI cut-off value of 2.1–3.1. High disease activity was found in 18% patients, marked by an ASDAS-CRP value of 2.1–3.5, corresponding to a BASDAI cut-off value of 3.1–3.7. Very high disease activity was found in 4.1% patients, marked by ASDAS-CRP values of 3.5, corresponding to a BASDAI cut-off value of 3.7. Notably, the optimal BASDAI values corresponding to ASDAS-ESR 1.3, 2.1, and 3.5 were 2.0, 2.6, and 4.8, respectively.

Tables 4, **5** reveal the degree of agreement of disease activity states based on the corresponding BASDAI cut-off values with those based on ASDAS-CRP and ASDAS-ESR cut-off values, respectively. **Figure 1** and **Supplementary Table 1** show the agreement between BASDAI ≥3, ≥4 and high and very high disease activity states according to ASDAS values.

As shown in **Supplementary Tables 2, 3**, the corresponding cut-off values of BASDAI in patients with a symptom duration > 16 years were larger than those in patients with a symptom duration ≤ 16 years. The mean ± standard deviation mSASSS was higher in AS patients with a symptom duration > 16 years than that in patients with a symptom duration of ≤ 16 years (24.19 ± 24.24 vs. 10.73 ± 16.43, $p < 0.001$).

DISCUSSION

The correlation coefficients between the BASDAI and ASDAS-CRP/ASDAS-ESR were 0.7203/0.7590 ($p < 0.0001$, both), which were consistent with those reported in prior studies (7, 16–18). Our data determined the disease activity state cut-offs for BASDAI at 2.1/2.0, 3.1/2.6, and 3.7/4.8 for inactive disease, low disease activity, high disease activity, and very high disease activity, while using ASDAS-CRP/ASCAS-ESR as references. These cut-offs are much lower than the cut-offs of BASDAI currently used to define disease states, which are at 3, 4, and 6, respectively (3). Although the corresponding BASDAI cut-off values seem to be larger in AS patients with a symptom duration > 16 years compared with those aged ≤16 years, they were still lower than the currently used BASDAI cut-offs. The higher corresponding cut-off values of BASDAI in patients with longer disease duration may be explained by greater structure damage in the spine. Given that the BASDAI cut-offs at 3, 4, 6 for disease activity states have not been validated, our estimated BASDAI cut-offs can be used as options to define AS disease activity states in real-world practice when ASDAS are not available.

It is difficult to define a state of remission in AS patients. Godfrin-Valnet M et al. found that the cut-off of ASDAS corresponding to the inactive disease based on patient-reported disease activity was 2.3 (7). However, in longitudinal studies, a significant proportion of AS patients with an ASDAS value of a1.3–2.1 still suffered from radiographic progression (19, 20). Therefore, ASDAS<1.3 is suggested to be the global therapeutic target considering long-term consequences (21). Our first finding that inactive disease, set at an ASDAS-CRP/ASDAS-ESR value <1.3, corresponding with a BASDAI cut-off value of 2.1/2.0, suggests that the treatment goals of remission/inactive disease might be set at BASDAI 2.1 or 2.0, as opposed to the traditional goals of BASDAI 3 values.

In patients with AS, ASDAS-CRP ≥2.1 and BASDAI ≥4 have been widely used to suggest a change of therapy, such as initiating biologics (3, 22). However, ASDAS-CRP ≥2.1 can take more patients eligible to initiate biologics than BASDAI ≥4 (2, 23, 24). Our second finding that high/very high disease activity, marked by an ASDAS-CRP value of ≥ 2.1, corresponds to a BASDAI cut-off value of 3.1, suggesting an inadequate response to prior therapy and a need to adjust treatment (3, 22, 25). In a Korean cohort of 333 patients with axial spondyloarthritis, Chan et al. found that the BASDAI values corresponding to ASDAS-CRP values of 1.3, 2.1 and 3.5 were 1.9, 3.5 and 4.9, respectively (11). Their defined BASDAI cut-off value for the inactive disease was consistent with our finding. However, their estimated BASDAI cut-offs for high disease activity and very high disease activity were higher than our findings. In a Turkish cohort of 396 patients with AS, the estimated BASDAI cut-off values corresponding to ASDAS-CRP values of 2.1 and 3.5 were 2.4 and 3.7, respectively (12). The data of this Turkish cohort were more consistent with our findings than those of the Korean cohort. Possible explanations of the discrepancies in the Korean cohort were the exclusion of fibromyalgia, the inclusion of patients

TABLE 4 | Degree of agreement between disease activity states based on BASDAI and ASDAS-CRP cut-off values.

	ASDAS-CRP < 1.3	1.3 ≤ ASDAS-CRP <2.1	2.1 ≤ ASDAS-CRP ≤ 3.5	ASDAS-CRP > 3.5
	n = 130	*n* = 180	*n* = 154	*n* = 25
BASDAI < 2.1	107 (82.3)	83 (46.1)	19 (12.3)	0 (0.0)
2.1 ≤ BASDAI < 3.1	19 (14.6)	57 (31.7)	30 (19.5)	0 (0.0)
3.1 ≤ BASDAI ≤ 3.7	2 (1.5)	22 (12.2)	33 (21.4)	2 (8.0)
BASDAI > 3.7	2 (1.5)	18 (10.0)	72 (46.8)	23 (92.0)

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C-reactive protein.
Weighted kappa: 0.464, p < 0.001.

TABLE 5 | Degree of agreement between disease activity states based on BASDAI and ASDAS-ESR cut-off values.

	ASDAS-ESR < 1.3	1.3 ≤ ASDAS-ESR < 2.1	2.1 ≤ ASDAS-ESR ≤ 3.5	ASDAS-ESR > 3.5
	n = 134	*n* = 184	*n* = 149	*n* = 22
BASDAI <2.0	113 (84.3)	64 (34.8)	14 (9.4)	0 (0.0)
2.0 ≤ BASDAI <2.6	13 (9.7)	51 (27.7)	12 (8.1)	0 (0.0)
2.6 ≤ BASDAI ≤ 4.8	8 (6.0)	67 (36.4)	87 (58.4)	5 (22.7)
BASDAI > 4.8	0 (0.0)	2 (1.1)	36 (24.2)	17 (77.3)

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; ESR, erythrocyte sedimentation rate.
Weighted kappa: 0.538, p < 0.001.

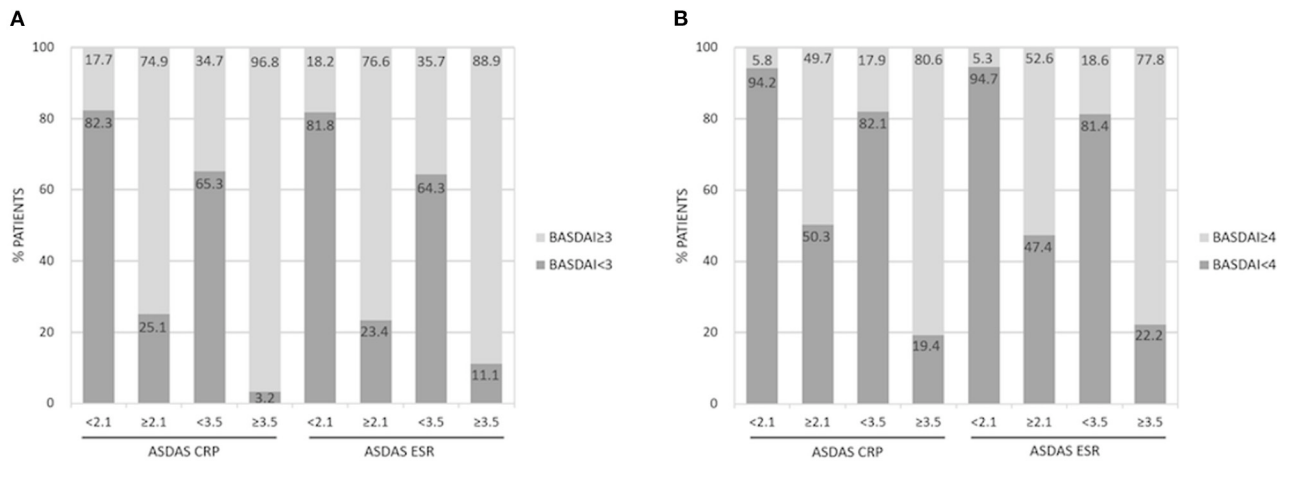

FIGURE 1 | Agreement between high and very high disease activity states according to ASDAS-CRP/ASDAS-ESR and BASDAI ≥3 **(A)** and BASDAI ≥4 **(B)**.

with non-radiographic axial spondyloarthritis and the inclusions of patients with a shorter symptom duration than our cohort. However, we also conducted a subgroup analysis based on the median value of symptom duration and found consistent results. Of note, ASDAS-ESR data were only available in our cohort and cannot be compared with the data of other studies.

One limitation of our study is that this is a single-center analysis in a Taiwanese cohort. The second limitation is that our patient population was enrolled as per convenience, which means no specific exclusion criteria have been used. The third limitation is that a close differential diagnosis of fibromyalgia, which could influence our statistical correlation analysis, was not screened in this cohort. Fourth, the long symptom duration and the small proportion of very high disease activity of our cohort limited the application of our findings on AS patients with short disease duration and very high disease activity. Finally, the findings in our Taiwanese cohort may not be generalized to other populations.

CONCLUSION

The cut-offs used by rheumatologists for escalating therapy is currently based on the 2016 ASAS EULAR management

recommendations for axial spondyloarthritis at ASDAS ≥2.1 vs. BASDAI ≥4. We propose a revision to a lower BASDAI cut-off based on our data, as BASDAI scores are more commonly used globally. A more reasonable and accurate, lower BASDAI cut-off to initiate or change biological therapy brings us one step closer to better decisions to treat AS patients with bDMARDs, ultimately reducing the disability due to progression in AS patients. In addition to the early initiation of therapy with highly effective treatments, long-term follow-up data on treatment outcomes with different agents can guide the selection of these agents. Further studies are warranted to evaluate the long-term outcomes of AS patients with various disease activity states based on the BASDAI cut-offs corresponding to the ASDAS cut-offs.

We must find the most accurate markers to predict prognosis, severe disease, and treatment response. For the next steps, our large, broad-based foundation cohort of AS patients is a great resource when complemented with more detailed follow-up information in the longer term to achieve treatment goals of slowing down AS disease progression.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board I of Taichung Veterans General Hospital (Approval number: CE18321A). The Ethics Committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

H-HC, Y-MC, and K-LL: conceived and designed the experiments. T-YH, W-TH, C-TL, C-WT, K-TT, Y-YC, Y-DW, C-WH, Y-JC, and H-HC: acquired data. Y-MC, K-LL, C-YH, and H-HC: contributed materials/analysis tools. H-HC, Y-HC, W-NH, Y-MC, and K-LL: wrote the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Toward Molecular Stratification and Precision Medicine in Systemic Sclerosis

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Systemic sclerosis (SSc), a complex multi-systemic disease characterized by immune dysregulation, vasculopathy and fibrosis, is associated with high mortality. Its pathogenesis is only partially understood. The heterogenous pathological processes that define SSc and its stages present a challenge to targeting appropriate treatment, with differing treatment outcomes of SSc patients despite similar initial clinical presentations. Timing of the appropriate treatments targeted at the underlying disease process is critical. For example, immunomodulatory treatments may be used for patients in a predominantly inflammatory phase, anti-fibrotic treatments for those in the fibrotic phase, or combination therapies for those in the fibro-inflammatory phase. In advancing personalized care through precision medicine, groups of patients with similar disease characteristics and shared pathological processes may be identified through molecular stratification. This would improve current clinical sub-setting systems and guide personalization of therapies. In this review, we will provide updates in SSc clinical and molecular stratification in relation to patient outcomes and treatment responses. Promises of molecular stratification through advances in high-dimensional tools, including omic-based stratification (transcriptomics, genomics, epigenomics, proteomics, cytomics, microbiomics) and machine learning will be discussed. Innovative and more granular stratification systems that integrate molecular characteristics to clinical phenotypes would potentially improve therapeutic approaches through personalized medicine and lead to better patient outcomes.

Keywords: systemic sclerosis, stratification, precision medicine, molecular, multi-omic analyses

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INTRODUCTION

Systemic sclerosis (SSc) is a multi-system immune-mediated disease characterized by vasculopathy and fibrosis of skin and internal organs (1). Early clinical manifestations include Raynaud's phenomenon, puffy swollen fingers and gastroesophageal reflux (2, 3). Later manifestations include musculoskeletal involvement, severe vasculopathy such as digital ulcerations, gastrointestinal (GI) complications, interstitial lung disease (ILD), pulmonary arterial hypertension (PAH) and scleroderma renal crisis (4). Although uncommon, SSc has one of the highest morbidity and mortality among autoimmune diseases, with cumulative 10-year survival of 62% from diagnosis (1, 5). Unmet needs in the management of SSc include risk stratification to prognosticate severity of disease complications and to predict treatment responses.

The heterogenous pathological processes that define SSc is a challenge to targeting appropriate treatment. SSc subset classification into relatively homogenous subtypes would have prognostic value to stratify patients for disease complications and treatment responses. The classification of SSc subsets have relied mainly on clinical features. Incorporation of laboratory (e.g., autoantibodies) and molecular gene signatures could lead to a more granular classification system. This is a promising approach toward precision medicine.

The most commonly used SSc classification system is based on the extent of skin involvement, specifically limited cutaneous (lc) and diffuse cutaneous (dc) SSc (6). A minority (<5%) of patients have clinical features of SSc and SSc-specific antibody without any skin involvement, and this group is classified as sine-scleroderma (7). This SSc classification system is suitable for clinical care as it is mainly based on clinical examination of skin with fairly distinct clinical associations and specific serum auto-antibody profiles (8). Nevertheless, due to the heterogenous nature of SSc, patients may present with similar initial clinical manifestations, but have different clinical outcomes and responses to treatment. Thus, SSc classification system needs to be refined to incorporate laboratory tools, such as auto-antibody profiles and gene expression signatures.

Molecular stratification allows segregation of different groups of patients based on pathogenetically homogenous subsets in relation to organ manifestations, prognosis and treatment response. The pathogenesis of SSc involves a complex interplay between immune activation and vascular damage, which leads to activation of fibroblast and excessive collagen deposition in the skin and internal organ (9). Better understanding of variable contributions from each process during the course of the disease could help tailor treatment approaches for different patients.

Therapies used for different clinical manifestations of SSc were shown to have varied efficacy (10). Evidence-based treatment guidelines published in 2017 by EUSTAR adopt an organ-based approach, rather than one based on the patients' clinical, laboratory or molecular subsets (11). The diverse natural course of SSc disease makes it challenging to predict which patients will benefit the most from particular treatment based on clinical manifestations alone. Molecular stratification of SSc will help to personalize treatment based on distinct molecular signatures.

In this review article, we will provide updates in SSc clinical and molecular stratification in relation to patient outcomes and treatment responses. We will also discuss promises of molecular stratification through advances in high-dimensional tools, including deep phenotyping of tissues to single cell analysis, network medicine, omic-based stratification (transcriptomics, genomics, epigenomics, proteomics, cytomics, and microbiomics) and machine learning.

SSC CLASSIFICATION SYSTEM

SSc classification system is a rapidly evolving field. Over the years, a combination of multi-system involvement, SSc-specific autoantibodies, and nail-fold capillaroscopy (NFC) patterns have emerged to supplement SSc classification.

SSc Classification Based on Cutaneous Involvement

The most commonly used system of SSc classification is a two-subset criteria by LeRoy et al., which dichotomizes patients into lcSSc and dcSSc based on extent and pattern of skin fibrosis (12). LcSSc, which includes patients with cutaneous involvement distal to the elbows or knees, is usually associated with anti-centromere antibody (ACA), telangiectasia and late onset of PAH (12). Whereas, dcSSc, which includes patients with cutaneous involvement proximal to the elbows or knees, is frequently associated with anti-topoisomerase I antibody (ATA), tendon friction rub, early internal organ involvement such as ILD, myocardial and diffuse GI tract involvement; hence, dcSSc is known to have poorer prognosis than lcSSc (12).

Although this classification system has a discriminatory value in the prognostication of patients, it has various limitations. There may be overlapping clinical features between the two subsets, e.g., ILD occurrence was 30% in lcSSc and 50% in dcSSc ($p = 0.16$) (13). A subgroup of patients may have serological, vascular and internal organ manifestations of SSc but without cutaneous involvement, and this subgroup has been classified as sine scleroderma (14). In addition, patients with very early diagnosis of systemic sclerosis (VEDOSS) may not have cutaneous involvement or internal organ involvement but have early SSc features such as Raynaud's phenomenon with vascular changes on NFC or SSc-specific autoantibody (2). A subset of patients may also display overlap syndromes with other connective tissue diseases (e.g., systemic lupus erythematosus and polymyositis), and be variably associated with anti-Ku, PM-Scl75 or anti-U1-ribonucleoprotein antibodies (15, 16).

SSc Classification System Based on More Novel Disease Attributes

Combining clinical data with laboratory tools may provide better prognostic value and be feasibly applied in routine clinical care. NFC patterns have been demonstrated to have prognostic value to inform disease activity and disease progression. The abnormal NFC patterns are classified as early, active and late (17, 18). In an international multi-center cohort study evaluating cross-sectional data in the EUSTAR registry, early/active NFC patterns were found in patients with mild/moderate skin involvement and low number of disease manifestations; whereas late NFC pattern was associated with more severe forms of SSc disease (17, 18). Moreover, the NFC pattern could also be an indicator of overall disease progression (18). Prospective cohort study of SSc patients ($n = 140$) over 3 years showed that reduced capillary density was associated with overall disease progression, progression of skin fibrosis, occurrence of new digital ulcers and new onset PAH (18). Furthermore, the severity of NFC patterns was shown to be predictive of future severe organ involvement with increasing risk from early to late pattern, after adjusting for disease duration, subset and vasoactive medications (19, 20).

SSc-specific autoantibodies are strong predictors of disease outcome and internal organ involvement (21). The 3 main SSc-specific autoantibodies are ACA, ATA and anti-RNA polymerase III antibody (anti-RNAP III), and they are usually mutually

exclusive (21, 22). SSc patients with ACA have better prognosis and are more likely to have limited cutaneous involvement and PAH (21). Patients with ATA represent a distinct subgroup with extensive cutaneous involvement and increased risk of ILD (21), and anti-RNAP III represents a subgroup with higher risk of malignancies and development of scleroderma renal crisis

TABLE 1 | Stratification in relation to clinical features.

	Subset and association with clinical features	**References**
Cutaneous	**Extent of skin involvement**	(12)
	lcSSc: higher prevalence of PAH and ACA	
	dcSSc: higher prevalence of ILD, less prevalence of ACA	
	Pre-fibrotic stage /very early disease	
	VEDOSS: (RP, puffy finger, ANA) AND (NFC or SSc-specific Ab)	(2)
NFC	Early/ active: mild/moderate skin involvement, low number of disease manifestations	(17)
	Late pattern: more severe disease	
	Reduced number of capillaries: overall disease progression, DU, PAH, ILD	(18–20)
SSc-Ab	ACA: lcSSc, PAH	(21)
	ATA: dcSSc, ILD	
	anti-RNAP III: lcSSc, SRC	
	anti-Th/ To: lcSSc, ILD, PAH	
	anti-U3RNP: dcSSc, muscle involvement, PAH	
	anti-PM-Scl: PM/DM overlap, arthritis overlap, ILD	
	anti-Ku: muscle and joint involvement	
	anti-U1RNP: overlap syndromes	
	anti-U11/ U12RNP: ILD	
Clinical features and prognosis	Cluster 1: female, older onset, GI involvement, lcSSc, ACA	(26)
	Cluster 2: ILD, PH, lcSSc, ACA, ATA	
	Cluster 3: younger onset, lowest mRSS, less aggressive, lcSSc, ACA > ATA	
	Cluster 4: older onset, DU, cardiac, lung, MSK, GI involvement, lcSSc, ATA > ACA	
	Cluster 5: male, younger onset, multi-organ involvements (cardiac, lung, GI, joint), dcSSc, ATA >ACA	
	Cluster 6: male, youngest onset, most aggressive, multi-organ involvement (cardiac, lung, renal, GI, MSK), dcSSc, ATA	
Intrinsic gene signature	Normal like	(27)
	Inflammatory	
	Fibroproliferative	
Monocyte subset	Cluster 1 (high CD16+ monocyte, low memory B cell subsets): lcSSc	(28)
	Cluster 2 (high classical monocytes): dcSSc, high mRSS	
	Cluster 3 (high memory B cells): often no skin involvement	
	Cluster 4 (low classical monocytes): often no skin involvement	
T-helper cells	Few immune abnormalities: gastrointestinal involvement, digital ulcer	(29)
	Treg-dominant group: anti-RNA polymerase III Ab, less digital ulcer and less gastrointestinal involvement	
	Tfh-dominant group: progressive skin sclerosis, gastrointestinal involvement, digital ulcer, late NFC pattern	
Gut microbiomes	**SSc cutanous subtypes and GI microbiome (species level)**	(30)
	LcSSc: ↓ *Firmicutes prausnitzii*	
	DcSSc: ↑ *Veillonella parvula, Klebsiella pneumoniae: dcSSc*	
	SSc GI involvement and GI microbiome (genus level)	(31)
	Milder GI symptoms: ↑ *Lactobacillus,* ↑ *Clostridium*	
	More severe GI symptoms: ↑ *Prevotella*	
	SSc disease duration and GI microbiome (genus level)	(32)
	Early SSc: ↑ *Lactobacillus,* ↑ *Streptococcus,* ↑ *Blautia,* ↓ *Bacteroides,* ↓ *Sutterella*	
	Long-standing SSc: ↑ *Lactobacillus,* ↑ *Streptococcus,* ↓ *Odoribacter,* ↓ *Sutterella*	
Proteomics	DcSSc with higher MRSS: upregulation of IGFBP-2, FSTL3, SPON1, ST2	(33)
	LcSSc with PAH: upregulation of FSTL3 and Midkine	(34)

lc, limited cutaneous; dc, diffuse cutaneous; ACA, anti-centromere antibody; ATA, anti-topoisomerase antibody; ANA, anti-nuclear antibody; SSc, systemic sclerosis; Ab, antibody; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; RP, Raynaud's phenomenon; SRC, scleroderma renal crisis; PM, polymyositis; DM, dermatomyositis; VEDOSS, very early diagnosis of SSc; NFC, nailfold capillaroscopy; MSK, musculoskeletal; GI, gastrointestinal; mRSS, modified Rodnan skin score; DU, digital ulcer.

(21, 23). Other SSc-specific autoantibodies identified to have prognostic values include anti-Th/To, which is associated with lcSSc and ILD but lower prevalence of PAH (21, 24).

Integration of autoantibody profiles with clinical phenotypes can provide a more robust and comprehensive classification system to risk stratify the patients better. Using cluster analysis of a combination of auto-antibodies and clinical features in 140 SSc patients, Boonstra et al. revealed 5 subgroups of patients with different prognosis and clinical outcomes (25). However, autoantibodies only partially contributed to risk stratification as not all ATA-positive patients had worse prognosis. Another cluster analysis of a large database using clinical and serologic variables (120 EUSTAR centers, $n = 6927$) showed that dichotomous classification of SSc patients were insufficient as significant proportion of patients with lcSSc (39%) and dcSSc (19%) clustered discordantly. By using data on the presence of organ damage to prognosticate risk of more organ damage/ death, the study identified 6 different clusters with more homogenous clinical phenotypes (**Table 1**) (26). Although cluster analyses have improved current classification system for better risk stratification, the analysis was driven by data readily available to physicians and none of the analyses included high-throughput molecular data (26). Moreover, the mean disease duration of patients in the EUSTAR study was 11 years (26). We believe that incorporation of high throughput molecular data could improve SSc stratification system.

SSC MOLECULAR STRATIFICATION

SSc Classification Based on Circulating Immune Cells

Van der Kroef et al. showed that prior to the onset of skin fibrosis and other organ manifestations, patients with Raynaud's phenomenon, positivity for SSc-specific autoantibodies and/ or specific NFC patterns, were shown to have different immune cell subset frequencies (**Table 1**) (28). Hierarchical cluster analysis showed that circulating immune cell population could be used to distinguish different SSc subsets into 4 different clusters, namely cluster 1 (high CD16+ monocytes and low memory B cells), cluster 2 (increased classical monocytes), cluster 3 (increased memory B cells), and cluster 4 (lower classical monocytes) (28). The different clusters were associated with different clinical features, for example limited cutaneous involvement in cluster 1 and no skin involvement in cluster 3 and 4. In contrast, cluster 2 was enriched in patients with ILD and diffuse cutaneous involvement (28). Future studies should further investigate the value of cellular phenotyping in relation to disease progression and treatment response.

SSc Classification Based on Intrinsic Gene Subsets

SSc classification based on gene expression phenotyping were previously described, namely the fibroproliferative, inflammatory, normal-like intrinsic gene subsets (**Table 1**) (27, 35). Serial biopsies of skin specimen showed that the intrinsic gene subsets were inherent and stable features of the disease, suggesting distinct pathogenic processes between patients (27, 35). More recent studies by Skaug et al. showed that immune cell and fibroblast signatures changed over time in early dcSSc (within 3 years of disease onset) with a tendency toward normalization as the immune cell and fibroblast signatures declined at follow up (36). This could inform future clinical trials to stratify patients in early disease.

Within an individual with SSc, the intrinsic gene subsets were shown to be consistent across the different skin biopsy sites regardless of the clinical involvement (thickened skin or morphologically normal skin) (27, 35). In addition, the intrinsic gene subsets were demonstrated to be conserved across tissues such as the esophagus and skin (37). This highlights common pathogenic processes in SSc across different tissues. Nevertheless, the tissue microenvironment plays an important role in the immune-fibrotic axes (38). By using functional genomic network analysis, Taroni et al. identified a distinct lung specific innate immune process which suggests certain gene pairs are more likely to interact in a particular tissue than the others (38).

Treatment Response in Relation to Intrinsic Gene Subsets

Molecular phenotyping of SSc patients has the potential to guide therapeutic approaches, specifically by selecting treatments that individual patients are most likely to respond based on their unique intrinsic gene subsets. Intrinsic gene subsets at baseline have been shown to potentially be predictive of therapeutic responses. Several studies have investigated the relationship between intrinsic gene subsets and specific treatment responses (**Table 2**).

SSc patients in the fibroproliferative, but not the inflammatory gene subsets were shown to respond to tyrosine kinase inhibitors (TKI). TKI were explored as therapies for SSc because of the central role of tyrosine kinases in the pathogenesis of fibrosis (46). Imatinib is a small molecule TKI that antagonize c-Abl, a downstream mediator of PDGF and TGFβ receptors (R) (46). Use of Imatinib as experimental drug in SSc was previously reported in patients with dcSSc and clinical response to imatinib showed reduced expressions of genes typically found in the fibroproliferative subset (39). Similarly, responses to nilotinib, another TKI, were seen in patients with higher baseline expression of genes associated with TGFβR and PDGFR signaling, which significantly decreased in the improvers ($n = 4$, out of 6 patients) (40). In a more recent trial analyzing response to dasatinib, improvers ($n = 3$, out of total of 12 subjects) mapped to the fibroproliferative or normal-like subsets, whereas most of the non-improvers ($n = 7$, out of 9 non-improvers) were in the inflammatory subsets (41).

Janus kinase (JAK), which is a non-receptor tyrosine kinase that transduce cytokine signals via phosphorylation of STATs, has been suggested in pre-clinical studies to play a role in the pathogenesis of SSc through either pro-inflammatory or pro-fibrotic signals to the target cells (47). Gene expression profiling analysis confirmed elevated IL6/JAK/STAT and tofacitinib gene signatures in skin biopsies from the previously defined inflammatory subset of dcSSc patients, as compared to healthy

TABLE 2 | Stratification in relation to treatment responses.

Medications	Improvers	Non-improvers	Study design^	Tissue specimens	References
Imatinib	Baseline high fibroproliferative related gene expression (phosphorylated PDGFRβ and Abl) that decreased post-treatment in improvers	N.A.	Longitudinal (*n*, 2)	Skin biopsy (lesional) at baseline and during therapy	(39)
Nilotinib	Baseline high expression of TGFβR and PDGFRβ signaling genes that decreased post-treatment in improvers	Baseline low expression of PDGFRβ signaling genes	Longitudinal (*n*, 6)	Skin biopsy (lesional) at baseline and during therapy	(40)
Dasatinib	Baseline normal like or fibroproliferative subset in improvers	Baseline inflammatory subset	Longitudinal (*n*, 12)	Skin biopsy (lesional and non-lesional) at baseline and during therapy	(41)
Fresolimumab	Baseline high TGFβ-regulated gene thrombospondin-1 expression that decreased post-treatment in improvers	Baseline high immune related genes	Longitudinal (*n*, 15)	Skin biopsy (lesional) at baseline and during therapy	(38), (42)
Mycophenolate mofetil	Baseline inflammatory subset in improvers	Baseline fibropliferative or normal like subset	Longitudinal (*n*, 9)	Skin biopsy (lesional and non-lesional) at baseline and during therapy	(43)
Abatacept	Baseline inflammatory subset with high levels of CD28 signaling in improvers	Baseline normal like subset with low levels of CD28 signaling	Longitudinal (*n*, 6)	Skin biopsy (lesional) at baseline and during therapy	(44)
Rituximab	N.A.	Variable subsets (inflammatory, fibroproliferative, normal like); no change in gene expression post-treatment	Longitudinal (*n*, 13)	Skin biopsy (lesional) at baseline and during therapy	(27)
Mycophenolate mofetil and cyclophosphamide	Baseline higher IFN-inducible protein score	Baseline lower IFN-inducible protein score	Longitudinal (*n*, 133)	Serum at baseline	(45)

^Longitudinal study designs: tissue specimens were obtained serially at baseline and during therapies; n, sample size defined as number of patients with treatment and tissue specimens; N.A, not available.

controls (48). A pilot, single-center study of patients ($n = 10$, case series) evaluated the use tofacitinib, which inhibits primary JAK1/3 signaling in dcSSc with refractory skin thickness (49). The results demonstrated significant modified Rodnan skin score (mRSS) improvement in the first month suggesting its role as an effective immunosuppressant in refractory dcSSc with progressive skin thickness. PhaseI/II randomized controlled trial (NCT03274076) by Khanna et al. is ongoing, with initial results showing safety and a trend toward mRSS improvement (50). Further studies are needed to confirm the efficacy of tofacitinib and to evaluate its response in relation to inflammatory and fibrotic gene signatures.

In contrast to improvers to TKI, improvers to immunosuppressive medications were likely to be in the inflammatory gene subsets. Responders to mycophenolate mofetil (MMF) which targets lymphocyte proliferation (51), mapped to the inflammatory gene subset ($n = 4$, out of 7 improvers), whereas the non-improvers mapped to the fibroproliferative gene subset (n=2 subjects) (43). Likewise, responders to abatacept, which inhibits T cell activation by blocking CD80/CD86 interaction with CD28 (52), were in the inflammatory gene subset ($n = 4$ out of 5 improvers) and had higher baseline levels of CD28 signaling. The non-improver ($n = 1$) was in the normal-like gene subset with lower baseline levels of CD28 signaling (44).

Fresolimumab targets TGFβ signaling, with high baseline levels of TGFβ-regulated gene thrombospondin-1 (THBS1) that declined in patients with improved skin scores (42). Taroni et al. performed functional genomic meta-analysis, specifically functional genomic networks and machine learning of publicly available gene expression data from clinical trials of different therapeutics, including MMF and fresolimumab (53). While improvers to fresolimumab had high baseline TGFβ-related genes, non-improvers had elevated baseline levels of immune-related genes (53). Conversely, MMF improvers had high baseline immune-related genes that decreased post treatment (53). This study highlights the significance of genome-wide gene expression data gathered in clinical trials, which provides insight into the functional consequences of treatment and may be used to tailor treatment approaches.

MULTI-OMIC STRATIFICATION OF SSC: TOWARD PRECISION MEDICINE

The intrinsic complexity of SSc with heterogeneous manifestations necessitates a more strategic approach to thoroughly understand the underlying molecular mechanisms and to guide therapeutic approaches. Multiple-omics approaches from individual patients should be the direction of future work. Integration of high dimensional data encompassing information from transcriptomics, as well as genomics, epigenomics, proteomics, cytomics and microbiomics could lead to a more granular stratification system.

Through transcriptomic analysis, molecular signatures of SSc patients have been identified as described above. In addition, transcriptomic analysis has also revealed potential biomarkers with cross-sectional relationship with mRSS and may shed light into the disease pathogenesis (54). Two of the genes, cartilage oligomeric protein (COMP) and thrombospondin-1 (THBS1), are known to be regulated by transforming growth factor-β (TGFβ), whereas the other two genes, interferon-induced protein 44 (IFI44), and sialoadhesin (SIGLEC1) are known to be regulated by interferon (IFN) (54). More recently, systemic gene expression profiling through high throughput unsupervised clustering analysis has identified multiple genes as potential pharmacodynamic biomarkers in SSc skin (55). The identified genes were not limited to TGF? and IFN-regulated genes, but also MHC class I, proteasome, antigen processing, macrophage and vascular marker genes (55). These results highlight the roles of macrophage driven and vascular injury pathways in driving the disease process leading to fibrosis (55).

Technological advances in genomics, such as genome-wide association study (GWAS) and candidate gene approach (CGA) have highlighted important SSc susceptibility genes and non-HLA susceptibility genes (56). The majority of SSc susceptibility loci were found to be involved in innate or adaptive immune system (56). In addition, meta-analysis of GWAS revealed molecular pathways potentially involved in vasculopathy and fibrosis, both of which are central in the pathogenesis of SSc (57).

Although genetics play an important role in SSc pathogenesis, genetic factors alone are not sufficient to explain the disease occurrence, as there is low concordance rate of SSc among monozygotic twins (58). It is believed that environmental factors play an important role in the disease pathogenesis possibly through epigenetic regulation mediated through modifications in DNA, histone and non-coding RNAs (ncRNAs) (58, 59). However, the underlying pathophysiology linking genetic factors, epigenetic and environmental factors are still not fully understood.

The cellular responses to genetic, epigenetic and environment factors are reflected in the proteomic profiles. Accumulating data in high throughput proteomics have pointed to number of proteins and pathways associated with SSc progression and pathogenesis (60). Through progress in aptamer-based proteomic technology, a large array of serum protein was identified and could potentially be used as biomarkers in SSc to assess clinical progress as a number of differentially expressed proteins were found to correlate with mRSS (33). Differential expressions of proteins (midkine and FSTL3) were found in SSc patients with PAH and could potentially serve as a PAH biomarker and promising drug target (34). Type I interferons (IFNs), which are key regulators of innate immunity, play a role in the pathophysiology of SSc (61). Type I IFN signature was found in patients with very early SSc (before overt skin fibrosis), ATA and anti-U1 RNP antibodies (62, 63). High IFN-inducible chemokine levels were correlated with more severe skin, lung and muscle involvement in SSc (64). IFN-inducible proteins were demonstrated to have promising prognostic value in predicting treatment response (45, 61, 65).

Phase I trial of anifrolumab for SSc showed suppressed IFN signature in whole blood and skin, and this finding corresponded to suppression of T cell activation and collagen accumulation (66, 67). These shed light to the promising potential of using peripheral markers (e.g. high or low IFN signatures) to stratify patients for targeted treatment. More recently, serum proteins were shown to potentially be useful to guide therapeutic approaches in SSc-ILD patients (45). SSc-ILD patients with higher score of serum interferon-inducible proteins (IFNγ-inducible10-kd protein, monokine induced by IFNγ, monocyte chemotactic protein 2, β_2-microglobulin, tumor necrosis factor receptor type II, and macrophage inflammatory protein 3β) responded better to immunosuppression (45). Future prospective longitudinal clinical studies are needed to evaluate the prognostic values of various candidate proteomic biomarkers in clinical practice.

In comparison to above techniques, cytomics allows simultaneous analysis of a number of parameters. It could be potentially used to shed light into the pathophysiology of SSc. High dimensional cytometry has proven to be a powerful tool to quantify large number of immune cell subsets and analyze their correlation with clinical markers (28, 68, 69). The frequency of monocyte subsets was found to be correlated with disease severity in SSc and changes in monocyte frequencies were already noted in the early phase of SSc disease in the pre-fibrotic stage. (28) Our group investigated blood mononuclear cells from SSc patients using mass cytometry and transcriptomic analysis (68). Unsupervised clustering analysis were performed to identify nodes composed of similar cells, and the results revealed significant differences in the frequencies of T and B cell subsets in SSc subsets, as well as compared to healthy controls (68). In patients with ILD compared to those without ILD, we found increased nodes representing CD4+ T cells expressing CCR4 and ICOS, but decreased nodes representing mucosal associated invariant T cells (68). In addition, based on peripheral blood immune cell phenotypes and organ involvement, Kubo S et al. stratified SSc patients into 3 groups: Treg-dominant group, Tfh-dominant group and fewer abnormalities group (**Table 1**) (29). Future studies could evaluate the potential role of immune cell phenotypes to prognosticate therapeutic response, e.g., role of targeted therapy for B cells by rituximab in Tfh-plasmablast dominant group. Despite these advances, identification of high dimensional biomarkers to clearly stratify patients with SSc remains an unmet need. The Extended Polydimensional Immunome Characterization (EPIC), a web-based discovery

tool could be deployed for comprehensive analyses of single cell dataset to identify high dimensional biomarkers in SSc patients in comparison to healthy datasets (69).

Dysbiosis of the GI microbiome is known to have systemic effect on the immune system in SSc (70). Two culture-independent metagenomic sequencing technologies have been used to characterize the GI microbiome. Most commonly reported technology is the 16S RNA sequencing, which enables bacterial identification (answers the question "who are they?"). Whole-genome shotgun sequencing enables the identification of the gene and their metabolic and enzymatic pathways (answers the question "what are they doing?"). In SSc compared to healthy controls, consistent observations using both approaches have shown reduced abundance of *Bacteroides* species (which protects host from mucosal inflammation), *Clostridium* and *Faecalibacterium* species (butyrate-producing organisms that enhance epithelial barrier function), and increased abundance of *Lactobacillus* (implicated in SSc GI dysmotility) and *Bifidobacterium* species (30, 70). Alpha diversity, which is the complexity of microbiome composition within individuals of a group, was suggested to be decreased with SSc patients with more severe disease, longer disease duration and dcSSc (**Table 1**) (30–32, 71–73). There have been a few small clinical trials on GI-microbiome therapeutic interventions in SSc (74–76). In a placebo-controlled trial of probiotics, Low et al. found that baseline microbiome composition and probiotics were independently associated with GI symptom improvement. (75) These suggest a potential role of GI microbiome modulation to improve GI symptoms. Longitudinal studies of GI microbiome in SSc are needed to understand the contribution of microbiome alteration to the development of GI and extra-intestinal manifestations of SSc.

With the tremendous amount of multi-omics data in SSc, advances in machine learning has made it possible to integrate high dimensional data with cutting-edge computational tools. Systems biology based approach has the potential to condense multiple-omics data to derive meaningful molecular interaction network and facilitate better capture of SSc complex pathogenesis (77). Although it is challenging to delineate the modular organization at the molecular level, multiple integration strategies have been developed to analyze regulatory relationships between each omic layer (78). Future integration of multi-omics data may improve our understanding of complex SSc pathogenesis and distinguish distinct patient subtypes.

CONCLUSION AND UNMET NEED

SSc is a complex multisystemic disease with heterogenous clinical manifestations and characteristics. Advances in transcriptomics have led to identification of distinct SSc molecular gene signatures. Tremendous amount of multi-omics data has emerged in SSc field and machine learning could be deployed to integrate and analyze multi-omic data in SSc to develop a more granular classification system.

Challenges in multi-omic data analysis include the rarity of SSc disease and hence its limited sample size. Furthermore, lack of clinical information, e.g., disease duration or treatment history, further hinders clinical phenotyping of the subjects in publicly available datasets. In the future, a more comprehensive longitudinal study of SSc patients and collaborative effort to integrate high dimensional data would be pivotal to create an SSc atlas at global level to gain more insights into disease etiology, prognosis and progression. This may help the characterization of heterogenous SSc patients and personalization of therapeutic approaches toward precision medicine.

AUTHOR CONTRIBUTIONS

AL and SA contributed to conception and design of the study. MN wrote the first draft of the manuscript. VC contributed to manuscript revision and editing. All authors contributed to manuscript revision, read, and approved the submitted version.

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Secular Trends of Incidence, Prevalence, and Healthcare Economic Burden in ANCA-Associated Vasculitis: An Analysis of the 2002–2018 South Korea National Health Insurance Database

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Objectives: The incidence and prevalence of AAV in Asia remain poorly understood, especially in a nationwide setting. This study investigated the incidence, prevalence, and healthcare burden of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) in South Korea by analyzing a national database.

Methods: This study included patients with AAV identified from the National Health Insurance Service Database of South Korea from 2002 to 2018. Patients were diagnosed with AAV in a general or tertiary hospital and were registered in the individual payment beneficiaries program or were prescribed glucocorticoids. A calendar-based meteorological definitions were adopted to assess the differences in the incidence of AAV according to season. The average healthcare expenditure and patient outcomes of mortality and end-stage renal disease (ESRD) in patients with AAV were compared to 1:10 age, sex and residential area matched controls.

Results: A total of 2,113 patients [708, 638, and 767 with microscopic polyangiitis (MPA), granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis, respectively] were identified. The annual incidence and prevalence of AAV increased continuously, and MPA being the most common disease subtype after 2015. The highest incidence and prevalence of AAV was 0.48/100,000 person-years (PY) and 2.40/100,000 PY in 2017 and 2018, respectively. There were no significant differences in monthly and seasonal incidence of AAV. The average expense of medical care, overall mortality, and ESRD rates of patients with AAV were higher in patients with AAV than in controls, especially in the case of MPA.

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Conclusion: An increasing trend of AAV diagnosis observed is consistent with the evidence that AAV is more common in recent years; however, a relatively lower incidence and prevalence was observed compared to that in Western countries. The higher medical cost and rates of mortality and ESRD in AAV emphasize the early recognition and implementation of optimal treatment for these patients.

Keywords: antineutrophil cytoplasmic antibody-associated vasculitis, South Korea, incidence, prevalence, healthcare burden

INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a potentially lethal systemic autoimmune disease affecting small-sized vasculatures, and comprises three subtypes: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) (1). Detection of circulating ANCAs against myeloperoxidase (MPO) and proteinase 3 (PR3), although undetectable in ∼20%−40% of patients (2), is a classic laboratory finding in AAV, and typical clinical and pathologic results are crucial for differentiating these disease subtypes (3). Judicious clinical suspicion is crucial in AAV diagnosis owing to the large heterogeneity of disease manifestations and absence of absolute pathognomonic features. Traditionally, AAV has been considered a rare disorder that usually occurs in the elderly population. However, an increasing number of evidence indicates that AAV is more common in recent years compared to the previous numerical estimates (4).

The incidence of AAV is largely variable according to the existing literature. A considerable disparity in its incidence has been reported based on the geographic region in which the research was conducted, with a higher incidence of AAV reported in Western countries and a much lower incidence in Asia (5). In addition, a predominance of GPA is observed in Northern Europe and Australia, whereas MPA has been reported as the most common disease subtype in Japan and Southern Europe (6). Meanwhile, US data demonstrated that MPA was the most frequent diagnosis among the incident cases of AAV (7). However, the prevalence of AAV appears to be similar to its incidence pattern in the corresponding region.

A previous study by Fujimoto et al., which investigated the incidence of AAV in regions of Asia, has shown that the average annual incidence of AAV was estimated to be comparable between Japan and the UK; however, MPA comprised most AAV cases in Japan, whereas GPA was predominantly found in the UK (8). Except for this study, no other studies have reported on the incidence and prevalence of AAV in Asia, especially in a nationwide population-based setting. Furthermore, as most epidemiologic studies of AAV were performed in Western countries and are scarce in other Asian countries, these data might insufficiently reflect the difference in global epidemiology; therefore, a better understanding is required. To address this issue, this study aimed to evaluate the incidence and prevalence in South Korea by analyzing a nationwide healthcare administrative database.

METHODS

The National Health Information Database and Case Selection

We searched the National Health Information Database (NHID) from 2002 to 2018 for data acquisition. The NHID, which is managed by the South Korean government, contains information on the utilization of national hospital care of over 50 million individuals (more than 97% of South Korean residents) that is covered by the National Health Insurance Service (NHIS). As inclusion of the NHIS is mandated by the South Korean government, utilization of the NHID enables the identification of a large population of rare diseases in a nationwide healthcare setting suitable for epidemiological studies (9, 10). The dataset available in the NHID is as follows, which is also described elsewhere: (i) general information of patients such as age, gender, the healthcare provider, the date of visiting the hospital, and diagnosis requiring hospital care (either primary or secondary), (ii) utilization of healthcare services of procedures, operations, and injection, and (iii) the prescription of drugs for treatment (10). However, collecting detailed information of an individual is fundamentally prohibited, as it could result in de-identification of a patient.

In selecting patients with AAV, the following definitions were applied: (i) patients diagnosed with AAV in a general or tertiary hospital according to the ICD-10 codes for AAV (M31.7 for MPA, M31.3 for GPA and M30.1 for EGPA); (ii) those registered in the payment beneficiary program for rare and intractable diseases provided by the South Korean government with the corresponding code (V code) of AAV (V135 for MPA, V238 for GPA and V134 for EGPA); and (iii) those prescribed with glucocorticoids (prednisone, prednisolone, triamcinolone, methylprednisolone, dexamethasone, betamethasone, deflazacort, hydrocortisone and budesonide). Patients who satisfied definitions (i) + (ii) or (i) + (iii) were included for AAV case ascertainment.

In the present study, the date of AAV diagnosis (index date) was defined as the initial date of fulfilling the case ascertainment criteria. In addition, the incidence of AAV was calculated after applying a 2-year washout period (estimated from 2004), and prevalent cases were defined as those who fulfilled the criteria for case ascertainment and received outpatient or inpatient hospital care (estimated from 2002). Furthermore, as the ICD-10 code for MPA was included in the NHID in 2008, the incidence and prevalence of MPA were calculated after 2008. This study was approved by the Institutional Review Board of the NHIS Ilsan

Hospital (Institutional Review Board No: 2022-01-003), and the requirement to obtain informed consent from the patients was waived due to the retrospective nature of the study.

Baseline Variables, Medical Costs, and the Definition of Season

Baseline variables that were investigated at AAV diagnosis included sex, age, income and the type of insurance. The comorbidity of patients was evaluated using the Charlson comorbidity index (CCI) within 1 year of disease diagnosis (11). The average medical cost of patients with AAV was calculated as the total out-of-pocket expenses incurred in the hospital per patient after diagnosis.

In addition, calendar-based meteorological definitions of spring (1 March to 31 May), summer (1 June to 31 August), autumn (1 September to 30 November), and winter (1 December to 28 February) were adopted to assess the differences in the incidence of AAV according to season.

Patient Outcomes and Selection of Controls

For patient outcomes, all-cause mortality and end-stage renal disease (ESRD) were investigated. Patient mortality was defined as the existence of a registered date of death after the onset of AAV, and those who were granted a medical expense reduction (V code) and treatment code (O code) for haemodialysis (V001 and O7020) and/or peritoneal dialysis (V003 and O7061) after AAV diagnosis were considered as developing ESRD (12). The follow-up duration was calculated as the time interval between disease diagnosis and the occurrence of death and ESRD in patients or the last follow-up date.

To compare the medical costs and patient outcomes between patients with AAV and the general population, 20,695 controls who received medical care at a general or tertiary hospital were randomly extracted by performing a 1:10 matching by age, sex and residential area.

Statistical Analysis

Statistical analyses were conducted using the SAS V.9.4 Enterprise Guide (SAS Institute). Continuous and categorical variables were presented as mean (SD) and frequencies (percentages) and were compared using Student's *t*-test and Chi-squared or Fisher's exact test as indicated. The annual and prevalence rate/100,000 persons were calculated using the number of cases identified in the corresponding year and the population registered in the middle of each year. Differences

TABLE 1 | Baseline characteristics of patients with ANCA-associated vasculitis and controls.

	Total (n = 22808)	Controls (n = 20695)	ANCA-associated vasculitis (n = 2113)	p-value
Sex				
Male	10420 (45.7)	9445 (45.6)	975 (46.1)	0.658
Female	12388 (54.3)	11250 (54.4)	1138 (53.9)	
Age, years	57.9 ± 15.8	57.8 ± 15.8	58.1 ± 15.8	0.484
Age distribution				
10–19	373 (1.6)	339 (1.6)	34 (1.6)	0.997
20–29	1165 (5.1)	1059 (5.1)	106 (5.0)	
30–39	1701 (7.5)	1545 (7.5)	156 (7.4)	
40–49	2804 (12.3)	2547 (12.3)	257 (12.2)	
50–59	4813 (21.1)	4371 (21.1)	442 (20.9)	
60–69	5961 (26.1)	5413 (26.2)	548 (25.9)	
70–79	5064 (22.2)	4588 (22.2)	476 (22.5)	
80–89	895 (3.9)	804 (3.9)	91 (4.3)	
≥ 90	32 (0.1)	29 (0.1)	3 (0.1)	
Income[†]				
<3 rd quintile	4977 (22.3)	4511 (22.3)	466 (22.4)	0.624
3~7 th quintile	7198 (32.2)	6509 (32.1)	689 (33.1)	
>7 th quintile	10165 (45.5)	9236 (45.6)	929 (44.6)	
Insurance type				
Employee	9117 (40.0)	8430 (40.7)	687 (32.5)	<0.001
Self-employment	13005 (57.0)	11658 (56.3)	1347 (63.7)	
Medical-aid	686 (3.0)	607 (2.9)	79 (3.7)	
CCI	2.1 ± 1.3	2.0 ± 1.2	3.0 ± 1.6	<0.001

[†] *Data are available for 22,340 patients.*

Data are expressed as mean (SD) or frequencies (percentages).

ANCA, antineutrophil cytoplasmic antibody; CCI, Charlson comorbidity index.

TABLE 2 | Comparison of characteristics between subgroup of patients with ANCA-associated vasculitis at diagnosis.

	MPA (*n* = 708)	GPA (*n* = 638)	EGPA (*n* = 767)	ANCA-associated vasculitis (*n* = 2113)	*p*-value
Sex					
Male	305 (43.1)	300 (47.0)	370 (48.2)	975 (46.1)	0.121
Female	403 (56.9)	338 (53.0)	397 (51.8)	1138 (53.9)	
Age, years	64.3 ± 14.1	57.5 ± 15.2	52.9 ± 15.9	58.1 ± 15.8	<0.001
Age distribution					
10–19	11 (1.6)	9 (1.4)	14 (1.8)	34 (1.6)	<0.001
20–29	15 (2.1)	34 (5.3)	57 (7.4)	106 (5.0)	
30–39	22 (3.1)	47 (7.4)	87 (11.3)	156 (7.4)	
40–49	34 (4.8)	70 (11.0)	153 (19.9)	257 (12.2)	
50–59	112 (15.8)	152 (23.8)	178 (23.2)	442 (20.9)	
60–69	218 (30.8)	179 (28.1)	151 (19.7)	548 (25.9)	
70–79	246 (34.7)	126 (19.7)	104 (13.6)	476 (22.5)	
80–89	48 (6.8)	21 (3.3)	22 (2.9)	91 (4.3)	
≥90	2 (0.3)	0 (0.0)	1 (0.1)	3 (0.1)	
Income[†]					
<3 rd quintile	153 (21.9)	163 (26.0)	150 (19.8)	466 (22.4)	0.010
3∼7 th quintile	212 (30.3)	213 (34.0)	264 (34.9)	689 (33.1)	
>7 th quintile	335 (47.9)	251 (40.0)	343 (45.3)	929 (44.6)	
Insurance type					
Employee	218 (30.8)	204 (32.0)	265 (34.6)	687 (32.5)	0.603
Self-employment	464 (65.5)	408 (63.9)	475 (61.9)	1347 (63.7)	
Medical-aid	26 (3.7)	26 (4.1)	27 (3.5)	79 (3.7)	
CCI	3.3 ± 1.6	2.8 ± 1.6	2.8 ± 1.4	3.0 ± 1.6	<0.001

[†] *Data are available for 700, 627, 757 and 2,084 patients with MPA, GPA, EGPA and ANCA-associated vasculitis, respectively.*

Data are expressed as mean (SD) or frequencies (percentages).

MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; ANCA, antineutrophil cytoplasmic antibody; CCI, Charlson comorbidity index.

in the trends of the incidence and prevalence of AAV were assessed using Poisson regression, and the influence of monthly and seasonal differences in disease incidence was calculated using a one-way analysis of variance test. A two-tailed *p*-value of <0.05 was considered significant in all statistical analyses.

RESULTS

Comparison of Patient Characteristics Between Patients With AAV and Controls

Table 1 describes the baseline characteristics of the patients with AAV and the controls. The proportion of women and the mean age of patients with AAV were 53.9% and 58.1 years, respectively. AAV was most common in those aged 60–69 years. There was no difference in income status between patients with AAV and the controls. However, concerning insurance type, self-employment was more frequent in AAV patients, and the mean CCI was also higher in the AAV group.

Baseline Characteristics of AAV Subgroups

Comparison of patient characteristics of the AAV subgroups revealed no significant differences in terms of sex among patients with MPA, GPA and EGPA. However, among the AAV subgroups, patients with MPA had the oldest age when diagnosed, frequently diagnosed in ages 70–79 years. Meanwhile, GPA and EGPA were the most common in the age groups of 60–69 and 50–59, respectively. In addition, differences were noted in terms of income in the AAV subgroups, while the insurance types of patients were comparable. Patients with MPA had the highest mean CCI scores compared to patients with GPA and EGPA (**Table 2**).

Annual Incidence, Prevalence, and Monthly and Seasonal Incidence of AAV

The annual incidence and prevalence of AAV increased continuously during the observed years (both $p < 0.001$), and the incidence of MPA was the highest after 2015 among the AAV subgroups. In particular, the highest incidence of AAV was 0.48/100,000 person-years (PY) in 2017 and 2.40/100,000 PY in 2018 (**Figures 1A,B**). Incident cases of AAV were most frequently observed in March and lowest in June, but no significant monthly and seasonal differences were noted ($p = 0.973$ and $p = 0.877$ for every month and season, respectively; **Figures 2A,B**).

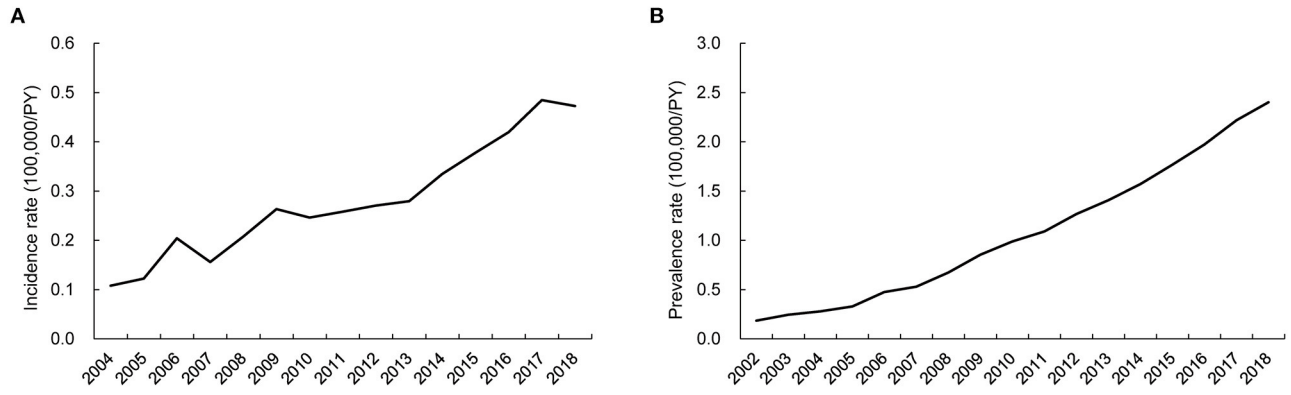

FIGURE 1 | Time-trends of incidence and prevalence rate of ANCA-associated vasculitis. Annual trends of **(A)** incidence and **(B)** prevalence of AAV showing a continuous increase during the investigation period. ANCA, antineutrophil cytoplasmic antibody; PY, person-year.

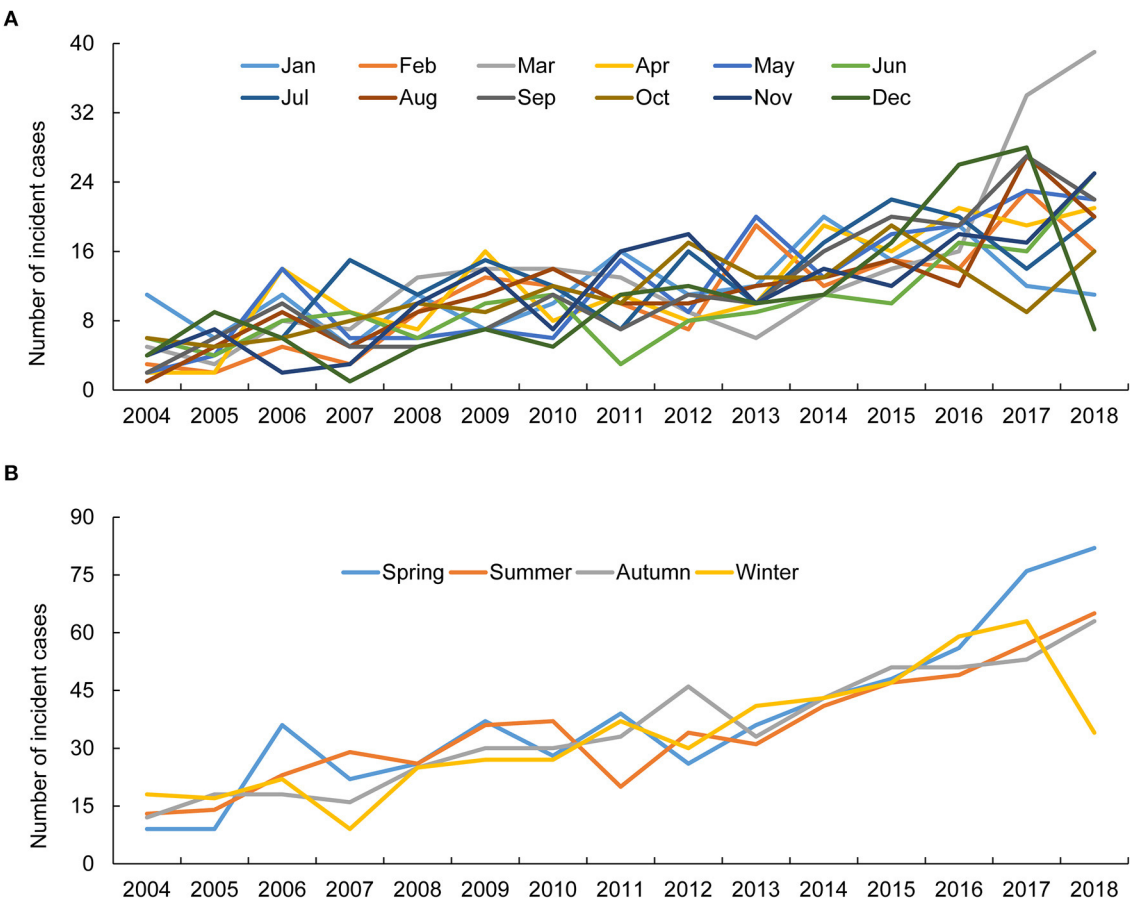

FIGURE 2 | Monthly and seasonal incidence of ANCA-associated vasculitis. The difference in AAV incidence was not apparent according to the **(A)** month and **(B)** season. ANCA, antineutrophil cytoplasmic antibody.

Healthcare Cost and Outcomes in Patients With AAV

Compared to the controls, the cost of medical care was greater in patients with AAV and highest in patients with MPA. This expenditure peaked in the first year of AAV diagnosis, rapidly declined in the second year, and was similar afterwards (**Figure 3**).

Furthermore, during the mean follow-up period of 58.7 months, the reported overall mortality and ESRD rates of patients with AAV were 26.8 and 8.2%, respectively, which were

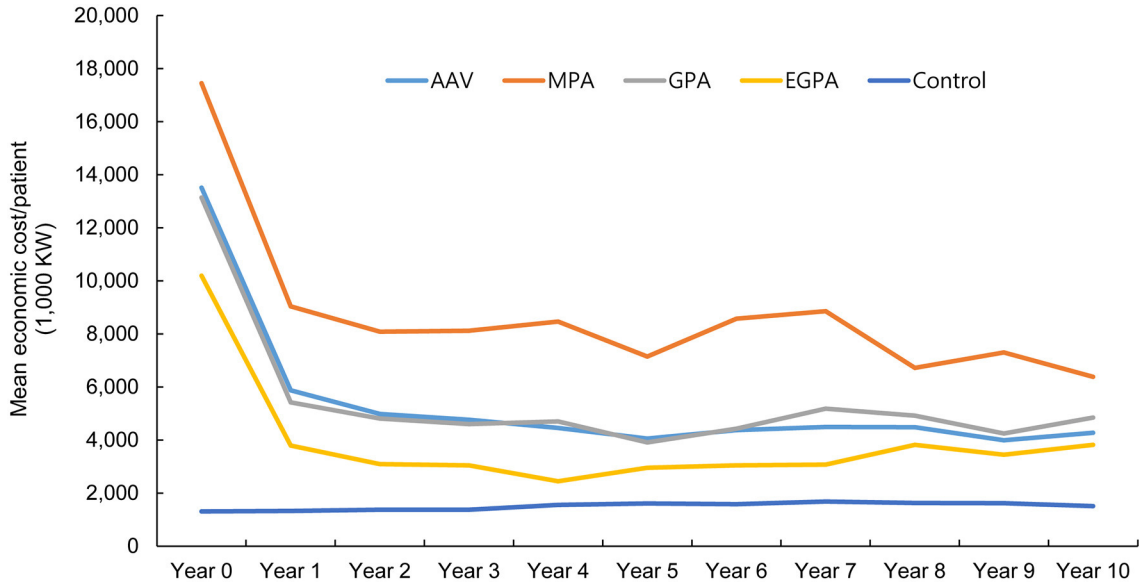

FIGURE 3 | Estimation of annual economic cost in patients with ANCA-associated vasculitis and controls. While the economic cost in healthy controls remained stable during the follow-up period, patients with ANCA-associated vasculitis showed a steep reduction in medical costs in the second year after diagnosis, and remained similar after the third year. ANCA, antineutrophil cytoplasmic antibody; ESRD, end-stage renal disease; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis.

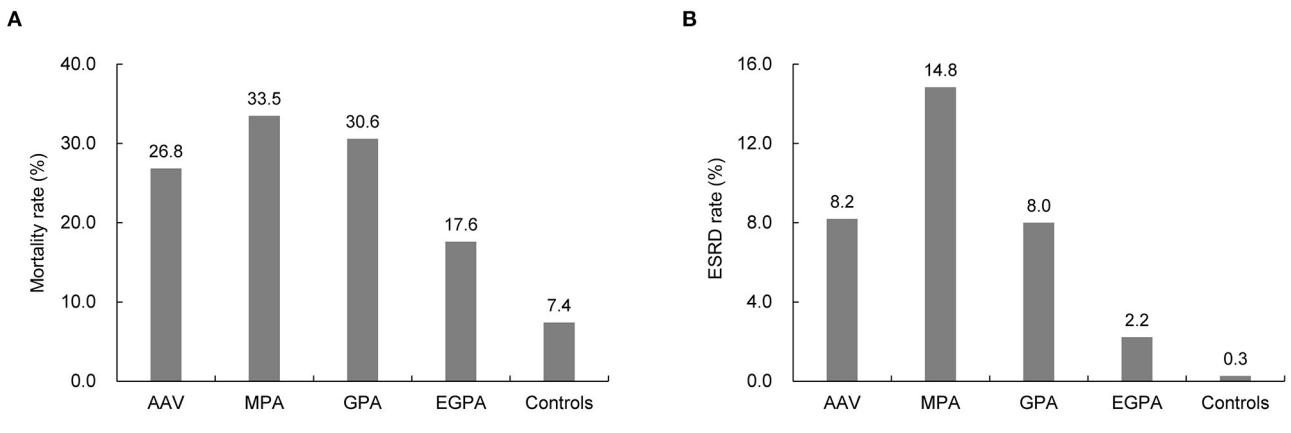

FIGURE 4 | Comparison of mortality and ESRD rate between patients with ANCA-associated vasculitis and controls. Compared to the controls, patients with AAV had higher rates of **(A)** mortality and **(B)** developing ESRD. ESRD, end-stage renal disease; ANCA, antineutrophil cytoplasmic antibody; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis.

significantly higher than those of the controls (all $p < 0.001$). The highest mortality and ESRD rates were found in patients with MPA (33.5 and 14.8%, respectively). The overall mortality and ESRD rates in patients with EGPA were the lowest in the AAV subgroups (17.6% and 2.2%, respectively) during the observation period (**Figures 4A,B**).

Basal Characteristics of Patients With Mortality and ESRD

Regarding the clinical characteristics of patients with AAV related to mortality, patients who died tended to be male, older, and were mostly diagnosed with MPA. Furthermore, the proportion of those who were covered by medical aid care and baseline CCI was higher in patients who died (**Table 3**). In contrast, compared with patients who did not develop ESRD, those who developed ESRD were older, were mostly diagnosed with MPA, and had a higher CCI at disease diagnosis (**Table 4**).

DISCUSSION

Although accumulating evidence indicates that AAV is more common than in the past decades, the incidence and prevalence

TABLE 3 | Baseline characteristics of ANCA-associated vasculitis patients suffering mortality and without.

	Patients with mortality ($n = 567$)	Patients without mortality ($n = 1546$)	*p*-value
Sex			
Male	307 (54.1)	668 (43.2)	<0.001
Female	260 (45.9)	878 (56.8)	
Age, years	67.2 ± 11.9	54.7 ± 15.8	<0.001
Diagnosis			
MPA	237 (41.8)	471 (30.5)	<0.001
GPA	195 (34.4)	443 (28.7)	
EGPA	135 (23.8)	632 (40.9)	
Income[†]			
<3 rd quintile	132 (23.7)	334 (21.9)	0.228
3∼7 th quintile	168 (30.2)	521 (34.1)	
>7 th quintile	257 (46.1)	672 (44.0)	
Insurance type			
Employee	194 (34.2)	493 (31.9)	<0.001
Self-employment	338 (59.6)	1009 (65.3)	
Medical-aid	35 (6.2)	44 (2.8)	
CCI	3.4 ± 1.6	2.8 ± 1.5	<0.001

[†] *Data were available for 557 and 1,527 patients with and without mortality, respectively. Data are expressed as mean (SD) or frequencies (percentages).*
ANCA, antineutrophil cytoplasmic antibody; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; CCI, Charlson comorbidity index.

TABLE 4 | Basal characteristics of ANCA-associated vasculitis patients with and without ESRD.

	Patients with ESRD ($n = 173$)	Patients without ESRD ($n = 1940$)	*p*-value
Sex			
Male	68 (39.3)	907 (46.8)	0.060
Female	105 (60.7)	1033 (53.2)	
Age, years	63.3 ± 14.9	57.6 ± 15.8	<0.001
Diagnosis			
MPA	105 (60.7)	603 (31.1)	<0.001
GPA	51 (29.5)	587 (30.3)	
EGPA	17 (9.8)	750 (38.7)	
Income[†]			
<3 rd quintile	37 (21.6)	429 (22.4)	0.633
3∼7 th quintile	52 (30.4)	637 (33.3)	
>7 th quintile	82 (48.0)	847 (44.3)	
Insurance type			
Employee	53 (30.6)	634 (32.7)	0.525
Self-employment	111 (64.2)	1236 (63.7)	
Medical-aid	9 (5.2)	70 (3.6)	
CCI	3.5 ± 1.7	2.9 ± 1.5	<0.001

[†] *Data were available for 171 and 1,913 patients with and without ESRD, respectively. Data are expressed as mean (SD) or frequencies (percentages).*
ANCA, antineutrophil cytoplasmic antibody; ESRD, end-stage renal disease; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; CCI, Charlson comorbidity index.

of AAV in Asia remain poorly understood. In the present study, the following results were obtained by searching through the NHID: (i) although AAV is still rare, the annual incidence and prevalence of AAV in South Korea showed a gradual rise, with the highest incidence and prevalence of 0.48/100,000 and 2.40/100,000 PY reported in 2017 and 2018, respectively; (ii) the economic healthcare burden of AAV was higher than that of the controls; this increased medical cost culminates in the first year of AAV diagnosis but remains stable after the third year of AAV diagnosis; and (iii) patients with AAV are at an increased risk of mortality and developing ESRD, which is significantly greater compared to the control group. To the best of our knowledge, this is the first study to evaluate the incidence, prevalence, and economic costs of AAV in Asia using a nationwide cohort.

The global incidence of AAV ranges 1.2–3.3/100,000 PY with a prevalence of 4.6–42.1/100,000 PY (4). While studies evaluating the incidence of AAV in Asia are rare, a single-district study from Japan demonstrated an average incidence of AAV of 22.6/million PY (8). Our results showed that the highest incidence and prevalence of AAV in South Korea were 4.8/million and 24.0/million PY, respectively, which is relatively lower than that reported in Japan. Meanwhile, a study performed in Taiwan using the National Health Insurance Database determined that the incidence of GPA was 0.37/million PY (13). The incidence of GPA in our study ranged 0.5–1/million PY, showing a similar numerical estimates with the study by Wu et al. In addition, in the present study, MPA was the most frequent diagnosis among the AAV cases after 2015, supporting that MPA is the most common disease subtype of AAV in Asia (14). Consistent with our data, an analysis of a national inpatient database from China demonstrated that MPA was the most common diagnosis requiring hospitalization, although the incidence and prevalence of AAV could not be calculated in the study by Li et al. (15). However, even in regions within Asia, an inconsistency of patient clinical characteristics has been also reported. For example, single-center studies from India reported a relatively lower age of onset in patients with GPA with a high positivity rate for anti PR3 (or c-ANCA), and a distinctive clinical feature was observed compared to the Western cohorts (16, 17). Altogether, it is apparent that there still is an uncertainty regarding the characteristics of patients with AAV in Asia (18), highlighting the needs for a further exploration.

A previous investigation indicated that ultraviolet radiation has an inverse correlation with the incidence of AAV, suggesting that seasonal variation could be related to disease incidence (19). Nonetheless, in our study, while the number of AAV cases was highest in March, the influence of season on the incidence of AAV was not significant, which was found similar in a recent national Scottish registry-based study, confirming a non-significant association between seasonality and AAV incidence (20). On our study, the overall incidence and prevalence of AAV seemed to increase gradually during the observation period. These results might have been attributed to increased disease awareness and patient management strategies among physicians attending to patient care. However, the incidence and prevalence

of AAV appeared to be lower than those reported in Western countries, implying that a geographic distinction exists.

Patients with autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, are subject to increased healthcare utilization and economic costs (21, 22). In line with this finding, a retrospective study from Italy revealed that healthcare costs in patients with AAV were noticeably high (23). On analyzing the trends of average medical costs of patients with AAV, we found that their economic expenditure was remarkably high in the first year after disease diagnosis and decreased sharply in the second year. Meanwhile, medical costs appeared to be similar after the third year of AAV diagnosis. Among the AAV subtypes, patients with MPA had the highest medical expenditure, which might be due to the fact that patients with MPA are more likely to undergo intensive medical care owing to the greater risk of death and development of ESRD, as described previously and in the present study (24, 25). Conversely, patients with EGPA had the lowest medical expenditure among AAV subtypes, which could be explained by the favorable prognoses of patients with EGPA (26). The medical cost of the control group remained constant in 10 years and was lower than that of patients with AAV, irrespective of disease subtypes. Finally, our study revealed a higher frequency of death in patients with AAV among medical-aid covered individuals. Taken together, our findings emphasize that programs that reduce the medical expenses of patients with AAV should be continuously implemented to facilitate adequate medical care.

Although significant advances have been made in the diagnosis and treatment of AAV, there is a heightened risk of mortality and ESRD in patients with AAV. The European Vasculitis Society (EUVAS) data showed that the 1- and 5-year survival rates in patients with AAV were 88 and 78%, respectively (27). Furthermore, long-term data from the EUVAS clinical trial registry reported that 18.6% and 13% of patients died and developed ESRD, respectively (28). Additionally, the analyses of the Glomerular Disease Collaborative Network inception cohort described a 5-year mortality and ESRD rates of 28 and 46%, respectively, in patients with AAV, although a substantial improvement in patient prognoses was also observed compared to previous years (29). In our study population, 26.8% of the patients died and 8.2% developed ESRD, which was significantly higher than that in the control group. Collectively, these findings indicate that AAV is a life-threatening disease that requires prompt diagnosis and timely treatment.

This study has some limitations. First, given that the results of laboratory tests were not recorded in the NHID, we were not able to investigate the proportion of ANCA-positive patients and the differences in ANCA specificity and laboratory features according to the AAV subtypes. Second, to increase diagnostic accuracy, we only included patients with AAV who had the corresponding ICD-10 code and V code for rare and intractable diseases or those who were treated with glucocorticoids, which might have led to disease underestimation. Third, due to the limitations of the NHID, disease-specific details of patients with AAV, such as the extent of disease (e.g. limited or systemic), organ involvement patterns, and the existence of organ damage, were not available. Fourth, the information and influence of medications, besides glucocorticoids, to treat AAV could not be evaluated. Fifth, the description of cause-specific death in our patients could not be provided, owing to the inherent limitation of the NHID. We believe that additional research are necessary in the future to better understand the epidemiology and characteristics of patients with AAV, especially in Asia.

In conclusion, the present study identified a secular trend of increasing incidence and prevalence in South Korea. It was also demonstrated that patients with AAV required greater expenditure in terms of medical care and conferred a higher risk of death and development of ESRD compared to the controls. Our findings provide valuable information for understanding the epidemiology of AAV in Asia and highlight the importance of early recognition and implementation of optimal treatment for AAV to improve patient outcomes.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available. Data are available from the Korea National Health Insurance Sharing Service (NHISS) for licensed researchers. However, data are restricted to be shared publicly owing to the sensitive nature of the collected data. Requests to access the datasets should be directed to contact at: https://nhiss.nhis.or.kr, contact: +82-33-736-2432, 2433.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the NHIS Ilsan Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: SA and J-SP. Methodology, validation, investigation, and project administration: SA, HL, and J-SP. Software and visualization: SA and HL. Formal analysis and data curation: HL. Resources: CL and Y-BP. Writing—original draft preparation: SA, CL, Y-BP, and J-SP. Writing—review and editing: SA, HL, CL, Y-BP, J-SP, and S-WL. Supervision: CL, Y-BP, and S-WL. Funding acquisition: S-WL. All authors have read and agreed to the final version of the manuscript.

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Serum ferritin as a crucial biomarker in the diagnosis and prognosis of intravenous immunoglobulin resistance and coronary artery lesions in Kawasaki disease: A systematic review and meta-analysis

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Background: Early identification and treatment are paramount for intravenous immunoglobulin (IVIG) resistance and coronary artery lesions (CALs) in patients with Kawasaki disease (KD). Unfortunately, there is no single crucial biomarker to identify these patients in a timely manner, which makes KD the most common cause of acquired heart disease in children in developed countries. Recently, many studies have focused on the association between serum ferritin (SF), IVIG resistance, and CALs in KD. We thus performed a systematic review and meta-analysis to ascertain the diagnostic and prognostic values of SF in predicting IVIG resistance and CALs in KD in the acute phase.

Methods: The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the receiver operating characteristic curve (AUC) were extracted from the data to evaluate the SF levels in KD. The hazard ratios (HRs) of related risk factors and their corresponding 95% confidence intervals (CIs) were applied to compute the pooled assessments of the outcomes.

Results: A total of 11 eligible articles were included in this meta-analysis, including twenty studies for diagnosis and five studies for prognosis. In terms of diagnostic values, SF could identify KD patients in the overall studies with a relatively high pooled sensitivity, specificity, PLR, NLR, DOR, and AUC of 0.76 (95% CI: 0.69–0.82), 0.82 (95% CI: 0.76–0.88), 4.33 (95% CI: 3.07–6.11), 0.29 (95% CI: 0.22–0.38), 15.0 (95% CI: 9.00–25.00), and 0.86 (95% CI: 0.83–0.89), respectively. In studies comparing KD patients and controls, there were a relatively high pooled sensitivity, specificity, PLR, NLR, DOR, and AUC of 0.79 (95% CI: 0.72–0.84), 0.84 (95% CI: 0.79–0.91), 4.61 (95% CI: 3.27–6.51),

0.26 (95% CI: 0.20–0.34), 20.82 (95% CI: 11.83–36.64), and 0.89 (95% CI: 0.86–0.91), respectively. For the prognostic values, we found poor survival outcomes based on KD patients (HR = 1.31, 95% CI: 1.07–1.59, P = 0.008).

Conclusion: Our meta-analysis suggests that SF may be used as a workable and critical biomarker for the diagnosis and prognosis of IVIG resistance and CALs in patients with KD. We also propose that maintaining the dynamic balance between iron, SF, and ferroptosis will be an important therapeutic strategy to reduce the morbidity of CALs.

Systematic review registration: [https://www.crd.york.ac.uk/prospero/], identifier [CRD42022279157].

KEYWORDS

serum ferritin, Kawasaki disease, coronary artery lesions (CALs), intravenous immunoglobulin resistance, ferroptosis

Introduction

Kawasaki disease (KD) is an acute systemic vasculitis with unknown etiology, which is the most common cause of pediatric acquired heart disease in developed countries and may result in long-term cardiac sequelae during adulthood (1). Moreover, according to the recent global epidemiology of vasculitis, Kawasaki disease occurs most frequently in East Asia, especially Japan, South Korea, and China, with a relatively equal distribution elsewhere (2). Virtually all deaths of patients with KD result from coronary artery lesions (CALs) (3). Many cases of fatal and non-fatal myocardial infarction (MI) in young adults have been attributed to "missed" KD in childhood (4). KD and KD-related cardiac sequelae create an enormous burden on individuals, families, and society. However, the diagnosis of KD mainly depends on patients' clinical symptoms, which directly lead to a delay in the diagnosis and treatment of many children with incomplete KD (i.e., patients who do not have sufficient principal clinical findings) and increase the incidence of CALs. Although the timely initiation of intravenous immunoglobulin (IVIG) has been found to reduce the prevalence of CALs to approximately 4%, the incidence of CALs in KD remains high, and 10–20% of patients are resistant to initial IVIG treatment (1). Many studies have proven that patients who are resistant to initial IVIG are at increased risk of developing coronary artery abnormalities (5, 6). Thus, exploring potential biomarkers to diagnose or predict IVIG resistance and CALs in patients with KD has become a very fascinating area, but the predictive values of recognizing biomarkers, for example, C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), are partly limited (1, 7).

Elemental iron is one of the most plentiful and widely used metals on Earth (8). As an important trace element, it plays an essential role in a wide range of key biological processes in all living organisms, including DNA synthesis, energy production (oxygen transport and ATP production), and immune function (9). These biological functions of iron are mainly dependent on its ability to reversibly gain or lose a single electron to participate in oxidation–reduction reactions, which also catalyze the generation of reactive oxygen intermediates (ROIs) (10). It is possible that the disruption of iron homeostasis tends to cause cell death and human diseases because it serves as a mediator to promote the production of reactive oxygen species (ROS); thus, iron homeostasis is tightly controlled by a series of finely tuned, complex mechanisms (11).

Of these elaborate regulatory mechanisms, ferritin is one of the most fascinating. Ferritin is a hollow iron storage protein that consists of a protein shell and an iron core. The protein shell is composed of 24 highly symmetrical subunits of ferritin heavy chain (FTH) and ferritin light chain (FTL), and in the cavity of the protein shell, ferritin can bind different amounts of Fe^{3+} in a redox-inactive form (12, 13). In addition to iron storage, ferritin functions as a protective agent preventing iron overload and decreasing oxidative stress (OS) (14, 15). Growing evidence has indicated that ferritin acts as a switch in the dynamic balance of iron, that is, it is synthesized in response to high cellular iron levels and is degraded to release iron when iron demand is increased (14, 15). Unfortunately, free excess iron may be an OS source that leads to cell damage if it is not correctly stored in ferritin cores. Specifically, excessive free iron induces OS through the Fenton reaction, thereby activating cell ferroptosis by the iron-induced accumulation of lipid peroxides and ROS (16–18). Ferroptosis, characterized by iron-dependent OS and lipid peroxidation, is another new mechanism underlying iron-induced cell death, which contributes to the pathophysiology of various diseases (17, 19).

Undoubtedly, maintaining homeostasis between iron availability and iron storage and steering ferroptosis orientation are extremely important to ensure normal physiological

function and improve the outcome of diseases, and SF plays a pivotal role in equilibrating holistic homeostasis. Elevated ferritin concentration is a marker of high levels of stored iron. The detection of ferritin concentration is not only an important indicator to diagnose diseases with iron overload or iron deficiency but also a marker of inflammatory conditions or autoimmune disorders (18). Therefore, an increasing number of scholars have focused on FS as a diagnostic or prognostic biomarker to distinguish targeted diseases early and in a timely manner and evaluate the major complications (20, 21). Given this compelling rationale, a series of clinical trials have assessed the association between SF and KD, IVIG resistance, and CALs. Recognizing that individual studies might not be able to provide sufficient data on their own to affect practice, we sought to objectively assess the diagnostic or prognostic value of SF as a crucial biomarker in IVIG resistance and CALs in KD. We therefore performed a meta-analysis to establish the relationship between SF and KD, IVIG resistance, and CALs.

Materials and methods

Study protocol

We performed this systematic review and meta-analysis according to the PRISMA guidelines (22, 23). The meta-analysis with systematic review described herein has been accepted by PROSPERO, an online international prospective register of systematic reviews curated by the National Institute for Health Research (PROSPERO number: CRD42022279157). The population, intervention, comparison, and outcome worksheet are shown in **Table 1**.

Search strategy

On 30 September 2021, PubMed, Web of Science, the Cochrane Central Register of Controlled Trials, Embase, and China National Knowledge Infrastructure (CNKI) databases were searched by using the following keywords to retrieve literature: ("mucocutaneous lymph node syndrome or Kawasaki syndrome or lymph node syndrome, mucocutaneous or Kawasaki disease") and ("ferritins or ferritin or isoferritin or basic isoferritin or isoferritin, basic"). We considered all potentially eligible studies for review, irrespective of the primary outcome or language. We also performed a manual search using the reference lists of key articles published in English.

TABLE 1 Participants, Intervention, Comparator, Outcomes, and Study design (PICOS) criteria for inclusion in the systematic review and meta-analysis.

Acronym	Definition	Application of the criteria
P	Population	Pediatric patients who had been diagnosed with KD
I	Intervention	SF was measured in all KD included patients
C	Comparison	Our study was evaluated the comparison between the included studies of the KD and all controls in SF levels
O	Outcome	The outcomes were the incidence and the risk factor of SF levels on KD
S	Study designs	Prospective and retrospective studies; case studies ($N \geq 20$)

KD, Kawasaki disease; SF, serum ferritin.

Study selection criteria

Studies were enrolled in our meta-analysis based on the following criteria: (1) the diagnosis of Kawasaki disease was based on KD diagnostic criteria; (2) the studies were case–control, retrospective, or prospective studies with sample sizes of at least 20 cases and investigated the relationship between SF and KD; (3) the studies provided adequate information to build up true positives (TPs), true negatives (TNs), false positives (FPs), and false negatives (FNs) for diagnostic meta-analysis, and assessed hazard ratios (HRs) or odds ratios (ORs) and 95% confidence intervals (95% CI) for prognostic meta-analysis; and (4) the studies included KD patients with non-CALs vs. CALs and IVIG-responsive vs. IVIG-resistant subjects related to SF.

The following studies were excluded: (1) reviews, meta-analyses, meeting abstracts, proceedings papers, case reports, case series, editorials, letters, animal articles, and conference abstracts; (2) studies with no quantitative data or incomplete or unavailable data for SF levels; (3) duplicate publications; and (4) studies with incorrect statistical methods, deficiencies in the necessary information of TPs, TNs, FPs, and FNs for diagnostic meta-analysis, and contradictions in the process.

Data extraction and quality assessment

Data extraction and quality assessment were incorporated into the exclusion and inclusion criteria by three authors (Marady Hun, Huai Wen, and Mingyi Zhao) independently: (1) the initial results were screened for titles, first author, year of publication, abstracts, study population, sample size, and study type; (2) TP, TN, FP, FN, area under the curve (AUC), and cutoff values were extracted for diagnostic meta-analysis, and related outcomes or risk factors for SF in HRs or ORs and 95% CIs were extracted for prognostic meta-analysis; (3) the self-designed data extraction table was used for included studies; and (4) the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS2) was utilized for diagnostic meta-analysis (24), and the Newcastle–Ottawa Scale (NOS) was utilized for prognostic meta-analysis (25) to assess the quality of the included literature. If there were conflicts in the quality evaluation procedure, the consensus was extended through debate by the fourth investigator (**Supplementary Figure 1** and **Supplementary Table 1**).

Statistical analysis

We study used ReviewManager (RevMan) version 5.4[1], Meta-DiSc 1.4[2], and STATA 15.0 software[3] to conduct statistical analysis of the included records.

For the diagnostic meta-analysis, to obtain accurate diagnostic components of SF for KD, including the pooled diagnostic odds ratio (DOR), sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), summary receiver operator characteristic (SROC) curve, and AUC, we calculated TP, FP, FN, and TN values by using sensitivity (SEN) = [TP/(FN + TP)], specificity (SPE) = [TN/(FP + TN)], and diagnostic odds ratio (DOR) = [(1-SPE)/SPE]. A bivariate boxplot was performed to distinguish heterogeneity. The potential publication bias for the prognostic meta-analysis was computed by the ES (95% CI) value for each inclusion logarithm OR ($\log^{OR}$) value as the productive value indicator and was identified by the evaluated variance of the $\log^{OR}$. The generic inverse variance process was used for weighting, and a P-value < 0.05 was considered a statistically significant difference between the groups or subgroups. Forest plots were designed for related factors. Cochran's Q test and the I^2 value were used to estimate the heterogeneity for each selected study; $I^2 > 50\%$ indicated the appearance of heterogeneity. The random-effects model was used for $I^2 > 50\%$; otherwise, the fixed-effects model was utilized (26). The funnel plot, Egger's test, and Begg's test were applied to determine the publication bias. A P-value < 0.05 was considered statistically significant (27).

Results

The flow diagram utilized to illustrate the process of inclusion and exclusion (PRISMA statement) is shown in **Figure 1**. A total of 428 related records for SF and KD were initially recognized from PubMed (72 records), Web of Science (110 records), Cochrane Library (4 records), Embase (111 records), and CNKI (131 records) databases updated through 30September 2021. Of these, 338 records were excluded; 235 were duplicates, 29 were reviews and meta-analyses, 52 were cases and case reports, and 72 were not related to the topic. After the full-text articles of the remaining 40 records were assessed for eligibility, 29 records were excluded (the reasons for exclusion are detailed in **Figure 1**); ultimately, 11 records (nine records (20, 21, 28–34) for diagnostic meta-analysis and four records (33–36) for prognostic meta-analysis) were included in this meta-analysis. Of these 11 studies, seven were from China, two were from Korea, and two were from Japan (the quality of all enrolled studies for diagnostic meta-analysis is presented in **Supplementary Figure 1**). For diagnostic assessment, 20 studies from nine articles were enrolled in our meta-analysis and were separated into three comparable groups: (1) KD vs. controls (including fever, healthy, and s-JIA (systemic juvenile idiopathic arthritis) groups), 11 studies from six articles (totaling 1023 KD cases and 661 controls); (2) KD-CAL vs. KD-non-CAL, five studies from five articles (totaling 152 KD-CAL cases and 716 KD-non-CAL cases); and (3) KD-IVIG resistance vs. KD-IVIG responders, four studies from four articles (totaling 151 KD-IVIG resistance and 570 KD-IVIG responders). For prognostic assessment, a total of 894 patients were included from five studies (four articles) relevant to SF and KD (the quality of all enrolled studies for prognostic meta-analysis is presented in **Supplementary Table 1**). Among the enrolled studies in the diagnostic meta-analysis, 19 were retrospective trials, and one was a prospective trial; in the prognostic meta-analysis, all five were retrospective trials. The baseline characteristics of all included studies are summarized in **Table 2** for the diagnostic meta-analysis and **Table 3** for the prognostic meta-analysis.

Diagnostic value of serum ferritin for Kawasaki disease

Study characteristics and quality assessment

The quality of the included studies is outlined in **Supplementary Figure 1**. Of the 20 included studies, nine articles (2,612 related KD patients with SF testing) (20, 21, 28–34) demonstrated a moderate to high quality of all diagnostic studies. The sample size of the included studies ranged from 27 to 271, and the studies were published from 2015 to 2021. The characteristic details of the included literature are shown in **Figure 1**. To improve the quality of all included studies, they were separated into three subgroups: (1) the first group included studies comparing SF values of KD patients and different controls (including fever, healthy, and s-JIA groups); (2) the second group included studies comparing SF values of endogenous KD patients with CAL vs. non-CAL; and (3) the third group included studies comparing SF values of endogenous KD IVIG responders vs. IVIG resistance. The baseline characteristics of all included studies are summarized in **Table 2**.

Data analysis

Forest plot results of related data from the 20 studies (nine articles) (20, 21, 28–34) on the sensitivity and specificity of overall SF in diagnosing KD are shown in **Figure 2**. Due to significant heterogeneity of sensitivity (I^2 = 84.81%, 95% CI: 79.05–90.58%, $P < 0.001$) and specificity (I^2 = 92.55%, 95% CI: 90.26–94.85%, $P < 0.001$), the random-effects model was used. The pooled outcomes of the included diagnostic studies

1 http://www.cochrane.org
2 http://www.hrc.es/investigacion/metadisc_en.htm
3 https://www.stata.com

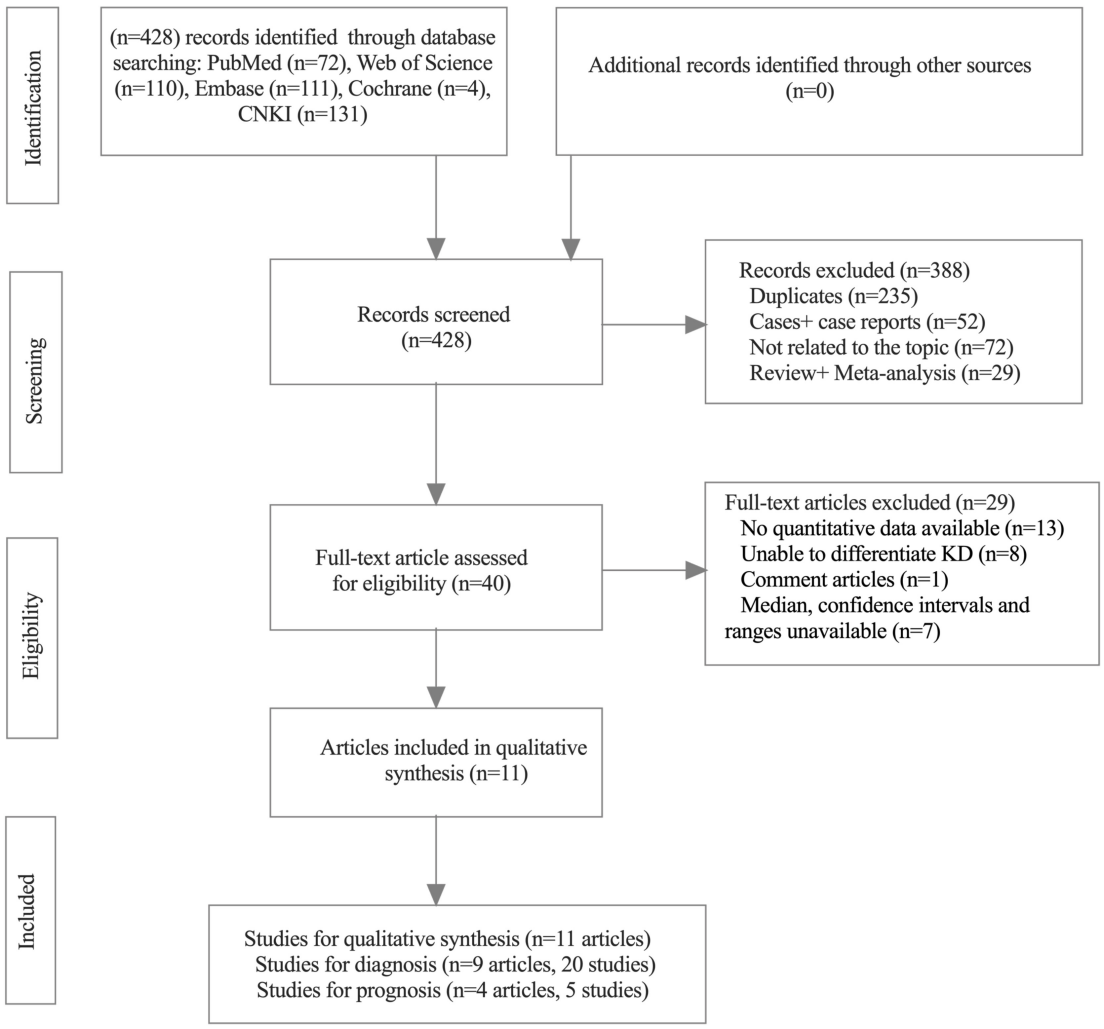

FIGURE 1
Flow diagram of study selection process.

were as follows: sensitivity, 0.76 (95% CI: 0.69–0.82); specificity, 0.82 (95% CI: 0.76–0.88); PLR, 3.90 (95% CI: 2.87–5.30); NLR, 0.29 (95% CI: 0.22–0.38); and DOR 15.0 (95% CI: 9.00–25.00) (**Supplementary Figure 2** and **Table 4**).

Heterogeneity and subgroup analysis

In addition, a summary receiver operator characteristic (SROC) curve (**Figure 3A**) was utilized in this meta-analysis of KD vs. overall studies of SF, and the value of AUC (0.86, 95% CI: 0.83–0.89) was computed. **Figure 3A** suggests that no typical shoulder arm exists, thereby proving the non-attendance of the threshold effect. Spearman's correlation coefficient value was –0.059, and the *P*-value was 0.805, demonstrating that there was no threshold effect. It could also demonstrate the fact that the threshold effect is not a source of heterogeneity. Although the bivariate boxplot and Galbraith plot are shown in **Figures 3B,C**, respectively, five studies fell outside the boxplot and Galbraith plot (30, 32, 33). Of these five studies, two studies were based on estimation between KD with SF values vs. controls with s-JIA (32, 33) and might be based on the analytic data (sensitivity = 60.40% vs. specificity = 98.80%) (32); two studies were based on estimation between IVIG responders and IVIG resistance [n = 271 and n = 29, respectively (30); n = 161 and n = 67, respectively (33)]; and another study was based on estimation between CAL (n = 48) and non-CAL (n = 252). (32) Moreover, Cook's distance plot was used for influence analysis (**Figure 3D**), suggesting that two studies had strong sensitivity (32, 33), and the other studies did not cause sensitivity of the arithmetic results. Overall, the results of this study are relatively stable, indicating that pattern should be the main cause of heterogeneity.

Our meta-regression analysis suggests that the sample sizes in the included studies may contribute to the cause

TABLE 2 Characteristics of studies in diagnostic meta-analysis.

Study	Years	Country	Design	Diagnostic criteria	Sample size	Mean age (month)	Mean ferritins levels (ng/ml)	Cut-off of ferritins (ng/ml)	TP	FP	FN	TN	AUC	Sensitivity	Specificity
KD patients vs. controls (including fever, healthy, and s-JIA groups)															
Guo et al. (28)	2021a	China	RCS	AHA	KD vs. Fever: 60 vs. 60	KD vs. Fever: 33.84 vs. 33.96	KD vs. Fever: 192.75 ± 76.98 vs. 117.15 ± 51.61	142.45	46	16	14	44	0.792	76.70%	73.30%
Guo et al. (28)	2021b	China	RCS	AHA	KD vs. Healthy: 60 vs. 60	KD vs. Healthy: 33.84 vs. 39.00	KD vs. Healthy: 192.75 ± 76.98 vs. 72.21 ± 21.57	142.45	46	16	14	44	0.792	76.70%	73.30%
Kim et al. (21)	2021	Korea	PCS	NA	KD vs. Fever:77 vs. 32	KD vs. Fever:22.8 vs. 25.2	KD vs. Fever:188.8 (25.5–750.5) vs. 106.8 (11.1–632.2)	120.80	63	1	14	31	0.83	81.82%	95.76%
Pan et al. (29)	2019a	China	RCS	JKDRC	KD vs. s-JIA: 53 vs. 53	KD vs. s-JIA: 42 ± 7.2 vs. 43.2 ± 6	NA	254.7	38	7	15	46	0.83	72.00%	87.5%
Pan et al. (29)	2019b	China	RCS	JKDRC	KD vs. Healthy: 53 vs. 53	KD vs. Healthy: 42 ± 7.2 vs. 39.6 ± 4.8	NA	254.7	38	7	15	46	0.83	72.00%	87.5%
Wen et al. (31)	2018a	China	RCS	AHA	KD vs. Healthy: 108 vs. 30	KD vs. Healthy: (2–137) vs. (4–120)	KD vs. Healthy: 227 ± 238 vs. 72 ± 101	133.35	76	10	32	20	0.793	70.10%	66.70%
Wang et al. (32)	2016a	China	RCS	JKDRC	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 77.04 vs. 78.96	KD vs. s-JIA: 232.35 ± 155.95 vs. 1017.66 ± 584.18	239.5	82	17	14	56	NA	85.40%	76.50%
Wang et al. (32)	2016b	China	RCS	JKDRC	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 96 vs. 73	292.5	76	11	20	62	NA	79.20%	85.20%
Wang et al. (32)	2016c	China	RCS	JKDRC	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 96 vs. 73	385.5	74	6	22	67	NA	77.10%	91.40%
Wang et al. (32)	2016d	China	RCS	JKDRC	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 96 vs. 73	KD vs. s-JIA: 96 vs. 73	929.5	58	1	38	72	NA	60.40%	98.80%
Mao et al. (33)	2016a	Japan	RCS	AHA	KD vs. s-JIA: 228 vs. 81	KD vs. s-JIA: 24 (1.2-168) vs. 84 (7.2-312)	KD vs. s-JIA: 147.5 (14–2,376) vs. 1189 (63–68,310)	369.6	215	14	13	67	0.939	94.30%	82.70%
CALs vs. non-CALs															
Guo et al. (28)	2021c	China	RCS	AHA	CALs vs. non-CALs: 6 vs. 54	CALs vs. non-CALs: 29.4 vs. 25.44	CALs vs. non-CALs: 235.48 ± 95.71 vs. 188.01 ± 74.18	NA	5	14	1	40	NA	76.70%	73.30%

(Continued)

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TABLE 2 Continued

Study	Years	Country	Design	Diagnostic criteria	Sample size	Mean age (month)	Mean ferritins levels (ng/ml)	Cut-off of ferritins (ng/ml)	TP	FP	FN	TN	AUC	Sensitivity	Specificity
Kong et al. (30)	2019a	China	RCS	AHA	CALs vs. non-CALs: 48 vs. 252	CALs vs. non-CALs: 19 (9–33) vs. 25 (14–42)	CALs vs. non-CALs: 146.9 (105.6–217.4) vs. 152.6 (111.7–208.9)	NA	21	28	27	224	NA	43.00%	88.80%
Kim et al. (34)	2019	Korea	RCS	AHA	CALs vs. non-CALs: 55 vs. 118	CALs vs. non-CALs: 36 (18–63) vs. 29 (14–55)	CALs vs. non-CALs: 69.9 (47.4–112.4) vs. 24.1 (19.7–28.5)	30.6	45	5	10	113	0.907	81.82%	95.76%
Wen et al. (31)	2018b	China	RCS	AHA	CALs vs. non-CALs: 31 vs. 77	NA	CALs vs. non-CALs: 340 ± 405 vs. 183 ± 99	160.2	23	37	8	40	NA	73.70%	52.10%
Mao et al. (33)	2016b	Japan	RCS	AHA	CALs vs. non-CALs: 12 vs. 215	NA	NA	NA	8	99	4	116	NA	62.70%	54.00%
IVIG resistance vs. IVIG responders															
Kong et al. (30)	2019b	China	RCS	AHA	IVIG-resistance vs. IVIG-responders: 29 vs. 271	IVIG-resistance vs. IVIG-responders: 25 (15–47) vs. 24 (13–41)	IVIG-resistance vs. IVIG- responders: 198.6 (129.7–411.6) vs. 146.6 (107.2–205.5)	269.7	12	30	17	241	0.663	43.00%	88.80%
Wen et al. (31)	2018c	China	RCS	AHA	IVIG-resistance vs. IVIG-responders: 27 vs. 81	NA	IVIG-resistance vs. IVIG- responders: 257 ± 287 vs. 215 ± 216	133.35	19	27	8	54	0.623	70.10%	66.70%
Mao et al. (33)	2016c	Japan	RCS	AHA	IVIG-resistance vs. IVIG-responders: 67 vs. 161	NA	NA	144.3	63	28	4	133	0.618	94.30%	82.70%
Yamamoto et al. (20)	2015	Japan	RCS	JKDRC	IVIG-resistance vs. IVIG-responders: 28 vs. 57	IVIG-resistance vs. IVIG-responders: 32 (5–112) vs. 29 (2–124)	IVIG-resistance vs. IVIG- responders: 214.9 (50.6–558.5) vs. 141.1 (34.5–428.4)	165	20	21	8	36	0.674	70.40%	63.20%

RCS, retrospective cohort study; PCS, prospective cohort study; TP, true positives; FP, false positives; FN, false negatives; TN, true negatives; AUC, area under the receiver operating characteristic curve; KD, Kawasaki disease; s-JIA, systemic juvenile idiopathic arthritis; AHA, American Heart Association; JKDRC, Japan Kawasaki Disease Research Committee; IVIG, intravenous immunoglobulin; CALs, coronary artery lesions; NA, not available.

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TABLE 3 Characteristics of studies in prognostic meta-analysis.

Study	Years	Country	Design	Diagnostic criteria	Sample size	Mean age (month)	Mean ferritins levels (ng/ml)	IVIG dosage	Aspirin (mg/kg/day)	OR (95%CI)
Tan et al. (35)	2021	China	RCS	AHA	IVIG-resistance vs. IVIG-responder: 15 vs. 77	IVIG-resistance vs. IVIG-responder: 31.56 ± 5.28 vs. 32.88 ± 5.64	IVIG-resistance vs. IVIG-responder: 190.62 ± 9.54 vs. 136.52 ± 8.97	2 g/kg	High-dose: 30–50 Low-dose: 3–5	1.21 (1.05–1.40)
Peng et al. (36)	2020	China	RCS	AHA	IVIG-resistance vs. IVIG-responder: 31 vs. 142	IVIG-resistance vs. IVIG-responder: 19.5 (9.0–40.5) vs. 17.0 (8.0–39.0)	IVIG-resistance vs. IVIG-responder: 133 (83–263) vs. 210 (151–277)	2g/kg	High-dose: 30–50 Low-dose: 3–5	1.19 (1.08–1.32)
Mao et al.*[1] (33)	2016a	Japan	RCS	AHA	IVIG-resistance vs. IVIG-responder: 67 vs. 161	NA	NA	2 g/kg	NA	1.98 (1.10–3.54)
Mao et al.*[2](33)	2016b	Japan	RCS	AHA	IVIG-resistance vs. IVIG-responder: 67 vs. 161	NA	NA	2 g/kg	NA	4.86 (1.50-15.78)
Kim et al. (34)	2019	Korea	RCS	AHA	CALs vs. non-CALs: 55 vs. 118	CALs vs. non-CALs: 36 (18–63) vs. 29 (14–55)	CALs vs. non-CALs: 69.9 (47.4–112.4) vs. 24.1 (19.7–28.5)	NA	NA	0.95 (0.93–0.97)

*[1]OR and 95% CI value when patients not needing plasma exchange. *[2]OR and 95% CI value when patients needing plasma exchange. RCS, retrospective cohort study; AHA, American Heart Association; IVIG, intravenous immunoglobulin; CALs, coronary artery lesions; NA, not available.

of heterogeneity. We also performed a subgroup analysis according to specimens in the included studies. The outcomes are presented in Table 4. The pooled AUC between SF levels in KD vs. all controls (11 studies) was 0.89 (95% CI: 0.86–0.91), corresponding to a sensitivity of 0.79 (95% CI: 0.72–0.84) and specificity of 0.86 (95% CI: 0.79–0.91). In addition, among the KD vs. all control groups, we separated the patients into three subgroups: (1) KD vs. healthy (three studies), (2) KD vs. fever (two studies), and (3) KD vs. s-JIA (six studies). Their pooled sensitivities vs. specificities were 0.72 vs. 0.77, 0.80 vs. 0.82, and 0.82 vs. 0.87, respectively. The subgroup of IVIG responders vs. IVIG resistance presented better diagnostic accuracy than that of the subgroup of CAL vs. non-CAL, with AUC values of 0.83 (0.79–0.86) and 0.79 (0.75–0.82) and with pooled DOR values of 9.40 (2.70–32.72) and 0.02 (0.00–0.11), respectively (Table 4).

Fagan's nomogram was applied to evaluate the posttest probabilities, and the related result is shown in Figure 3E. We found that when 20% was chosen as the pretest probability, the posttest probability of SF was 52% of LR-positive and 7% of LR-negative.

Deek's funnel plot asymmetry test was considered a useful tool for potential publication bias of diagnostic studies. The results showed that there was no significant publication bias, with a P-value of 0.01 (Figure 3F).

Meta-analysis serum ferritin levels of Kawasaki disease patients vs. controls

Forest plot results of related data from the 11 studies (six articles) (21, 28, 29, 31–33) on the sensitivity and specificity of SF in diagnosing KD are shown in Figure 4. Due to significant heterogeneity of sensitivity (I^2 = 84.11%, 95% CI: 75.76–92.46%, $P < 0.001$) and specificity (I^2 = 74.74%, 95% CI: 59.74–89.75%, $P < 0.001$), the random-effects model was used. The pooled outcomes of the included diagnostic studies were as follows: sensitivity, 0.79 (95% CI: 0.72–0.84); specificity, 0.86 (95% CI: 0.79–0.91); PLR, 4.61 (95% CI: 3.27–6.51); NLR, 0.26 (95% CI: 0.20–0.34); and DOR 20.82 (95% CI: 11.83–36.64) (Supplementary Figure 3).

Meta-analysis serum ferritin levels of Kawasaki disease patients versus controls

In addition, a summary receiver operator characteristic (SROC) curve was utilized in this meta-analysis of SF levels of KD patients vs. controls (Figure 5A) and computed the AUC value (0.89, 95% CI: 0.86–0.91). Figure 5A suggests that no

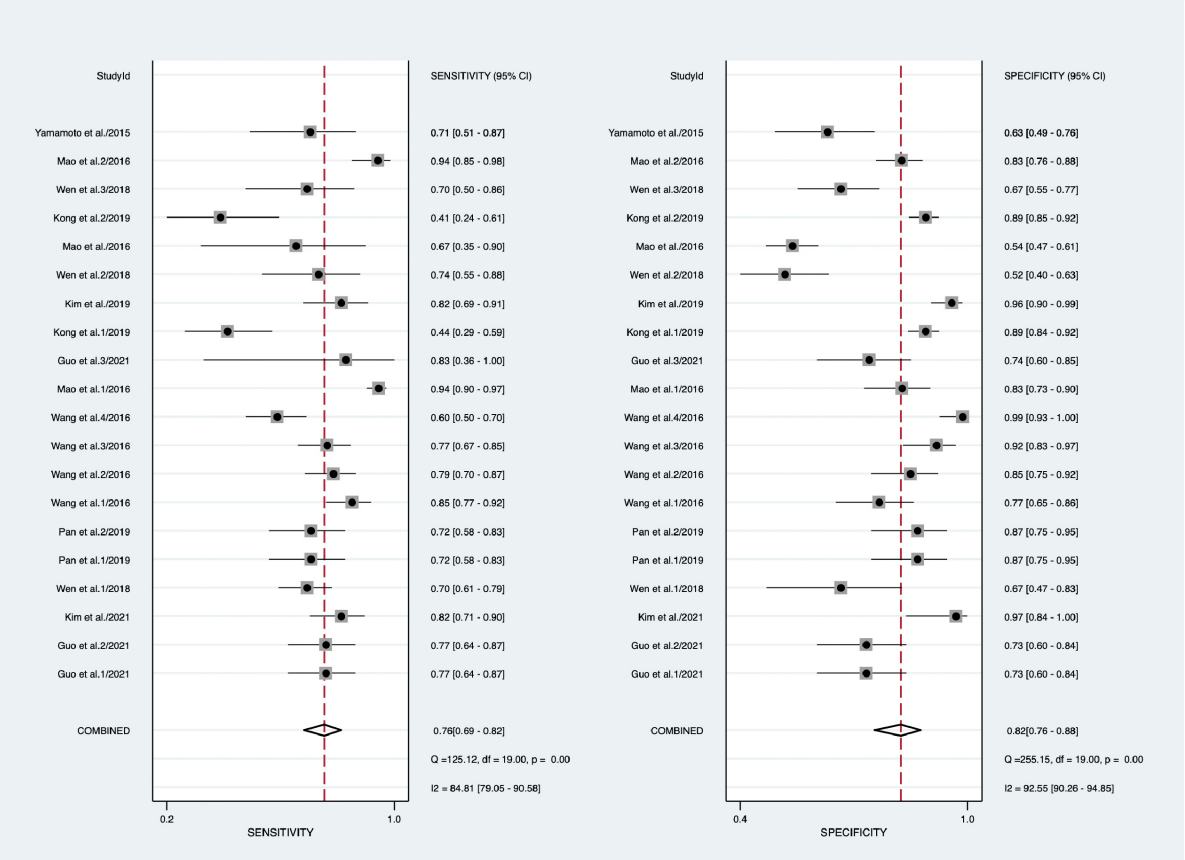

FIGURE 2
Forest plots of sensitivity and specificity of overall SF in the diagnosis of KD.

TABLE 4 Subgroup analysis of diagnostic accuracy of overall SF for KD.

	No	SEN	SPE	PLR	NLR	DOR	AUC	Heterogeneity	
		(95%CI)	(95%CI)	(95%CI)	(95%CI)	(95%CI)	(95%CI)	I^2	*P*-value
Overall	20	0.76 (0.69–0.82)	0.82 (0.76–0.88)	4.33 (3.07–6.11)	0.29 (0.22–0.38)	15.0 (9.00–25.00)	0.86 (0.83–0.89)	81.2%	0.000
Subgroup									
KD vs. all controls	11	0.79 (0.72–0.84)	0.86 (0.79–0.91)	4.61 (3.27–6.51)	0.26 (0.20–0.34)	20.82 (11.83–36.64)	0.89 (0.86–0.91)	73.0%	0.000
KD vs. healthy	3	0.72 (0.66–0.78)	0.77 (0.69–0.84)	3.04 (1.90–4.87)	0.37(0.29–0.47)	8.65 (4.37–17.14)	–	43.2%	0.172
KD vs. fever	2	0.80 (0.72–0.86)	0.82 (0.72–0.99)	7.66 (0.47–124.27)	0.24 (0.15–0.41)	30.32 (1.88–488.79)	–	84.1%	0.012
KD vs. s-JIA	6	0.82 (0.79–0.85)	0.87 (0.83–0.90)	5.72 (3.89–8.40)	0.22 (0.14–0.35)	31.93 (18.02–56.58)	0.92 (0.89–0.94)	53.1%	0.058
CAL vs. non-CAL	5	0.70 (0.70–0.70)	0.78 (0.78–0.78)	0.23 (0.09–0.56)	10.05 (3.10–32.59)	0.02 (0.00–0.11)	0.79 (0.75–0.82)	42.0%	0.136
IVIG responders vs. IVIG resistance	4	0.74 (0.49–0.90)	0.78 (0.65–0.87)	3.02 (1.77–5.14)	0.34 (0.14–0.82)	9.40 (2.70–32.72)	0.83 (0.79–0.86)	85.3%	0.000

KD, Kawasaki disease; IVIG, intravenous immunoglobulin; CALs, coronary artery lesions; s-JIA, systemic juvenile idiopathic arthritis; SEN, sensitivity; SPE, specificity; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating characteristic curve; NA, not available.

typical shoulder arm exists, thereby proving the non-attendance of the threshold effect. Spearman's correlation coefficient value was 0.064, and the *P*-value was 0.851, thereby demonstrating that there was no threshold effect. Furthermore, it could also demonstrate the fact that the threshold effect is not a source of heterogeneity. Although the bivariate boxplot and Galbraith plot are shown in **Figures 5B,C**, three studies fell outside the boxplot and Galbraith plot (31–33). Of the three studies, the sample size of one study was 108 KD patients vs. 30 healthy controls (31), the sample size of the second study was 228 KD patients vs. 81

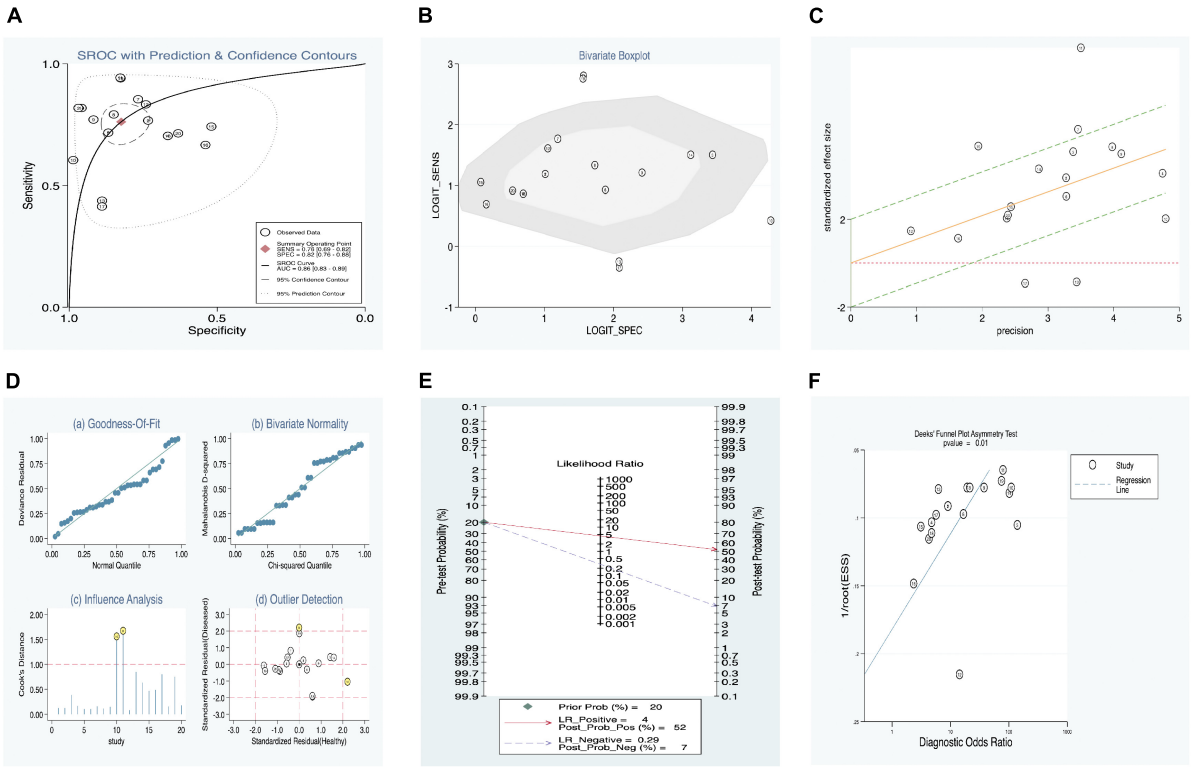

FIGURE 3
Estimation of diagnostic accuracy of overall SF in KD. **(A)** Summary receiver operator characteristic (SROC) curve; **(B)** bivariate boxplot; **(C)** Galbraith plot; **(D)** Cook's distance plot; **(E)** Fagan's nomogram; **(F)** Deek's funnel plot.

s-JIA controls (33), and the third study was based on sensitivity (60.40%) vs. specificity (98.80%) (32). Moreover, Cook's distance plot was used for influence analysis (**Figure 5D**), indicating that the pattern should be the cause of heterogeneity.

Fagan's nomogram was applied to evaluate the posttest probabilities, and the related result is shown in **Figure 5E**. We found that when 20% was chosen as the pretest probability, the posttest probability of SF was 58% LR-positive and 6% LR-negative.

Deek's funnel plot asymmetry test was considered a useful tool for potential publication bias of diagnostic studies. The results showed that there was no significant publication bias, with a *P*-value of 0.12 (**Figure 5F**).

Prognostic value of serum ferritin for Kawasaki disease

Study characteristics and quality assessment

A total of 894 patients were included from five studies (four articles) (33–36) relevant to SF levels in KD. A total of four studies examined the relationship between the SF level and IVIG responders vs. IVIG-resistant groups, and the fifth study examined the relationship between the SF level and CAL vs. non-CAL groups. One study contained two ORs and 95% CI values [the first value was from patients who did not need plasma exchange (PE), and the second value was from patients who needed PE] (33). The sample sizes of the included studies ranged from 92 to 228, and the prognostic studies were published from 2016 to 2021. The detailed characteristics of each included study and NOS score are shown in **Supplementary Table 1**. All OR values were obtained directly from publications. The baseline characteristics of all included studies are summarized in **Table 3**.

Data analysis

After performing the heterogeneity test on five studies, we obtained the following results: $\chi^2 = 41.93$, df = 4, $I^2 = 90\% > 50\%$, and $P = 0.0001 < 0.1$ (random-effects model) in the *Q*-test, indicating that there was strong significant publication bias between the five studies. The pooled hazard ratio (HR) of the five records reached 1.21 (95% CI: 0.99, 1.48), and the results were significant ($Z = 1.84$, $P = 0.07 > 0.05$), indicating that the risk in SF may contribute to the development of KD in children (**Figure 6**). However, the literature has a great influence on the results, which indicated that the results of this study are not relevantly steady (**Supplementary Figures 4A,B**). Kim's study may have a particular impact on the stability of the results, and after

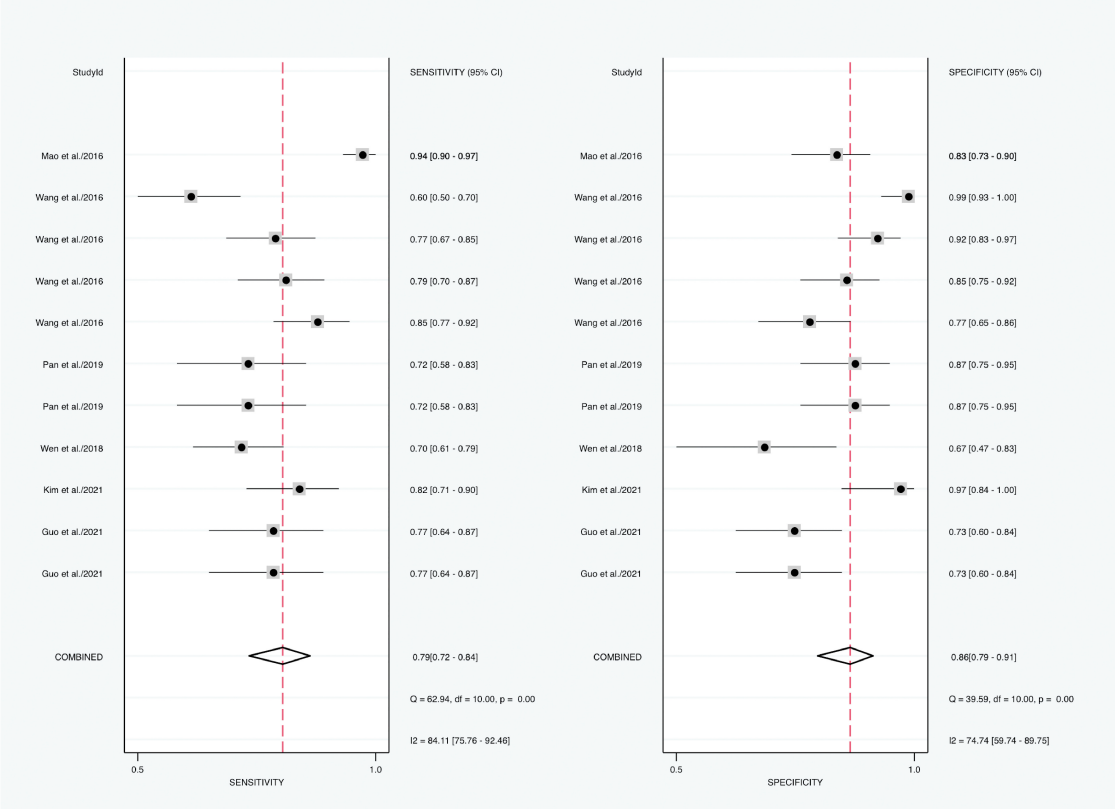

FIGURE 4
Forest plots of sensitivity and specificity of SF in the diagnosis of KD (KD vs. controls).

withdrawing Kim's study (34), the remaining four studies obtained better results. The related outcomes were as follows: χ^2 = 8.10, df = 3, I^2 = 63% > 50%, and P = 0.04 < 0.1 (random-effects model) in the Q-test, demonstrating that there was significant publication bias between the four records. The pooled HR of the four records reached 1.31 (95% CI: 1.07, 1.59), and the results were significant (Z = 2.66, P = 0.008 > 0.05), illustrating that the risk in SF could contribute to the development of KD in children (**Figure 7**). The sensitivity analysis of the study is shown for SF and illustrated that the results of this study are relatively stable (**Supplementary Figure 6A**). However, Begg's test results (P = 0.09 > 0.05) and Egger's test results (P = 0.015 < 0.05) based on the funnel plot (**Supplementary Figure 5B**) were still within the acceptable range.

Discussion

Not only is ferritin a ubiquitous intracellular protein characterized by storing iron, but it is also an acute-phase reactant widely utilized in clinical practice (37, 38). Under inflammatory conditions, ferritin synthesis is markedly induced by TNF-α and IL-1α, which are released from activated macrophages (39, 40). Emerging research has proven that serum ferritin levels are elevated in patients with certain inflammatory conditions, including rheumatoid arthritis, systemic lupus erythematosus, chronic kidney disease, COVID-19 caused by the virus SARS-CoV-2, thyroiditis, and others. (41–45). Meanwhile, an increasing number of studies have shown that SF is aberrantly elevated in KD and related complications (20, 21, 39, 40), and hyperferritinemia (i.e., a ferritin level above 500 mg/L) is considered one of the diagnostic criteria in HLH-2004 (41). More interestingly, with the deepening research on ferroptosis, we realized that ferroptosis, a new type of programmed cell death, plays a crucial role in the pathophysiological process of various diseases (19, 38), and ferritin might be an important switch in cell ferroptosis (18). All signs indicate that SF seems to be a good prospective ideal biomarker for the diagnosis or prognosis of IVIG resistance and CALs in KD when compared to other biomarkers. Therefore, our study conducted a comprehensive and systematic meta-analysis to assess the relationship between the SF level and diagnostic and prognostic efficiency in IVIG resistance and CALs in KD based on 3506 patients.

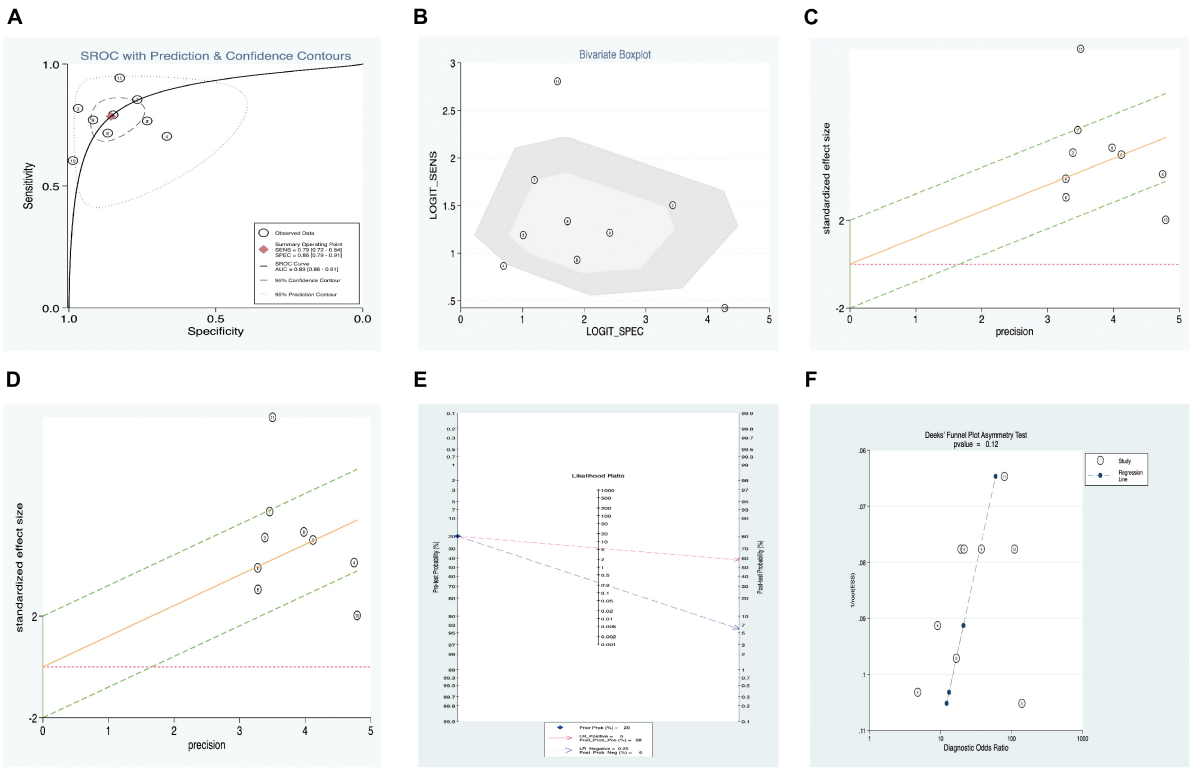

FIGURE 5
Estimation of diagnostic accuracy of SF vs. control (fever, healthy, and s-JIA) in KD. **(A)** Summary receiver operator characteristic (SROC) curve; **(B)** bivariate boxplot; **(C)** Galbraith plot; **(D)** Cook's distance plot; **(E)** Fagan's nomogram; **(F)** Deek's funnel plot.

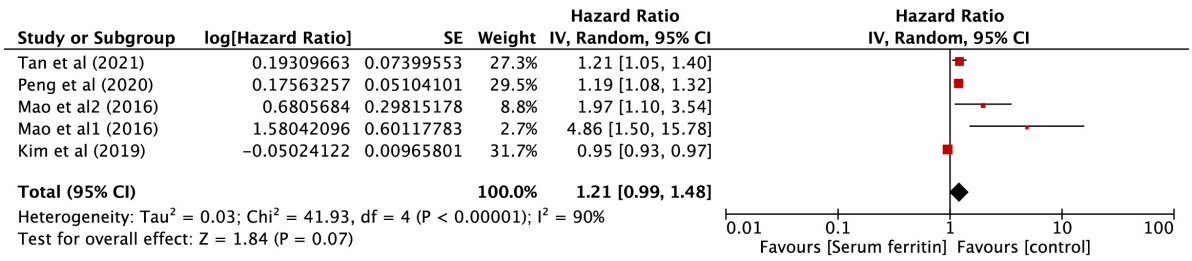

FIGURE 6
Forest plots of hazard ratios for the association between expression of SF for KD in five studies.

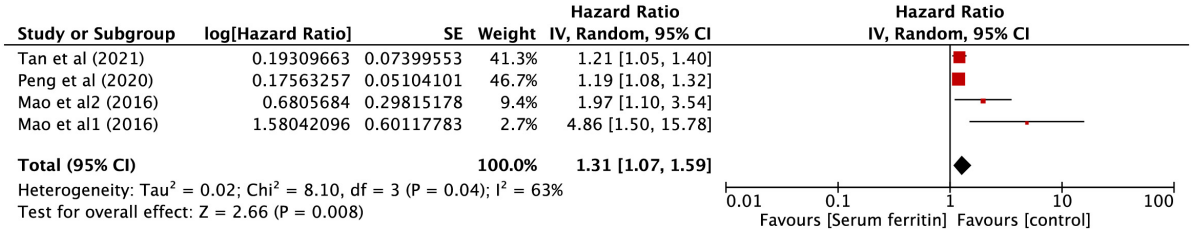

FIGURE 7
Forest plots of hazard ratios for the association between expression of SF for KD in four studies.

Our study found that SF has relatively high diagnostic efficiency in discriminating KD patients from overall controls. In general, the minimum value of DOR is 0, and the maximum value is infinity; a higher value of DOR suggests a better discriminatory test performance (46). All these results suggested that SF might be suitable as a potential biomarker for diagnosing KD, which is consistent with conclusions from other studies (20, 47). Ferritin is an acute-phase reactant that is utilized in clinical practice as a serum biomarker (37), which regulates the homeostasis of serum iron, and elevated ferritin concentration signifies high levels of stored iron (48). Excessive free iron induces cell ferroptosis characterized by the iron-dependent accumulation of lipid ROS and leads to cytological changes (49); growing evidence has proven that activating or blocking the ferroptosis pathway alleviates the progression of the disease, which provides a promising therapeutic strategy for many diseases (17, 19).

Generally, KD has a good prognosis, and clinical symptoms improve if diagnosed and treated early. However, if the diagnosis or treatment is not made in time, CAL sequelae or even death may occur, with an approximately 0.02–1% mortality rate, especially when combined with IVIG-resistant patients (1, 50, 51). However, IVIG resistance and CALs adversely cause life-threatening conditions in pediatric KD (1, 52). Numerous studies have investigated CALs as a more common occurrence of approximately 15–25% in KD (1, 52, 53). However, the specific factors or main causes of developing CALs in childhood KD remain unclear. Recent studies of KD related to IVIG resistance and CALs in children may be the causes of SF levels (20, 21, 28–33). Moreover, elevated SF is a biomarker of IVIG resistance and CALs in KD, as reported by Kong et al. (30). In addition, the survey by Kim et al. (21) also shows that SF may be a diagnostic biomarker for KD. The aforementioned results and our meta-analysis confirmed that SF may be used as a workable and critical biomarker for the diagnosis of patients with KD and IVIG resistance. Although our subgroup analysis based on CALs vs. non-CALs presented a poorer diagnostic accuracy than that based on IVIG responders vs. IVIG resistance, its corresponding sensitivity and specificity values were still greater than 0.70. A series of current published studies from 2015 to 2021 investigated the SF level as a useful marker for the prediction of resistance to initial IVIG therapy (20, 30, 31, 33). Our group considered that SF may still be a potential biomarker to predict CALs in KD with sufficient research. To the best of our knowledge, our meta-analysis is the first to assess the diagnostic roles of SF in KD, IVIG resistance, and CALs and innovatively proposes that maintaining the dynamic balance between iron, SF, and ferroptosis will be an important therapeutic strategy to reduce morbidity in pediatric acquired heart disease. Moreover, it is a known fact that SF could be elevated in KD (with abundantly available research). The issue occurs when one tries to make a cutoff value that predicts IVIG resistance and that predicts CALs. Various RCTs propose various cutoffs regarding the same. In that situation, it is difficult for the physician to rely on a single value. Still, this meta-analysis could add to the available research.

Although numerous studies have investigated KD continuously, the particular risk factors for SF in developing childhood KD remain unknown (33–36). Elevated serum ferritin levels are significantly related to IVIG resistance in KD (30, 54). Moreover, SF levels are notably increased in KD with CALs (30, 33, 34). In our meta-analysis, we found that SF was vital to the prognosis of IVIG resistance and CALs in KD. To our knowledge, our article is the first meta-analysis of the relationship between SF and the prognostic roles of IVIG resistance and CALs in KD. The aggregated results confirmed that SF may be a potential prognostic biomarker for IVIG resistance and CALs in KD.

Strength and limitations

Most of the included studies are systematic reviews to conduct the biomarker in the diagnosis and prognosis associated with SF in pediatric KD. To our knowledge, this is by far the most comprehensive research on the biomarker in the diagnosis and prognosis associated with SF in pediatric KD by using the meta-analysis. Nevertheless, there are still several limitations to our current meta-analysis. First, the number of included studies was small (n = 11), and all of the studies were conducted in Asian populations (China, Korea, and Japan). There were no data from other countries or regions. Thus, our conclusions are only applicable to Asian populations, which may affect the external applicability of our results in different regions. However, according to the recent global epidemiology of vasculitis, Kawasaki disease occurs most frequently in East Asia, especially Japan, South Korea, and China, with a relatively equal distribution elsewhere (2). Second, all of the follow-up times of the included articles were unclear, which may lead to the deviation of CAL diagnosis and could further affect the accuracy of FS in predicting CALs of KD. Third, although we conducted hierarchical analyses, heterogeneity still existed in some subgroups.

Conclusion

In summary, this meta-analysis identified SF as a workable and critical biomarker for the diagnosis of KD patients, including the prediction of CALs and IVIG resistance in patients. In addition, our study demonstrated a significant association between SF and the prognosis of KD, signifying that SF may have an essential role in the occurrence and prognosis of KD, but we suggest multicenter (even multi-national) approaches or more powerful research in obtaining larger sample sizes to prove. We also propose that maintaining

the dynamic balance between iron, SF, and ferroptosis will be an important therapeutic strategy to reduce morbidity in pediatric acquired heart disease. However, more comprehensive studies with large scale, more regions, and high quality should be performed to elucidate the roles of SF in diagnosing and predicting IVIG resistance and CALs in KD.

Data availability statement

All datasets generated for this study are included in the article/**Supplementary material**.

Author contributions

HW and MH conceived the idea for the study. HW, MH, and MZ selected studies for inclusion and abstracted data. MH and PH conducted the statistical analyses. MH and HW interpreted the data. HW and MH wrote the first draft. MZ and QH critically revised the manuscript for important intellectual content. All authors have read and approved the content of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.941739/full#supplementary-material

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Advances in Takayasu arteritis: An Asia Pacific perspective

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Takayasu Arteritis (TA) is a rare form of chronic granulomatous large vessel vasculitis that is more common in Asia compared to other parts of the world. There have been several developments in the field of Takayasu arteritis in relation to genetics, classification, clinical features, imaging, disease activity assessment and management and much of these works have been done in the Asia Pacific region. We will be discussing selected few in the current review.

KEYWORDS

Takayasu arteritis, HLA B52, ITAS, PET-VAS, PET/MRI, biologicals, granulomatous vasculitis

Introduction

Takayasu Arteritis (TA) is a rare form of chronic granulomatous large vessel vasculitis that is more common in Asia compared to other parts of the world. However the frequency is increasing in other parts of the world, partly due to high immigration rates (1). The Annual incidence rate ranges from 0.4–3.4 per million individuals and the prevalence differs from region to region with Japan having the highest prevalence at 40 per million to 9.0 per million in USA (2). TA commonly presents in second to third decade of life, with a female predominance. Disease may have its onset in childhood and children with TA have significant differences in their clinical presentations from adults (3, 4). Recent studies from Japan and Korea identified differing patterns of arterial involvement in females and males; they found females to have more frequent involvement of thoracic aorta and its branches whereas males were more likely to have abdominal aorta involvement (5, 6).

Genetics

The first known genetic association with TA was identified by Isohisa et al. (7) who found an increased frequency of HLA-B5 - Bw52 in their 65 patients of Japanese descent. HLA-Bw52 serotype corresponds to HLA-B*52:01 genotype in these patients. This was replicated by other independent groups making this association significant in Japanese population (8, 9). The association between HLA-B*52:01 genotype and Asian Indians with TA was documented by Rose et al. (10) in 50 patients and Mehra et al. (11) in 80 patients. Two Korean studies (12, 13) and a Turkish study (14) also documented the association in their

respective populations. Genetic associations with severity of disease, aortic regurgitation (AR) and glucocorticoid (GC) requirements were also reported for HLA-B52 allele (15).

Chen et al. (16) conducted a meta-analysis with inclusion of 20 studies, 1,864 TA patients and 6,973 controls. HLA-B*52 allele was found to be significantly associated with TA with a pooled OR of 3.91. Statistically significant association was also found with TNF-α-308A/G polymorphism.

Non-HLA and other non Bw52 HLA genes

IL12B region on Chromosome 5 was revealed to have a significant association with TA and the Single nucleotide polymorphism (SNP) rs6871626 in the IL12B region was associated with increased risk and severity of AR (17) and vascular damage (18). Matsumura et al. (19) demonstrated in their study, a strong association between the number of risk alleles in IL12B region and disease severity, pointing to a potential diagnostic use and goal oriented therapy. Yang et al. (20) however did not find any association with polymorphisms of IL 12/23 axis in Chinese population.

An SNP in *IL12B* region (rs755374) was identified as a shared genetic susceptibility loci between Giant cell arteritis (GCA) and TA with the help of Immunochip genotyping data from 1,434 Large vessel vasculitis patients and 3,814 controls (21).

GWAS (Genome-wide association study) in Turkish, North American, Japanese, and Han Chinese patients reported an association with a polymorphism of the genes encoding Fc γ receptor IIA and IIIA (FCGR2A/ FCGR3A) (22). FCGR2A/3A encodes for receptors of Fc portion of immunoglobulin and thereby, is associated with the mechanisms involved in removal of pathogenic antigen antibody complexes (22).

Terao et al. (23) conducted a GWAS study in 633 TA cases and 5,928 controls and reported four previously undocumented loci namely rs2322599, rs103294, rs17133698, and rs1713450, in PTK2B, LILRA3/LILRB2, DUSP22, and KLHL33, respectively. They found a significant epistasis association between HLA-B*52 and rs103294 in LILRA3. They also found a novel SNP in the MICB (MHC Class I Polypeptide-Related Sequence B region), which showed a strong linkage disequilibrium with HLA-B52. This SNP contributes to upregulation of MICB on blood vessels, activation of Natural killer cells, and vasculitis.

MLX gene encodes Max like Protein X transcription factor and a single nucleotide polymorphism, rs665268, was associated with severity of disease in the form of AR and the number of arterial lesions. The Q139R mutation in MLX gene leads to suppression of autophagy and enhanced Inflammasome activation leading to cellular oxidative stress (24).

A recently published multi ancestral meta-analysis comprising of TA patients from five different populations (Turkish, Northern European descendant, Han Chinese, South Asian, and Italian), reported independent association with five different variants in HLA region: Three in *HLA-B* susceptibility locus (rs12524487-*HLA-B/MICA*, rs17193507-*HLA-B/MICA*, and rs12526858-*HLA-B*), and 2 other newly described ones namely, rs2844678-*MUC21* and rs28749167-*HLA-G*; in addition, they also identified four non-HLA susceptibility loci in *VPS8*, *SVEP1*, *CFL2*, and chr13q21. The study also fortified the association of *IL12B*, *PTK2B*, and chr21q22 with TA across ancestries (25).

Pathogenesis

The core of pathophysiology involves inflammation of the aorta, its major branches and coronary and pulmonary vasculature, which leads to arterial wall remodeling and thickening leading to a stenotic or aneurysmal disease (26).

Inflammation begins around the vasa vasorum in early stages of TA, that progresses to cause an inflammatory cell infiltration of adventitia. Eventually, granulomas form adjacent to media, with infiltration of monocytes, lymphocytes, neutrophils, epithelioid cells, and giant cells, eroding the media from outside. Intimal hyperplasia occurs as a consequence of damage to internal elastic lamina by granulomatous inflammation. This, along with adventitial fibrosis leads to stenosis of the arterial lumen causing ischemic symptoms (27). Whereas, a rapidly progressing & aggressive cascade of inflammatory events far overtaking a slower & lagging fibrotic healing process in the vessel wall leads to aneurysm formation (28). The frequency of aneurysms is variable, ranging from 4–45%, among different ethnicities, with highest numbers reported from Japan (29).

Innate immunity

Vasa vasorum of adventitia is postulated to be the site of primary injury, triggering a sequence of events leading to activation of innate and adaptive immunity (30).

Dendritic cells

T cells have been found to co-localize with dendritic cells (DCs) within adventitia in TA (31). Dendritic cells are activated by toll like receptors (TLRs) *via* an unknown trigger. Pryschep et al. (32) demonstrated that there is differential expression pattern of TLRs 1–9 in medium and large vessel walls, which probably explains the pattern of vessel wall involvement in TA.

Immature dendritic cells migrate to secondary lymphoid structures on activation. However, in Large Vessel Vasculitis, the activated DCs produce chemokines CCL18, CCL19, and CCL21 that bind the chemokine receptor CCR7, and get trapped within the vascular wall, stimulating local immunity. They express CD 86 and co-localize with T cells in inflamed vessel walls, indicating their role in linking innate and adaptive immunity (30).

Natural killer T cells

Seko et al. (33) demonstrated an increased infiltration of aortic tissue with killer cells, including NK (Natural killer) cells, γδT cells (Gamma delta T cells), and CD8+ T cells. These cells were found to release perforin directly onto the surface of arterial vascular cells. They further analyzed the expression of costimulatory molecules, and found that pro-apoptotic pathways 4-1BB/4-1BBL, MICA/NKG2D and Fas/FasL (Fas ligand) play important roles in vascular wall injury (34).

MICA or Major Histocompatibility Class I Chain-Related A, is induced by cellular stress like bacterial or viral infections. NKG2D, an activating receptor on NK cells and γδT cells binds to its ligand MICA, which initiates cytotoxic responses against the target cells expressing MICA, along with stimulation of antigen specific effector T cells (30).

A recent study demonstrated that TA patients had decreased numbers of NK cells in the peripheral blood compared to healthy controls and the expression of granzyme and perforin was also less compared to healthy controls. This supports the hypothesis that decreased NK cells in the peripheral blood of TA patients is probably due to trafficking to aortic tissues (35).

Adaptive immunity

Role of T lymphocytes

CD4+ Th 1 type cells play a key role in pathogenesis, upon antigen presentation by dendritic cells. These cells promote granuloma formation by releasing interferon-γ (IFN-γ), which in turn activates macrophages and formation of giant cells. Activated macrophages in turn release VEGF (Vascular endothelial growth factor) and PDGF (Platelet derived growth factor) promoting neovascularization and intimal proliferation (36, 37). Studies have demonstrated an increase in activated CD4+ T cells in peripheral blood of TA patients (38) and *in vitro* experiments demonstrated that the peripheral lymphocytes were sensitized against aortal antigen (38, 39).

CD4+ T cells in TA demonstrated ability to spontaneously differentiate into pro inflammatory phenotype. Zhang et al. (40) identified a pathway involving hyperactive mTORC1 (mechanistic target of rapamycin complex 1) leading to spontaneous differentiation into Th1 and Th17 subsets and targeting this pathway in a human artery-NSG (NOD SCID gamma) chimeras led to amelioration of disease.

Role of T cell subsets other than Th1 type in TA is gaining momentum. Weyand et al. (30) demonstrated increase in IL 17 producing T cells before treatment with corticosteroids. Another study from India, recently showed that there is significant expansion of Th17 cells and elevated serum IL-17 and IL-23 levels in 30 TA patients compared to 20 healthy controls (41).

Pan et al. (42) showed elevated IL9 levels in patients with active TA along with increased numbers of Th9 lymphocytes in peripheral blood, suggesting a role in pathogenesis of TA.

Treg cells (T regulatory cells) are key players in maintenance of peripheral tolerance; however under certain circumstances, Treg cells can transform into Th-like cells with loss of tolerance function leading to autoimmunity. Gao et al. (43) have identified increased numbers of Th2 like T regulatory cells in peripheral blood of patients with TA, along with increased levels of IL 4 and IL 13, which correlated with IL 6 levels.

Interferon -γ is a major player in pathogenesis of TA and a recent study by Ren et al. (44) reported that, CD8+ T lymphocytes were a significant source of interferon -γ, with peripheral blood showing higher number of $CD3^+CD8^+IFN\text{-}\gamma^+$cells and a lower ratio of $CD3^+CD4^+IFN\text{-}\gamma^+$/$CD3^+CD8^+IFN\text{-}\gamma^+$ in TA compared to controls.

There is an imbalance between pro inflammatory (Th1 and Th17) and Treg cells in TA, and upregulated JAK STAT (Janus kinase/signal transducers and activators of transcription) pathway has been identified to have a crucial role. Régnier et al. (45) demonstrated that blockade of this pathway with Jak inhibitors (Ruxolitinib, Baricitinib and Tofacitinib) led to decrease in Th1 and Th17 cells and increase in Treg cells, thereby, restoring homeostasis.

Role of B lymphocytes

The role of B cells in pathogenesis of TA is unclear and the evidence is conflicting. A few studies have shown increased frequency of anti-aorta antibodies (46) and anti-endothelial antibodies (47–49) in TA patients vs. controls, however it was not replicated in other studies (50).

Two target proteins, endothelial protein C receptor (EPCR) and scavenger receptor class B type 1 (SR-B1) have been identified for anti-endothelial antibodies in TA. These proteins have anti-inflammatory properties, including impairment of Th 17 function, hence blocking antibodies against them promotes a pro inflammatory state (51).

Tertiary lymphoid structures were demonstrated by Clements et al. (52) in aortic adventitial tissue, however their role in pathogenesis of TA needs to be further studied.

In comparison to Giant cell arteritis patients and healthy controls, TA patients had an upregulated T follicular helper cell signature in another study, which help B cells proliferate and differentiate to produce antibodies. This study also found higher frequency of Tertiary lymphoid structures in inflamed aorta of TA vs. GCA supporting a role for B cell in vascular inflammation (53).

Role of pro-inflammatory mediators

Patients with TA have been found to have increased serum levels of IL-6, which correlates with disease activity (54). Seko et al. (55) on amplification of cDNAs (complementary DNA) from the aortic tissue for cytokine transcripts, found increased levels of IL-6 expression in aortic tissue, suggesting

an intravascular synthesis. IL 6 has multiple roles, as it activates B cells, enhances T cell cytotoxicity, promotes NK cell activity, activates MMPs (matrix metalloproteinases), fibroblast proliferation and acute-phase protein synthesis (30).

Goel et al. (56) analyzed the serum cytokine profile in Asian Indian patients with active vs. stable Takayasu arteritis and revealed higher serum IFN-γ in active disease than stable disease, and cytokines like Interleukin (IL)-6, IL-23, IL-17, IL-10 and transforming growth factor- β levels did not differ between both groups.

In addition, other cytokines found elevated in TA were IL-8, IL-9, IL-17, IL-18 and TNF (Tumor necrosis factor) (57).

Triggers of inflammation–environmental factors

The triggering event that leads on to a persistent inflammation of vascular walls is largely unknown. Molecular mimicry to certain peptides of micro-organisms has been suggested as one of the mechanisms. The most notable evidence available is for mycobacterial peptides—mycobacterial heat shock proteins (HSP). Aggarwal et al. (58) reported a heightened response to mycobacterial 65kDa HSP protein in TA patient's vs. healthy controls implying a role for this antigen in pathogenesis of TA. Chauhan et al. (59) demonstrated that there was increased cellular and humoral response to mycobacterial mhsp65 and its human analog Hhsp60, with higher CD4+ T cell proliferation and increased IGG isotype to both antigens, suggesting an infection induced autoimmunity. The link between TA and Tuberculosis (TB) has been hypothesized, due to higher prevalence of TB in Asia, Africa, and South America; the same geographic locations are also home to TA more than rest of the world. In addition, TA is also a granulomatous disease like TB and there have been incidences where TA and TB have occurred together in the same host (30). However, Arnaud et al. (60) did not find any evidence of mycobacterium infiltration in tissue biopsies of TA patients, although this does not rule out a cross reactivity with mycobacterial antigens.

Disease classification

TA has been traditionally classified into subgroups, based on angiography findings. Most frequently used classification is the Numano classification (also known as Hata's classification) which divided TA patients into six subgroups based on involvement of aorta, as a whole and its main branches (61).

Recently Goel et al. (62) using computer-based cluster analysis strategy, classified TA patients into 3 different subsets based on angiographic patterns, in an Indian cohort of 581 patients. These findings were replicated in 3 independent cohorts from North America. Cluster 1 predominantly involved abdominal aorta and its branches including mesenteric and renal arteries. Cluster 2 had high prevalence of disease affecting aortic arch and its branches and Cluster 3 had asymmetrical arterial involvement with less territorial involvement compared to other clusters. They also observed that out of 92 patients followed up with angiogram, 91 of them stayed in the initial cluster over a median time interval of 3.3 years. Comparison with Numano classification revealed that all three clusters could be represented by Type 5 and 5 % of Indian and 6.7% of North American cohorts were not classifiable according to the Numano classification.

Clinical features

The clinical spectrum of TA is quite heterogenous, ranging from asymptomatic and incidentally detected hypertension to acute-onset stroke or cardiac failure (3). TA can progress from a prepulseless phase with non-specific systemic features to vascular inflammatory phase, which rarely ends in burnt out fibrotic stenotic phase. However, patients may not conform to this triphasic pattern of disease (3, 63), as ongoing inflammation & damage are the rules rather than exceptions in vast majority of cases with TA in absence of immunosuppressive therapy.

Clinical presentation depends on the pattern of arterial involvement, which is different in different regions of the world. Moriwaki et al. (64) retrospectively compared the clinical manifestations of TA in India and Japan. This study with 102 Indian and 80 Japanese patients documented significant differences in clinical presentation. Indians were most likely to have Type IV disease, with hypertension being the most common clinical manifestation. Japanese TA patients predominantly had type I and IIa disease, with a more severe and prolonged course than Indians.

Goel et al.'s (62) recent work on cluster analysis based angiographic classification revealed that Indians more commonly had renal involvement and predominantly belonged in Cluster 1, whereas most of the North Americans had involvement of left carotid and subclavian arteries and fell within the cluster 3.

Clinical features from various cohorts in the world have been presented in Table 1.

In addition to vascular manifestations, extravascular manifestations can also be seen. Most frequently noted features include arthritis/arthralgia with or without sacroiliitis, oral ulcers, inflammatory bowel disease, ocular conditions like scleritis, episcleritis, uveitis, and skin lesions including pyoderma gangrenosum and erythema nodosum (71).

Childhood onset TA, is a subset that can affect any age group ranging from infants to adolescents, with significant differences in clinical presentation compared to adult TA (72). Danda et al. (65) compared clinical characteristics of 108 patients with childhood onset TA (c-TA) vs. 447 patients

TABLE 1 Clinical features of patients with TA in large cohorts across the world.

Clinical features	India Danda et al. (65)	Japan Watanabe et al. (5)	China Yang et al. (66)	Mexico Soto et al. (67)	France Comarmond et al. (68)	Turkey Bicakcigil et al. (69)	US Schmidt et al. (70)
Number of centers	Single center	Multicenter	Single center	Single center	Single center	Multi center	Single center
Number	602	1,372	566	110	318	248	126
women n (%)	466 (77.4%)	1,150 (83.8%)	448 (79.2%)	94 (85%)	276 (86.8%)	221 (89.1%)	115 (91%)
Age of onset	26 (21–33)§	35 (22–56.8) §	36.13 ± 13.4¥	26 ± 9¥	36 [25–47] §	40.1 (19–76) §	29.2 (20.5–34.5) §
Fever	119 (19.2%)	476 (34.7%)	52 (9.2%)	22 (20%)	55 (17%)	68 (27%)	30 (29%)
Hypertension	319 (53%)	54 (4%)	62 (11%)	58 (53%)	98 (31%)	106 (43%)	41 (38%)
Ocular symptoms	70 (11.7%)	45 (3.3%)	58 (10.2%)	64 (70%)	14 (4%)	57 (36%)	11 (12%)
Headache	125 (20.8%)	113 (8.7%)	NA*	77 (70%)	NA	119 (48%)	50 (45%)
Syncope	66 (11%)	36 (2.6%)	60 (10.6%)	39 (35%)	NA	47 (19%)	51 (49%)
Stroke	46 (7.7%)	181 (13.2%)	28 (4.9%)	10 (9%)	39 (12%)	44 (18%)	11 (11%)
Cardiac$	155 (25.8%)	153 (11.1%)	247 (43%)*	35 (32%)	21 (6%)	141 (57%)	39 (39%)
Lung	160 (26.7%)	92 (6.1%)	NA	17 (15%)	NA	NA	NA
Abdominal pain	NA	NA -	- NA -	NA	NA	NA	15 (16%)
Renal	42 (7.7%)	154 (11.2%)	6 (1.1%)	NA	NA	NA	NA
Carotidynia	5.6 (33%)	133 (9.7%)	25 (4.4%)	23 (21%)	33 (10%)	NA	15 (15%)
Limb claudication	310 (51.5%)	287(20.9%)	161 (28.4)	NA	NA	119 (48%)	64 (52%)
Pulse abnormalities	418 (69.6%)	UL 67 (4.9%)	124 (21.9%)	NA	NA	218 (88%)	88 (70%)
Vascular bruit	311 (51.7%)	NA	NA	51 (46%)	52 (16%)	190 (77%)	81 (65%)
Most frequent angiography type	Type V (51.3%)	Type I (28%)	Type III (37.8%)	Type V (69%)	Type V (49%)	Type V (51%)	Type V (57%)

*NA = "Not available" in the cited reference. $ = chest pain,or palpitations, or ischemic heart disease, or aortic regurgitation or heart failure or pericarditis. *Inclusive of aortic regurgitation in the China series.
§ = Median with Inter-quartile range.
¥ = Mean ± Standard deviation. UL = upper limb.

with adult TA. They observed lesser female predominance, higher frequency of systemic involvement in the form of deranged creatinine, hypertension, cardiomyopathy, abdominal pain, fever and headache in c-TA compared to patients with adult onset TA; the latter were more likely to present with claudication symptoms. When classified according to Hata's angiographic classification, Type IV disease was more frequent in childhood onset TA and Type I was more likely to occur in adult onset TA, although type V was the commonest variety in overall in this series (65).

Diagnosis

Diagnosis of TA is based on clinical features and supported by imaging and laboratory results. To help differentiate patients with TA, from healthy controls or other similar vasculitic disorders, Ishikawa diagnostic criteria, Sharma's modification of Ishikawa diagnostic criteria and ACR 1990 classification criteria are useful in clinical practice and clinical trials (73). Newly proposed DCVAS-ACR-EULAR classification criteria is awaited.

Imaging

Color doppler ultrasound

With benefits of low cost, easy access, absence of radiation exposure, and ease of repeatability, color Doppler ultrasonography (CDS) has an important role to play especially in a resource-poor setting and in disease assessment in pregnancy. It can detect vessel wall thickening, pulsatility, luminal stenosis, occlusion and calcification (74).

Macaroni's sign is a characteristic feature of TA on CDS, which implies a long segment of moderately echoic circumferential vessel wall thickening which is diffusely homogeneous. Carotid intima-medial thickness (IMT) is useful to assess disease activity with a sensitivity of 82% and specificity of 60%, using common carotid artery wall thickening (75).

CDS has a limited role in assessment of disease in obese patients, and cannot be used to assess origins of greater vessels and abdominal vessels and requires high expertise (74).

Contrast enhanced ultrasound

Contrast enhanced ultrasound (CEUS), a technique that is used to assess intraplane neovascularization of carotid artery atherosclerotic plaque, has recently found a place in assessment of disease activity in TA (76). In a retrospective study done on Chinese TA patients, CEUS vascularization score was found to positively correlate with vascular ^{18}F-FDG PET/CT (Fluorine-18 - Fluoro-Deoxy-glucose [^{18}F-FDG] Positron Emission Tomography [PET]/Computed Tomography [CT]) uptake (77). Ma et al. demonstrated the usefulness of this technique for monitoring response to treatment with reduction in parameters like thickness and vascularization, that were specific to TA (78).

Superb microvascular imaging (SMI), is a novel Doppler technique that can depict low velocity blood flow and microvascular blood flow without the use of a contrast agent (79). This technique has shown arterial wall vascularization in active TA patients, which regressed after treatment suggesting a possible role for it in disease assessment (79, 80).

PET-CT

Offering a combination of both anatomical and metabolic imaging, PET-CT has a unique place in diagnosis, especially for patients with atypical presentations, those with normal inflammatory markers (81) and presumably for monitoring of treatment response and predicting relapses (82). In a meta-analysis, from 10 studies, pooled Sensitivity and Specificity of ^{18}F-FDG-PET for TA activity was 81 and 74%, respectively (83). It is however influenced by treatment with glucocorticoids, and should not be performed more than 3 days after starting glucocorticoids as it was found to reduce uptake in vessel wall (84). On the contrary, a few studies have suggested that it has a limited value during the follow-up due to persistence of the ^{18}F-FDG uptake in a clinically silent disease (85).

PETVAS or PET Vascular Activity Score is a qualitative summary score derived by summation of the semi-quantitative PET visual scores (0 = no ^{18}F-FDG uptake; 1 = less than liver; 2 = equal to liver; 3 = greater than liver) of nine specific arterial territories (ascending aorta, aortic arch, descending thoracic aorta, abdominal aorta, innominate artery, right/left carotid arteries, and right/left subclavian arteries). A score of more than or equal to 20 was able to differentiate active from inactive disease with a sensitivity of 68% and a specificity of 71% and was able to predict clinical relapses in a prospectively followed-up cohort. It was also found to have a moderate correlation with acute phase reactants (86, 87).

PET/ MRI

PET/MRI (Magnetic resonance imaging) combines the quantitative measurement of vessel wall radiotracer uptake of ^{18}F-FDG PET along with the anatomic assessment of MRI. This modality has an added advantage of lower radiation exposure compared to PET-CT and hence can be used for follow up scans in younger individuals (88).

Laurent et al. defined three PET/MRI patterns, in their retrospective study of 13 patients with large vessel vasculitis: (a) inflammatory (with abnormal ^{18}F-FDG uptake with SUVMAX 4.8 [range: 3–8.6] and abnormal MRI), (b) fibrous (with normal ^{18}F-FDG uptake with SUVMAX 1.9 [range: 1.8–2.1] and abnormal MRI), and (c) normal (normal ^{18}F-FDG uptake with SUVMAX 2.2 [range: 2–4.5] and normal MRI) (88).

MRI

It allows a high-resolution characterization of both vessel wall and lumen, while avoiding radiation exposure. It also has the added advantage of assessing cardiac activity. MRI detects vessel wall thickening and contrast enhancement, that are presumed to reflect inflammation (89). EULAR (currently, European alliance of associations for Rheumatology) task force recommends MRI as a first choice for imaging a patient suspected with Takayasu arteritis (90). T1-weighted, fat-suppressed, contrast-enhanced sequences as well as black blood imaging are preferentially used (91). MRI has a doubtful role in follow up and monitoring of disease activity, as Tso et al. revealed 56% of their study patients in clinical remission had persistent wall oedema and there was no correlation between wall oedema and development of new lesions (92). However, in a study conducted in India in 20 patients with TA, MRI assessment of disease activity significantly correlated with ITAS (Indian Takayasu clinical arteritis score) (93).

CT angiography

It is most readily available, preferred diagnostic modality to assess the full extent of arterial involvement, especially when the lumen is unaffected, and the only finding is vessel wall thickening. It has a sensitivity and specificity of 95 and 100%, respectively.

A characteristic feature found in CT angiography (CTA), post contrast is known as double ring enhancement pattern, which is a poorly enhanced ring within indicating a swollen intima and a well enhanced ring outside indicating active inflammation in media and adventitia (94). CTA can depict structural changes including mural thickening, luminal occlusion, aneurysms, thrombosis and end organ damage (95).

CTA is limited by high radiation exposure, restricting its use in follow up and use of contrasts that may have contraindications too. It can delineate anatomical abnormalities, but cannot differentiate between active and inactive disease.

Biomarkers

CRP (C reactive protein) and ESR (Erythrocyte sedimentation rate) are neither sensitive nor specific for assessment of disease activity, as there can be persistent vessel wall inflammation and angiographic progression of disease in spite of normal acute phase reactants (96). So, there have been a number of studies over time looking for the ideal biomarker for TA.

Goel et al. have looked at the serum cytokine profile and observed that IFN-γ correlated best with disease activity, compared to other cytokines studied including interleukin (IL)-6, IL-23, IL-17, IL-10 and transforming growth factor - β levels (56). They also found a direct correlation between IL-23 and disease duration.

In a Turkish cohort of 51 patients, F. Alibaz-Oner et al. found significantly elevated IL-6, IL-8 and IL-18 levels in patients with TA compared to healthy controls, with higher IL-18 levels in active disease (57).

Nair et al. studied the utility of serum amyloid A (SAA) in assessment of disease activity and observed that SAA levels were higher in TA compared to controls and in active vs. stable disease (97).

Goel et al. explored the usefulness of serial monitoring of serum myeloid related protein 8/14 (MRP8/14) as a marker of disease activity and angiographic progression in (TA). The study revealed that the levels were higher in active disease which decreased in responders to treatment with no change in non-responders. In patients who had angiographic progression on follow up, 66% had elevated MRP8/14 levels compared to 26% in non-progressors. MRP8/14, therefore, could have prognostic implications (98). The same group also looked at soluble HLA E levels as a potential biomarker of disease activity as it is shed from endothelium in response to inflammation. Soluble HLA E levels were higher at baseline and follow up visits of patients with active disease (99).

NMR (Nuclear magnetic resonance) based metabolomics in TA patients revealed a reduced circulatory glutamine to glucose ratio, suggesting increased glutaminolysis and reduced glycolytic activity in active disease which may serve as a surrogate marker for disease activity (100).

The role of B cells in pathogenesis of TA is yet to be explored. A study from India measured levels of APRIL (a proliferation-inducing ligand) and BAFF (B-cell activating factor) in TA patients vs. healthy controls and concluded that serum APRIL levels were elevated in TA patients, but they did not correlate with disease activity (101).

Assessment of disease

Disease extent

DEI TAK (Disease extent index in Takayasu arteritis):

DEI TAK is a validated tool developed by Indian Rheumatology Association Core Group for Vasculitis (IRAVAS), based on Birmingham Vasculitis Activity Score (BVAS). It is a weighted score that quantifies the extent of disease at assessment, based on clinical findings only (102). Aydin et al. in their Turkish cohort of patients, found that the agreement between DEI TAK and NIH (National institute of health) criteria was 94% ($\kappa = 0.85$). However, the agreement between physician global assessment and DEI TAK was lower when compared to NIH criteria (103). One of the limitations is that, imaging is not taken into account and patients with slow progression of disease did not demonstrate change in DEI TAK (103).

Disease activity

To assess disease activity in TA, Misra et al. (104) developed a composite scoring system based on serial assessment of DEI TAK, which allowed selection of items reflecting active disease in recent past (104). ITAS 2010 and ITAS.A with acute phase reactants (ESR or CRP) have been extensively validated (104, 105) with inter-rater variability better than PGA (102). ITAS has been shown to respond to therapy and predict relapses in two different cohorts (106, 107).

Disease damage

TADS (Takayasu Damage Score), also derived from DEI TAK is used to assess damage caused by TA. It has 42 items in seven systems, scoring features persistent for at least 3 months. TADS can detect clinically-relevant outcomes, like pulse loss, stent patency and mortality (102). Rajappa et al. in their cohort of 82 TA patients, demonstrated a good correlation between TADS scores and disease duration and higher TADS scores in fatal disease (108). There was a positive correlation between duration of disease and DEI TAK and TADS scores in an Indian cohort of 602 patients with a median disease duration of 32 months for childhood TA and 27 months for adult TA (65).

Ma et al. in their recently published paper, developed a new disease assessment model that combines PET/CT sum of SUV mean, ESR and soluble IL 2 receptor levels. The new model was found to be superior to NIH scoring system in a cohort of 91 Chinese patients. However, this multimodel scoring system needs further validation before its use in clinical practice (109).

Treatment

Conventional synthetic DMARDS (Disease-modifying antirheumatic drugs)

Methotrexate

A pilot study by Hoffman et al. showed that 80 percent of their cohort of patients (13/16) achieved remission with weekly methotrexate on an average dose of 17mg. However, during a

mean follow up of 2.8 years, 44 percent of them relapsed on tapering of glucocorticoids (110).

Leflunomide

A prospective open label study from Brazil of 15 difficult-to-treat TA patients, who were started on leflunomide 20 mg per day, had 80 percent of them achieve remission at a follow up period of 9 months. Two patients had angiographic progression on treatment, one of whom was however still in clinical remission (111). An extended follow up study of 12 patients in remission was published in 2016 with a mean follow up period of 43 months, out of which 5 patients remained on leflunomide, rest had to change therapy in view of relapse or adverse drug reactions (112).

Recently, a case series from China by Cui et al. reported a response rate of 83 % at 6 months and 69% at 12 months from a cohort of 56 active TA patients, and at the end of 14 months, 85 percent were still on leflunomide with good tolerability. Also 9 out of 15 patients refractory to cyclophosphamide, responded to leflunomide (113).

Cyclophosphamide

A meta-analysis of 10 observational studies, revealed that at least 48 percent of patients on cyclophosphamide achieved partial response (114). A recent study put forth this observation that low-dose cyclophosphamide extended event free survival in a cohort of Chinese patients, and helped improve outcomes in high risk patients (115).

Azathioprine

Valsakumar et al. in their cohort of 65 newly diagnosed, treatment-naïve patients, found that a combination of azathioprine and prednisolone brought improvement in clinical symptoms and laboratory markers within 3 months in all patients, and it was observed that the disease was angiographically stable (116).

Calcineurin inhibitors

Multiple case reports have shown their efficacy in treating TA, especially in cases associated with pyoderma gangrenosum (117–119).

Mycophenolate mofetil

A retrospective observational study from India, with 21 TA patients on MMF noted a significant improvement in disease activity as evidenced by a drop in Median (range) ITAS from 7 (0–19) to 1 (0–7); and these patients had a median follow up duration of 9 months. The authors also reported a significant decrease in glucocorticoid dosage at the last follow up (106). Danda et al. in their cohort of 602 TA patients, had 251 patients with a follow up data of more than 12 months. One hundred and sixty (63%) of these patients had been on MMF. Compared to other DMARDS, numerically higher proportion of patients on MMF had sustained inactive disease, with no serious adverse events (65).

A prospective study from China observed that MMF alone or in combination with glucocorticoids, methotrexate or azathioprine was effective in controlling disease activity and retarding angiographic progression, with an effective rate of 80% (120).

Biological DMARDS

Tocilizumab

A Phase 3 randomized control trial from Japan, The TAKT study, conducted by Nakaoka et al. randomized 36 patients to receive either tocilizumab (TCZ) or placebo with background glucocorticoids. The primary endpoint of time to relapse was longer in the tocilizumab group, even though it barely missed statistical significance (121). In the Long term extension study, with all 36 patients receiving weekly subcutaneous TCZ, most demonstrated clinical improvement, glucocorticoid sparing effect and radiological stabilization at 96 weeks (122).

An observational study by Goel et al. with 10 "difficult-to-treat" TA patients, who had active disease in spite of glucocorticoids and multiple DMARDS with median treatment duration of 27 months, reported that 100 % of the patients receiving monthly intravenous Tocilizumab had achieved clinical response as reflected by ITAS of 0. Six out of ten of these patients maintained response till sixth infusion, with no angiographic progression. They also had an important observation that these patients relapsed on stopping tocilizumab, so the benefit was not sustained (123).

In a systematic review and meta-analysis of observational studies of patients receiving TCZ treatment, it was revealed that 87% achieved at least a partial clinical remission, 88% achieved angiographic stabilization, 62 % had reduced uptake on PET-CT and 94% had reduction in acute phase reactants. Patients on TCZ were able to reduce the median prednisolone dose by 83%. However, the results were heterogeneous (114).

Abatacept

In a multicentric double blinded randomized control trial, 36 patients received abatacept at 8 mg/kg, and 26 patients who achieved remission at week 12 were randomized to monthly abatacept or placebo with a background glucocorticoid taper. Primary outcome of relapse- free survival was achieved by 22% in abatacept arm at 12 months compared to 40% by placebo arm. The study revealed that there was no benefit of addition of abatacept to standard regimen (124).

TNF inhibitors

Ferfar et al. in their review article, documented 13 studies with 96 TA patients who were treated with TNF inhibitors (infliximab, etanercept and adalimumab). Clinical improvement was reported in 61%, Glucocorticoids were stopped in 39%, and 3 patients showed regression of lesions on MR angiography. Twenty-eight relapses were reported in a follow up period of 24 months (125). A point of concern with TNF inhibitors, however is the high risk of tuberculosis reactivation, especially in endemic countries.

A meta-analysis of 18 observational studies was conducted and the pooled data revealed that, in patients on TNF inhibitors, 81% could achieve partial response in clinical features, and 86 % had angiographic stabilization, with relapses in 32%. Results were however significantly heterogenous across the various studies (114).

Rituximab

In a retrospective study of seven TA patients, who were refractory to glucocorticoids and various conventional synthetic and biological DMARDS, Rituximab 2 grams was given as induction therapy followed by maintenance doses every 6 months. Three out of seven patients achieved complete remission, but remaining four patients still had persistent disease and radiographic progression. Results of this study do not support use of rituximab in TA. In contrast to this, prior case reports showed eight out of nine patients had positive results in terms of clinical and imaging criteria (126).

Jak inhibitors

Upregulated JAK STAT signaling pathways have been reported recently, contributing to the pathogenesis in TA and inhibition of these signaling pathways with Jak inhibitors is a promising new therapeutic avenue (45).

Tofacitinib

Multiple Case reports have been published recently describing patients with TA who did not respond to csDMARDs, TNF inhibitors or tocilizumab, had good clinical and imaging responses with Tofacitinib at a dose of 5 mg twice a day (127–130).

An observational study by Li et al. reported 5 consecutive patients with refractory TA, who were started on tofacitinib at 5 mg twice a day; all patients experienced clinical improvement within 4 weeks of starting the JAK inhibitor. Three out of these five patients also had stabilization of radiological disease (131).

Kong et al. in their prospective observational study of 53 patients, compared efficacy of tofacitinib vs. methotrexate (with tapering glucocorticoids in both groups) over a period of 12 months. At 6 months and 12 months, tofacitinib group had a higher complete remission rate and fewer relapses. They did not, however, observe any difference in disease progression on imaging (132).

Two randomized control trials from China, one comparing tofacitinib with adalimumab and the other comparing tofacitinib with methotrexate, are currently ongoing, with results expected in 2025 (133, 134).

Upadacitinib

Select–TAK, a phase 3 multicenter randomized control trial, comparing upadacitinib vs. placebo, is currently active, with estimated primary completion date in 2022 (135).

Pilot study on ustekinumab

Terao et al. conducted a pilot study and assessed the safety and efficacy of ustekinumab in three TA patients and reported response in clinical symptoms and acute phase reactants. These patients however, did not show any change in vessel wall enhancement on MRI (136).

Comparative studies

Methotrexate vs. leflunomide

When an observational study compared these two drugs over a 12-month duration, there was no difference in the proportion of patients achieving complete remission at 9 months and 12 months. There were however lesser number of relapses in the leflunomide group than in methotrexate group; 7.24% vs. 16.67% ($p = 0.03$) (137).

TNF inhibitors vs. tocilizumab

A retrospective observational study, analyzed drug retention rates in 50 TA patients and found that TNF inhibitors had higher drug retention rates than tocilizumab and using concomitant csDMARDs had a positive effect on the same (138).

Another retrospective multicenter study from France investigated outcomes in 49 TA patients and found no difference in efficacy between TNF inhibitors and tocilizumab (139).

Similarly, a multi - center retrospective cohort showed similar remission rates, relapses, glucocorticoid doses and mortality with TNF inhibitors and Tocilizumab (140).

Endovascular and surgical interventions

The main indications for interventions in TA include critical ischemia with risk of end organ damage, uncontrolled hypertension, coarctation of aorta, aortic aneurysm and aortic regurgitation (141–146).

Interventions should ideally be performed during periods of remission, as the complication rate and mortality is higher when the disease is active (147, 148). Saadoun et al. from their cohort of 79 patients, observed that the likelihood of complications was 7 times higher when procedures were done in the presence of active inflammation (148). A retrospective study by Perera et al. observed that the rates of procedural failure were lower in patients with well-controlled disease (142).

Vascular interventions can be by endovascular approach, or *via* open surgery. A meta-analysis by Jung et al. on 770 patients compared endovascular interventions vs. open surgery. They reported that patients who underwent endovascular interventions had a higher rate of restenosis, especially in coronary artery, supra-aortic branches, and renal artery in both active and inactive disease. The risk of stroke was instead higher in open surgical procedures (149). In a 10-year retrospective study by Diao et al., both types of procedures were found to be safe and had similar primary and secondary patency rates (150).

Joseph et al. published their data on 401 TA patients, who underwent 1,516 percutaneous interventions. Early outcomes were successful in 1,044 interventions. In follow up, on repeated percutaneous interventions in patients who developed restenosis, 83 % success rate was achieved at a mean follow up duration of 33 months from the last procedure (143).

Open surgery that is most commonly performed is the aortic valve surgery with (Bentall procedure) or without aortic root replacement. Other lesions where open surgery is indicated is for repair of thoracic and abdominal aneurysms, although endovascular stents are fast gaining popularity. Surgical bypass procedures are done in occlusive or stenotic lesions (144). Most frequent complications with open surgeries are postoperative bleeding, cerebrovascular accidents, and anastomotic aneurysm (151).

Endovascular interventions, like primary angioplasty can be performed with or without stents. Balloon angioplasty is commonly used in patients with stenotic or occlusive lesions. Stenting is indicated in cases of arterial dissection or persistent stenosis post angioplasty (152). Stents can be drug eluting stents, self-expanding stents or covered stents which are used in aortic aneurysms (153). Thoracic endovascular aortic repair (TEVAR) and endovascular abdominal aorta repair (EVAR) are life-saving interventions in patients with dissections and aneurysms of aorta (154, 155). The efficacy of percutaneous intraluminal angioplasty in renal artery involvement was evaluated by Sharma et al. in 66 TA patients with 96 stenosis. With uncontrolled hypertension being the most common indication, 83 % showed clinical benefit post procedure, however 16 % of them developed restenosis in a median follow up duration of 22 months (156).

Chacko et al. used their in-house developed CO_2 angiography guided interventions in renal insufficiency patients, where the use of contrast is considered detrimental to the renal functions. The retrospectively attained data showed that the procedure was safe and effective in this subset of patients (157).

In patients with severe renal artery stenosis, who have failed endovascular interventions, renal auto transplantation is a viable treatment option; it is also a great procedure to ameliorate refractory renovascular hypertension in TA in spite of multiple drugs (158).

Prognosis

Survival rate at 5 years, ranges from 67–100 % in various studies (66, 141, 159). A recent population based study from Korea comprising 2,731 TA patients, the 10 year survival rate was 85% (160). A French multi-center study reported a 96% overall survival rate at 10 years. The survival and mortality rates varied depending on ethnicity and have improved with time (161). Poor prognostic factors affecting survival are progressive disease course, thoracic aorta involvement and retinopathy (68, 161). Mortality rates range from 3–21%, and the most common causes of death are heart failure, stroke, infections and post-procedure complications (162). Goel et al. developed a model using ESR, CRP, Type 4 TA and Low DEI.TAK score, to predict sustained inactive disease with a sensitivity and specificity of 70 and 61.1% respectively. Sustained inactive disease was observed in 34.6% of their patients over the median follow up period of 42 (IQR: 24–81) months (163).

Establishing criteria for remission and introducing Treat to target therapy, could possibly pave the way for better survival. A Treat to Target algorithm developed by Sugihara et al. is a beginning in this direction (164). A practical treatment algorithm proposed by Jha A and Danda D is another recent, flexible and comprehensive therapeutic guide (165).

Conclusion

Diagnosing TA early remains to be a challenge even today, efforts in this direction for developing new biomarkers are subjects of ongoing research.

With advances in medical and surgical treatments, we hope that the mortality and morbidity of TA can be reduced further by targeted management of disease.

Author contributions

DD conceptualized the review. PM and DD reviewed the literature and drafted the manuscript. DD, XT, and MH reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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