Arthritis & Rheumatology

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Cover image: The figure on the cover (from Liping Tan et al, pages 423–438) depicts the periodic acid–Schiff (PAS) staining of kidney tissue from lupus mice with wild-type (*FoxO1*$^{f/f}$) and myeloid-specific FoxO1 deficiency (m*FoxO1*$^{-/-}$). This image illustrates a significant phenomenon: myeloid FoxO1 deficiency markedly exacerbates glycogen deposition and glomerular basement membrane thickening, leading to more severe renal damage (lower left). Administration of capmatinib significantly alleviated the renal injury in m*FoxO1*$^{-/-}$ lupus mice (lower right).

In this Issue

Highlights from this issue of *A&R* | By Lara C. Pullen, PhD

Epigenetic Myeloid Dysregulation in Systemic Sclerosis

In this issue, Martinez-Lopez et al (p. 439) report that patients with systemic sclerosis (SSc) have impaired epigenetic regulation that affects gene expression. The team's comprehensive analyses of the largest sample size reported in SSc revealed significant disparities in the epigenetic control of genes associated with key features of SSc, including immune dysregulation, vasculopathy, and fibrosis. Their findings enhance the knowledge of SSc pathogenesis and identify new molecules with potential clinical applications.

p. 439

The investigators conducted an epigenome-wide association study of whole blood from 179 patients with SSc and 241 unaffected controls. They obtained information on 352,036 CpG sites. Comparing patients with SSc and controls allowed them to identify 525 differentially methylated positions, suggesting both a proinflammatory and a profibrotic response in patients with SSc. The researchers then explored functional mechanisms associated with changes in methylation patterns seen in patients with SSc. They found that the patients had enriched immune-related pathways, with leukocyte cell–cell adhesion as the most significant. The team did not, however, see any evidence of accelerated epigenetic aging in patients with SSc. The investigators next asked whether methylation differences could be driving changes in the transcriptome of these patients; they found by integrating methylome and transcriptome data that integrins may play a role in disease.

The researchers analyzed genes by expression quantitative trait methylation and observed an enrichment of neutrophil-related pathways. The most significant term enriched was neutrophil degranulation, which included 47 genes, such as *PTX3* and *CD63*. They then investigated whether these changes in the methylation pattern occurred within transcription factor binding sites and identified myeloid CCAAT/enhancer-binding proteins (CEBPs) transcription factor methylation and expression signatures in patients with SSc. Since CEBPs are crucial for myeloid lineage development, the investigators concluded that myeloid cells contribute to SSc pathogenesis.

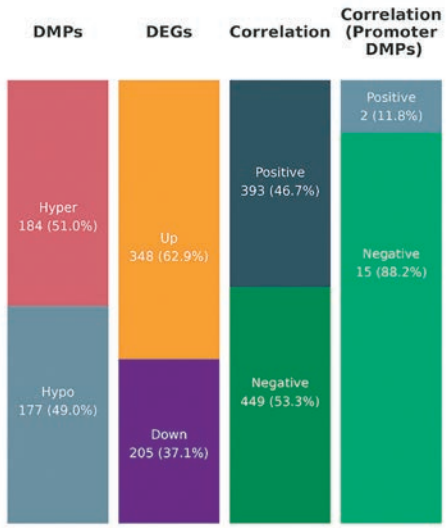

Figure 1. Bar plot characterization of CpG–gene interactions concerning differentially methylated positions (DMPs), differentially expressed genes (DEGs), and the direction of the correlation.

In Norway, Incidence of JIA Increases with Latitude

Previous studies have reported a high risk of juvenile idiopathic arthritis (JIA) in indigenous populations of North America, Australia, and New Zealand, as well as higher rates of JIA in the northern region of the UK compared with the southern region. In this issue, Hestetun et al (p. 458) report results from a nationwide study from Norway that found a higher incidence of JIA with increased latitude. While genetic factors modified the association, the investigators found no evidence that any of the available environmental factors could explain the observed gradient.

p. 458

The researchers used data from the nationwide registers of Norway to document an incidence rate (IR) for JIA of 14.4/100,000 person-years, in line with previous studies from the Nordic countries and southeast of Norway. The database included high-quality data encompassing all regions, and the researchers used the same method across all regions to calculate an IR for each region. After adjusting for perinatal factors, socioeconomic status, and antibiotic exposure from 0 to 24 months, they found these factors could not explain the observed north–south gradient. Genetic data from the Norwegian Mother, Father and Child Cohort Study (MoBa) were included in the same study. When the team adjusted for polygenic risk scores, the observed association between region North and JIA was weakened. When they included the 10 genetic principal components (PCs), the association was no longer significant. The team concluded that while genetic factors may explain some of the regional differences, these observations may also be attributed to environmental factors that are more common in certain regions, but were not included in the study.

The study included children born in Norway only, and the investigators had no data on children who immigrated during the study period. Moreover, with a positive predictive value of 93.4 in their case definition, they acknowledge that ~7% of children might be misclassified as having JIA, while others who did not seek medical care may not have been captured.

FoxO1 Deficiency in Monocytic Myeloid-Derived Suppressor Cells Exacerbates B Cell Dysfunction in Systemic Lupus Erythematosus

Although researchers have reported that FoxO1 regulates various immune cells, its regulatory effect on monocytic myeloid-derived suppressor cells (M-MDSCs) is not fully understood. Likewise, the role of M-MDSCs in the treatment of lupus remains unclear. In this issue, Tan et al (p. 423) report that the effective inhibition of B cells mediated by the ALKBH5/FoxO1/Met axis in M-MDSCs could represent a novel therapeutic approach to managing systemic lupus erythematosus (SLE).

p. 423

The investigators found that the imbalances of M-MDSCs were closely associated with the onset and progression of SLE. Previous research has revealed that N^6-adenosine methylation (m^6A) modification regulates the coding sequence (CDS) region and 3' untranslated region (3'UTR) of *FoxO1* mRNA, thereby influencing FoxO1 expression posttranscriptionally. The data from the current study supports the hypothesis that FoxO1 binds directly to the promoter region of Met, thereby exerting transcriptional regulation. ALKBH5 guides m^6A modification of FoxO1 at CDS and 3'UTR regions, wherein decreased levels lead to *FoxO1* mRNA degradation. FoxO1 deficiency subsequently activates Met transcription, disrupting downstream COX-2 and PGE_2 secretion.

Journal Club

A monthly feature designed to facilitate discussion on research methods in rheumatology.

Body Pain Diagram Data to Identify and Describe Evolution of Nonarticular Pain in the First Year of RA Diagnosis

Meng et al, *Arthritis Rheumatol.* 2025;77:405–413

Pain is a critical symptom of rheumatoid arthritis (RA), and underlying causes are not always accurately identified. Pain may manifest outside the joints as nonarticular pain (NAP) and, in tandem with articular pain, can contribute to the overall pain experienced in RA, potentially influencing patients' global assessment and clinical classification of disease activity. Accurately identifying and treating specific pain patterns is challenging but essential for effective treatment. The body pain diagram (BPD) is a commonly accepted tool for measuring pain location and distribution and may facilitate rapid identification of NAP in patients with RA in practice. Meng et al examined longitudinal data from BPDs collected from a real-world, prospective, protocolized, early RA cohort study during the first year of RA diagnosis to identify NAP patterns, prevalence, and evolution over time.

Standardized assessments were performed during clinical encounters and embedded in the participants' routine RA care. Participants completed a BPD, indicating areas of non-joint pain at baseline and at 6- and/or 12-months' follow-up. Prespecified definitions for regional and widespread NAP were generated based on published data of pain etiologies and patterns relevant to RA. This allowed identification of patterns of patient-reported pain on the BPD at each visit as no NAP, regional NAP, or widespread NAP. Frequencies and evolution of different NAP patterns over time were calculated. The presence of NAP at study entry was high. Over time, NAP could evolve by resolving, recurring, persisting, or evolving into another class of NAP (e.g., widespread to regional NAP). Summary statistics and descriptive statistics were applied. Last, the authors used clinical assessments such as joint counts and disease activity collected at the same visits as the BPDs to assess associations between NAP and remission using adjusted multivariable longitudinal analysis.

Questions

1. **What is currently known about the prevalence and evolution of NAP in patients with RA?**
2. **How have studies using BPD been reported in the literature, both in patients with RA and other populations?**
3. **What other measures of NAP could have been used?**
4. **What are the advantages and challenges in conducting studies from observational, real-world cohorts?**
5. **How might the study's findings influence how you approach identifying and managing NAP in RA patients in practice?**

Clinical Connections

Neutrophil Activation Markers and RA Treatment Response to JAK1/2 Inhibitor

Kuley et al, *Arthritis Rheumatol.* 2025;77:395–404

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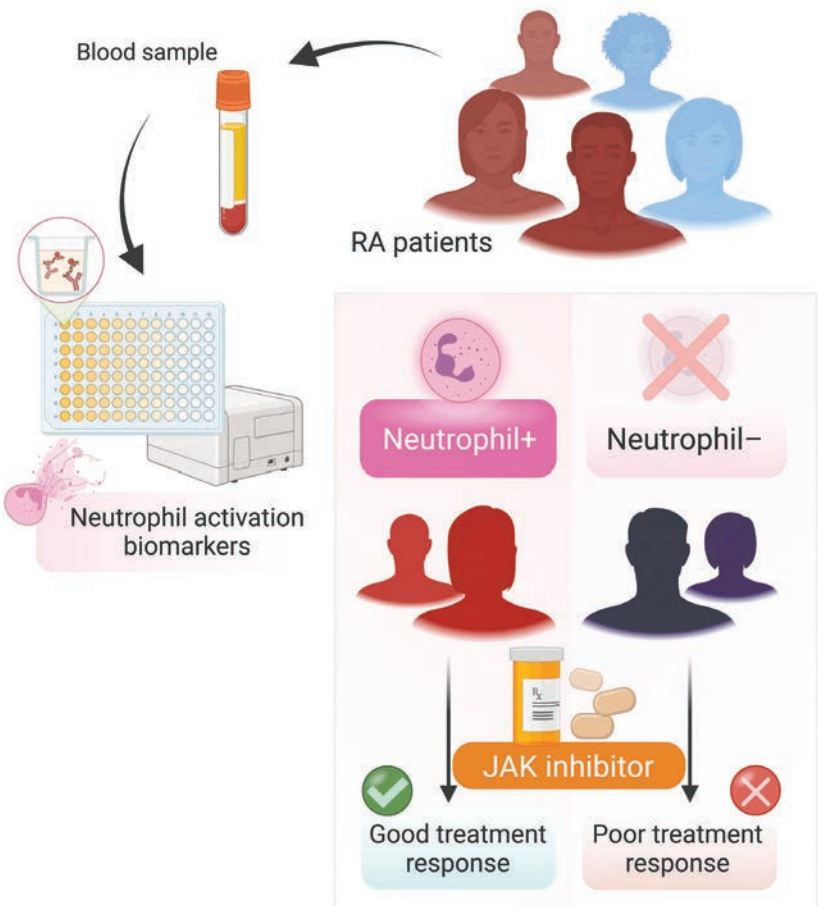

KEY POINTS

- Neutrophils are activated in rheumatoid arthritis.
- Neutrophil activation markers are reduced upon treatment with a JAK inhibitor.
- Neutrophil activation markers at baseline predict treatment response.

SUMMARY

Neutrophils are essential white blood cells that protect against invading pathogens. They also play an important role in regulating inflammation and tissue damage in rheumatoid arthritis (RA). Previous work found that markers of neutrophil activation tracked with disease activity and could predict disease progression, including development of erosive disease and extraarticular disease. The current study expanded on those findings to address another important clinical question, namely, whether the heterogeneity of RA disease course results in variable response to immunosuppressive treatment.

Kuley et al tested the hypothesis that treatment with the JAK inhibitor baricitinib would decrease markers of cytokine-mediated neutrophil activation and cell death in patients with RA, subsequently alleviating disease. Results showed that baricitinib treatment reduces overall neutrophil activation in RA. More importantly, only a subset of patients, namely those with increased neutrophil activation, had good treatment response, whereas patients lacking neutrophil activation had poor treatment response. The findings of this study emphasize the heterogeneity of disease course in RA, and provide evidence toward personalized medicine. Using a panel of neutrophil biomarkers to define current disease activity and determine disease progression may improve treatment response.

Clinical Characteristics of Anti-Synthetase Syndrome: Analysis from the CLASS Project

Faghihi-Kashani et al, *Arthritis Rheumatol.* 2025;77:477–489

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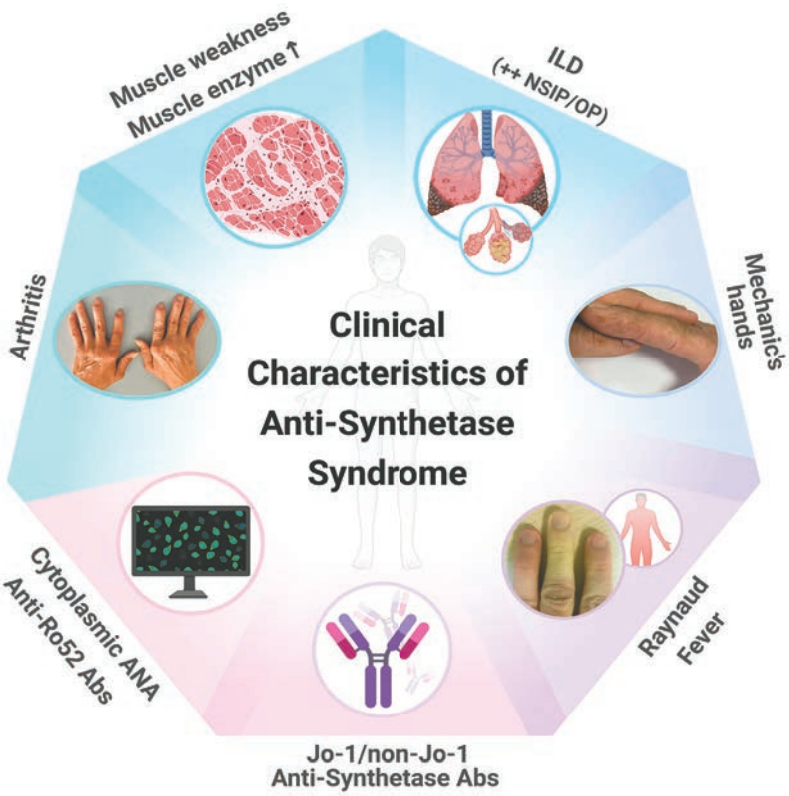

KEY POINTS

- Analysis of a large, international, multicenter CLASS database identified key variables associated with ASSD to aid in the development of data-driven classification criteria.
- Clinical features associated with ASSD included arthritis, muscle weakness, ILD, mechanic's hands, Raynaud phenomenon, and fever.
- Laboratory/serological markers including muscle enzyme elevation, anti-synthetase autoantibodies, ANA with cytoplasmic pattern, and anti-Ro52 autoantibodies were also associated with ASSD.

SUMMARY

Anti-synthetase syndrome (ASSD) is a rare and heterogeneous systemic autoimmune rheumatic disease (SARD) encompassing myositis, interstitial lung disease (ILD), and arthritis, and still lacks shared classification criteria. The Classification Criteria for Anti-synthetase Syndrome (CLASS) project is an international collaborative study to develop and validate the first data- and consensus-driven classification criteria for ASSD. The CLASS database comprises ASSD cases and controls, namely other SARDs and/or idiopathic forms of ILD, submitted from 92 centers across 30 countries worldwide. We employed univariable and multivariable regression analyses to identify clinical and serological features associated with ASSD and calculate their relative weights.

Our analysis included 948 ASSD cases and 1,077 controls. The clinical variable most associated with ASSD was ILD with nonspecific interstitial pneumonia/organizing pneumonia (NSIP/OP) pattern, followed by mechanic's hands, myositis, arthritis, unexplained fever, and Raynaud phenomenon. The serological variables most closely linked to ASSD were Jo-1/non–Jo-1 anti-synthetase autoantibodies, followed by antinuclear antibodies (ANAs) with cytoplasmic pattern and anti-Ro52 autoantibodies. This study offers a comprehensive set of variables and their weights aimed at establishing data-driven classification criteria for ASSD.

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American College of Rheumatology
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REVIEW

Macrophage Activation Syndrome

Peter A. Nigrovic

Macrophage activation syndrome (MAS) is a state of immune hyperactivation that can result in life-threatening multisystem end-organ dysfunction. Often termed a "cytokine storm," MAS occurs among the rheumatic diseases most typically in Still's disease but also in systemic lupus erythematosus and Kawasaki disease. MAS can also accompany infection, malignancy, and inborn errors of immunity. This review provides a practical, evidence-based guide to the understanding, recognition, and management of MAS in children and adults, with a primary focus on MAS complicating Still's disease.

Case presentation

A previously healthy 17-year-old male patient presented with three weeks of daily fever to 39°C, spiking in the afternoons and accompanied by transient rash and arthralgias. Physical examination showed a nontoxic but uncomfortable young man, with a temperature 38.5°C; the spleen tip was palpable, and a macular and linear erythematous rash was noted on the trunk, but the examination was otherwise normal. Laboratory test results revealed a white blood cell count of 15,000/μL (75% neutrophils), hematocrit 37%, platelets 453,000/μL, erythrocyte sedimentation rate (ESR) 83 mm/h, C-reactive protein (CRP) 8 mg/dL, ferritin 850 ng/mL, and normal aspartate transaminase (AST) and alanine transaminase (ALT). Interleukin (IL)-18 was 36,000 pg/mL (normal <800 pg/mL), and CXCL9 was 230 pg/mL (normal <121 pg/mL). Infectious and oncologic evaluation results were negative, including viral serologies. He was started on naproxen awaiting prior authorization for the recombinant IL-1 receptor antagonist anakinra, which was declined by his insurer. While taking naproxen, his fevers improved partially, appearing every few days for two weeks before worsening abruptly. On re-evaluation, he was pale and ill, with temperature 40°C, pulse 130, and blood pressure 107/65 mm Hg. His hematocrit level had decreased to 31%, and his platelets were now 230,000/μL, with ESR 45 mm/h, CRP 16 mg/dL, ferritin 43,000 ng/mL, AST 450 U/L, ALT 430 U/L, IL-18 180,000 pg/mL, and CXCL9 3,200 pg/mL. Polymerase chain reaction testing for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) was negative. He was treated with pulse glucocorticoids and anakinra 3 mg/kg per day intravenously (IV) twice daily, improving gradually over two weeks before being discharged with a prescription for anakinra 100 mg subcutaneously (SC) twice daily and a slow glucocorticoid taper. He was transitioned to the anti–IL-1β antibody canakinumab 300 mg SC monthly and remains in clinical remission off glucocorticoids and with normal CRP, ferritin, and CXCL9, although IL-18 remains elevated at 8,300 pg/mL.

What is macrophage activation syndrome?

The term "macrophage activation syndrome" (MAS) was coined in 1993 to describe an intensely inflammatory syndrome observed in children with rheumatic diseases and characterized by the presence of bone marrow macrophages engulfing other hematopoietic cells.[1] The term is now considered to encompass forms of hemophagocytic lymphohistiocytosis (HLH) that arise in the context of systemic rheumatic diseases rather than from a primary monogenic cause in children or adults.[2] More generally, the term describes a clinical syndrome characterized by a set of hallmark features, irrespective of underlying cause. These features, as they emerge in routine clinical tests, are summarized in the 2016 American College of Rheumatology (ACR)/EULAR classification criteria for MAS in the setting of Still's disease (a term we employ here to include both systemic juvenile idiopathic arthritis and adult-onset Still's disease, as per recent consensus recommendations) (Table 1), as well as in an ACR/EULAR consensus report on MAS.[3–5] Developed and tested in pediatrics, the ACR/EULAR criteria have also been employed for adults with Still's disease.[6] Patients with MAS exhibit severe systemic inflammation, usually with fever, together with the following.

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Table 1. 2016 ACR/EULAR classification criteria for MAS in the setting of Still's disease*

Criteria
Both of:
Known/suspected Still's disease with fever
Ferritin >684 ng/mL
Plus any two of:
Platelet count ≤81 × 10^9/L
AST > 48 U/L
Triglycerides > 156 mg/mL
Fibrinogen ≤360 mg/dL

* Modified from Ravelli et al.[3] The criteria were developed for Still's disease beginning before age 16 years (systemic juvenile idiopathic arthritis) but have been applied in patients older at onset. These criteria were developed for classification, not diagnosis, and may not be met by some patients with evolving or established MAS who would benefit from treatment. ACR, American College of Rheumatology; AST, aspartate transaminase; MAS, macrophage activation syndrome.

Elevated ferritin. Ferritin is an iron-binding protein released by activated macrophages, but also by other sources, including hepatocytes. An acute-phase reactant, ferritin is often elevated in inflammation; however, disproportionate ferritin elevation is a key marker of MAS, potentially reflecting release from erythrocyte-consuming hemophagocytes and other activated macrophages.[7, 8] There is no threshold at which ferritin becomes diagnostic for MAS, but very high values should trigger consideration of the diagnosis.[9] For example, in one large series, patients with Still's-associated MAS exhibited median ferritin levels >5,000 ng/mL, 10-fold higher than patients with active Still's disease without MAS.[10]

Low platelet count and other markers of disseminated intravascular coagulation. Ordinarily, systemic inflammation induces thrombocytosis, reflecting the induction of thrombopoietin by IL-6, among other factors. A platelet count lower than expected for the extent and duration of inflammation suggests either lack of marrow response (eg, from leukemic infiltration) or peripheral consumption, as in disseminated intravascular coagulation (DIC). MAS usually features at least subclinical DIC, so relative thrombocytopenia is an important clinical clue. Related features include elevated D-dimer and low fibrinogen, the latter translating into a paradoxical decline in ESR, because depletion of this positively charged hepatic acute-phase reactant prevents negatively charged red blood cells from forming the stack-like clusters (rouleaux) that drive ESR elevation.

Transaminase elevation. Hepatitis is a common manifestation of MAS. Although less specific than hyperferritinemia or markers of DIC, transaminase elevation retains diagnostic value, with transaminase values commonly several fold above the upper limit of normal.[3,11]

Further hallmarks of MAS are discussed below. However, the clinician should be on the alert for MAS in any patient with intense systemic inflammation, relative thrombocytopenia, and transaminitis, measuring ferritin as the first additional marker of MAS. Serial laboratory studies can provide additional evidence, in particular rising ferritin and transaminases and falling platelets.[11,12] Critically, failure to satisfy classification criteria should not delay initiation of treatment for patients in whom trends in clinical and laboratory parameters suggest progression toward MAS.[13]

Disease context is central to the suspicion for MAS. In pediatric rheumatology, MAS is encountered most often in patients with Still's disease, which is accompanied by subclinical MAS in up to one-third of patients.[14,15] Active Still's disease is considered the trigger of MAS in more than half of patients, with infections (most commonly EBV) implicated in approximately one-third.[11] MAS is also observed in patients with Kawasaki disease, systemic lupus erythematosus (SLE), severe infectious illness (bacterial or viral, including HIV and influenza), immunodeficiency or other immunoregulatory disorders, and malignancy, especially leukemia and lymphoma.[5,16] In adults, infection and malignancy are dominant causes of MAS, such that MAS should be suspected when severe inflammation arises in such patients, and should be considered carefully in patients presenting with MAS in the absence of another known predisposing condition.[5,17] In children, malignancy accounts for an estimated ~5% to 8% of HLH.[5,16] Figure 1 depicts an approximate distribution of causes of MAS in children and adults.

Pathogenesis of MAS

There is an unusually direct connection between the pathogenesis of MAS and the strategies used to diagnose and treat it. Particularly critical have been insights gained from primary HLH, a set of rare inborn errors of immunity that present as MAS in infancy or early childhood, often from defects in cell-cell killing mediated by perforin, a protein expressed by $CD8^+$ T cells, NK cells, and other lineages. Perforin helps terminate immune responses through a critical negative feedback mechanism depicted in Figure 2. Antigen-presenting cells (APCs) such as macrophages and dendritic cells present antigen to T cells, and these in turn promote further APC activation, including enhanced expression of antigen-presenting major histocompatibility complex (MHC) molecules, through cytokines such as interferon (IFN)γ. A vicious cycle is avoided because activated T cells kill APCs, limiting further antigen presentation and cytokine production. Perforin also allows NK cells to kill activated T cells and virus-infected cells. In primary HLH, genetic deficiency of perforin or of the molecules required to deliver it to the cell surface leads to a vicious cycle in which progressively larger numbers of APCs and T cells become activated, contributing to a toxic cytokine storm.

Abundant evidence supports this vicious cycle model of MAS. Mice lacking perforin develop lethal systemic inflammation

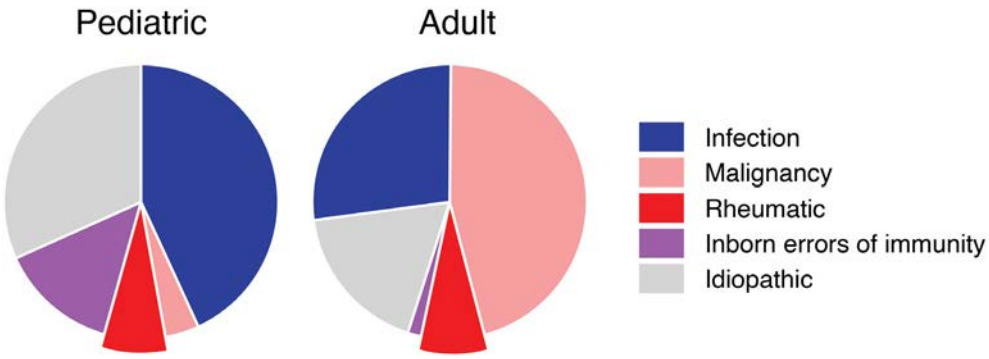

Figure 1. Causes of macrophage activation syndrome in children and adults. Data from a systemic literature search by Shakoory et al of a published series of ≥30 patients in which a single etiology was provided for each patient. Pediatric: <18 years at diagnosis, adult: ≥18 years at diagnosis. Adapted from Shakoory et al.[5]

when infected by virus, rescued by interference with IFNγ or $CD8^+$ T cells, the main source of IFNγ.[18] Interestingly, viral titers are similar in animals with and without perforin early in disease, when IFNγ levels are rising most sharply, indicating that uncontrolled viremia itself is not the main culprit; instead, perforin deficiency results in excessive antigen presentation by APCs and in persistent cytokine production from cytotoxic lymphocytes in prolonged contact with cells they are unable to kill.[19–21] Perforin also contributes to activation-induced cell death of $CD8^+$ T cells and to NK cell–mediated cytotoxic control of activated lymphocytes.[22,23] Finally, exuberant expression of the high-affinity IL-2 receptor alpha chain CD25 by activated $CD8^+$ T cells deprives regulatory

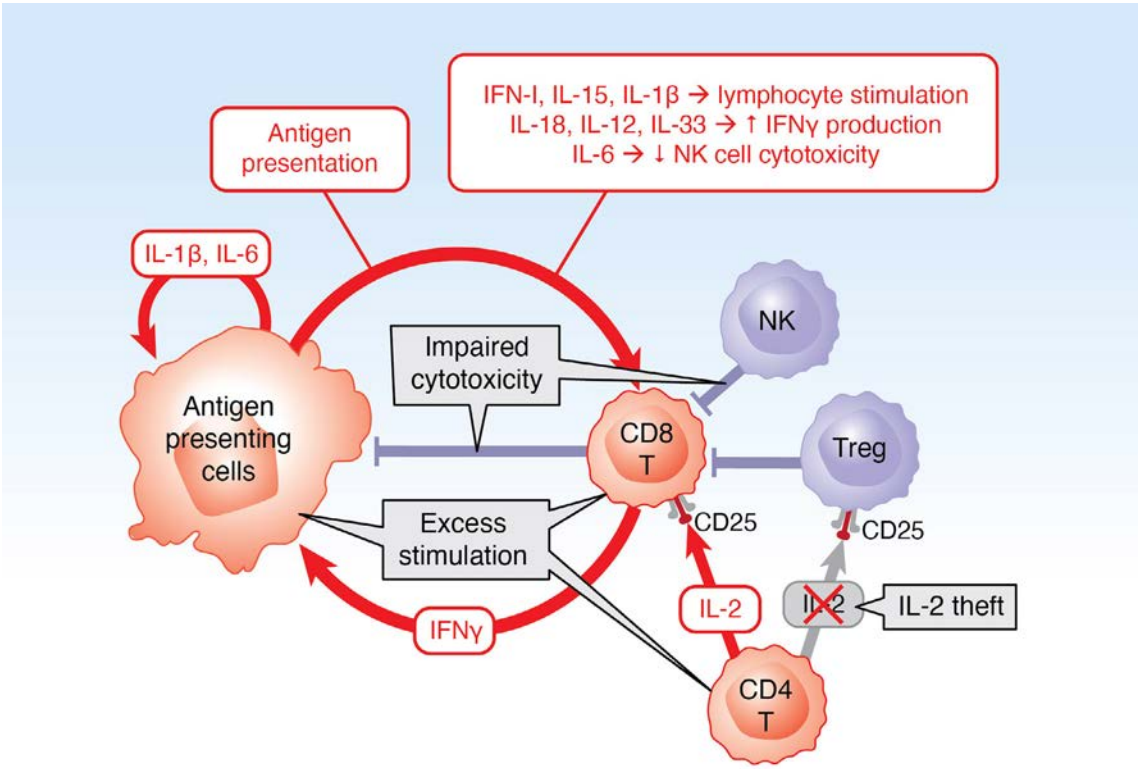

Figure 2. The vicious cycle of macrophage activation syndrome (MAS). Antigen-presenting cells (macrophages and dendritic cells) and lymphocytes (primarily $CD8^+$ T cells) form a cytokine-generating vicious cycle driven by multiple activation pathways (red arrows). Under normal conditions, this positive feedback loop is stopped by inhibitory pathways (blue), including killing of activated antigen-presenting cells and lymphocytes, Treg cells, and elimination of triggers such as viruses (not shown). In MAS, factors leading to uncontrolled activation of this vicious cycle (gray boxes) include genetic and acquired defects in cell-cell killing, excess stimulation (Still's disease activity, infection, malignancy), and "IL-2 theft" by highly activated $CD8^+$ T cells expressing the high-affinity IL-2 receptor α chain CD25. IFN, interferon; IL, interleukin. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43052/abstract.

T cells of the IL-2 they need to survive ("IL-2 theft"), reducing their numbers and contributing to Teff cell dysregulation.[24,25]

MAS can also be induced in mice by repeated administration of the Toll-like receptor ligand CpG, especially together with blockade of the immunoregulatory protein IL-10.[26] In this system, $CD8^+$ T cells are not required, demonstrating that hyperstimulation of innate immune cells can be sufficient to generate a cytokine storm.

Human data also support this explanatory model. Excess IFNγ activity, as assessed by measurement of the IFNγ-induced chemokine CXCL9, marks MAS in patients with Still's disease.[27–29] IL-18, likely from a hematopoietic source, promotes IFNγ release by T cells, in conjunction with IL-12 and IL-15; correspondingly, patients with Still's disease with high IL-18 levels are predisposed to MAS.[30,31] Exuberant T cell activation is evident in high levels of soluble CD25 (sCD25).[15] MAS is characterized not only by IFNγ (a type II IFN) but also by high levels of type I IFNs (IFN-I), a large family of cytokines including IFNα and IFNβ. IFN-I acts together with IL-15 (also elevated in MAS) to expand a population of highly activated circulating lymphocytes, termed cycling lymphocytes because they express the dividing cell marker Ki67; these lymphocytes, predominantly $CD8^+$ T cells but also $CD4^+$ T cells and NK cells, are recognizable by the surface markers CD38 and HLA-DR (MHC II) and are likely an important source of IFNγ in MAS.[32–34] The cytokine IL-33 is also elevated in both human and murine MAS, promoting T cell activation and IFNγ production.[35–37]

Therapeutic interventions support the vicious cycle model. Most informatively, blockade of IFNγ with emapalumab ameliorates both primary HLH and severe MAS associated with Still's disease, confirming a pivotal role for this mediator in human disease.[38,39] Less specific but also informative is the efficacy of interventions directed at myeloid cell activation (the IL-1 inhibitor anakinra), T cell activation (tacrolimus, cyclosporine, etoposide, JAK inhibitors), and IFN signaling, both type I and type II (JAK inhibitors) (discussed further below). Interestingly, humans lacking the IFNγ receptor and mice genetically deficient in IFNγ can still develop MAS, indicating that this cytokine, although often pivotal, is only one of multiple pathways to cytokine storm.[40]

Mechanisms downstream of these key mediators remain to be fully elucidated. One participant in this process is mechanistic target of rapamycin complex 1 (mTORC1). This protein complex assembles in response to multiple different types of signals, including nutritional status, hormones, and cytokines. Patients with Still's disease exhibit mTORC1 activation, amplified markedly during MAS, including in bone marrow hemophagocytes.[41] Drivers of mTORC1 activation include the proinflammatory cytokines IL-1β, IL-6, IFNγ, and IL-18, suggesting likely contributors to mTORC1 engagement during MAS. Interestingly, mTORC1 overactivation is sufficient to convert both murine and human monocytes into hemophagocytes. In murine models, mTORC1 blockade with rapamycin attenuates MAS, whereas mice in whom mTORC1 is constitutively activated due to deletion of its endogenous inhibitor TSC2 develop MAS spontaneously, showing that mTORC1 is both necessary and sufficient for MAS in mice.[41] Rapamycin has been employed successfully in a patient with refractory Still's disease but has yet to be reported in MAS.[42]

Diagnosis of MAS

The pathophysiologic model outlined highlights additional opportunities for diagnosis and monitoring of MAS, and to differentiate MAS from its clinical mimics. Of special value are the following.

1. **CXCL9**. A chemokine released in response to IFNγ, CXCL9 serves as a reliable measure of IFNγ activity. Typically normal even in active Still's disease, CXCL9 rises during MAS to levels ranging from a few fold to a log or more above normal.[27,28] Active MAS in the presence of a normal CXCL9 is quite unusual. The monocyte product adenosine deaminase 2 (ADA2) is a sensitive marker of IFNγ activity, with a greater dynamic range than CXCL9 and thus potentially of even better discriminative value, but is rarely available for clinical use.[28] Circulating IFNγ is elevated in MAS, but the magnitude of the change is smaller than with CXCL9, and highly sensitive tests identify measurable levels of this cytokine even in active Still's disease without MAS, limiting specificity.[27,37]
2. **IL-18**. Unlike CXCL9 and ADA2, IL-18 is often elevated in clinically inactive Still's disease, rising further with disease activity even in the absence of MAS; however, during MAS, elevation is often extreme, providing considerable sensitivity and specificity.[9,28,43] IL-18 elevation is especially characteristic of MAS in Still's disease and in a monogenic autoinflammatory disease associated with hyperactivity of the NLRC4 inflammasome; elevation is less marked, although generally still detectable, in primary HLH and in MAS associated with infection or malignancy.[43–45] Other conditions associated with high IL-18 are autoinflammatory diseases due to mutations in *CDC42*, *PSTPIP1*, *WDR1*, and *XIAP*.[30] In Still's disease, IL-18 often remains elevated even after disease control, although marked elevation is likely a marker of risk to relapse into MAS and so justifies caution in medication tapering.
3. **sCD25**. Activated T cells express and then shed CD25, the alpha chain of the IL-2 receptor that confers high sensitivity to this prosurvival and pro-proliferative cytokine. Levels of sCD25 (also called soluble IL-2 receptor) rise markedly as a reflection of T cell hyperactivation in MAS.[15] Infectious, autoimmune, and neoplastic conditions also raise the level of sCD25, limiting the specificity of this marker.[46,47]

4. **Cycling lymphocytes**. Marked elevation of $CD38^+HLA\text{-}DR^+$ cycling lymphocytes is readily identified by flow cytometry and represents a hallmark of MAS.[32–34]

Typically, the laboratory abnormalities of MAS emerge together, such that elevation in one value in the absence of others should trigger consideration of other entities. For example, isolated elevation of sCD25 can be seen in leukemia and lymphoma, whereas hyperferritinemia out of proportion to sCD25 can be observed in primary T cell immunodeficiency complicated by infection.[2,48]

In young children, primary HLH is a key consideration in patients presenting with MAS.[5] Features favoring this diagnosis, most importantly early age at onset (≤1.6 years) and low neutrophil count ($\leq 1.4 \times 10^9$/L), are summarized in the MAS/HLH score.[49] Prompt recognition of primary HLH is important because these children can deteriorate rapidly and require close management in preparation for bone marrow transplantation.[2] Certain causes of primary HLH are amenable to direct testing; for example, expression of perforin, SAP, and XIAP proteins.[2] Interestingly, genetic testing of children with Still's disease and MAS identified heterozygous variants in HLH-associated genes in 30% or more of patients (compared with 10% of children with Still's disease without MAS and healthy controls), further underscoring the similarity between these diseases and suggesting a threshold model of genetic susceptibility to MAS under inflammatory stress.[50] Genetic testing for HLH-associated gene variants is essential early in the evaluation of patients who may have primary HLH and should be considered in patients with atypical or refractory MAS.

CRP elevation is essentially ubiquitous in MAS but offers no specificity compared to clinical mimics of MAS such as Still's disease flare.[10,28] Nevertheless, CRP does meaningfully reflect systemic inflammation in patients with MAS and therefore is a useful marker to monitor improvement. Because IL-6 blockade directly antagonizes CRP release, measurement of CRP in patients receiving tocilizumab is less informative.[51]

Other tests can be useful on a case-by-case basis. Dysfunctional cell-cell killing by NK cells is evident in both primary and secondary HLH, either through an underlying genetic defect or induced transiently by IL-6.[52,53] This defect can be quantitated by examining in vitro NK cell killing of target cells and by testing expression of CD107a, a protein brought to the cell surface by perforin-containing vesicles. However, lymphopenia and the impact of glucocorticoid therapy render such tests of limited utility in many clinical contexts. Triglyceride levels rise in MAS, potentially reflecting suppression of macrophage-derived lipoprotein lipase.[54] IL-33 elevation is relatively specific for MAS among other causes of fever in children but is rarely available clinically.[37]

Although hemophagocytosis is characteristic of MAS, bone marrow biopsy is not a regular part of the diagnostic evaluation, except as necessary to rule out leukemia or other diagnoses. The reason is that both sensitivity and specificity are limited; almost 40% of bone marrow biopsy samples from patients with MAS lacked detectable hemophagocytosis, whereas patients with Still's disease can have hemophagocytes in the absence of overt MAS.[11,14] Imaging can demonstrate enlarged liver, spleen, and lymph nodes but is directed primarily at assessing end-organ involvement.

Additional testing in patients with MAS is related to alternate underlying diagnoses or disease triggers. A common trigger of MAS is viral infection, including EBV, CMV, adenovirus, HHV6/8, HIV, and SARS-CoV-2; testing for these viruses can be useful and may have therapeutic implications.[2,30] In patients with SLE and MAS, MAS is commonly part of the initial presentation, such that SLE and MAS are often diagnosed concurrently; typical SLE autoantibodies are present.[55] A peripheral blood smear can identify evidence of leukemia, including leukemic cells and teardrop red blood cells; lactate dehydrogenase and uric acid levels are nonspecific but suggestive markers of enhanced leukocyte turnover in leukemia and lymphoma. Hepatosplenic T cell lymphoma and intravascular lymphoma may require tissue biopsy for diagnosis.

Vigilance is required for patients with Still's disease receiving treatment with biologic agents, because the hallmarks of MAS may be less evident. IL-6 blockade with tocilizumab markedly attenuates CRP elevation and reduces the prevalence of fever and the level of ferritin; IL-1β blockade with canakinumab somewhat lowers ferritin but does not appear to abrogate fever.[51]

Therapeutic options in MAS

Treatment seeks to address the axes of the MAS vicious cycle (Figure 3) and is reviewed in the ACR/EULAR consensus report on MAS.[5] Key agents in routine use are as follows (please see Table 2 for details of use).

Glucocorticoids. These agents rapidly suppress inflammation, through suppression of lymphocyte proliferation, NF-κB signaling, and other mechanisms, and are the foundation of MAS treatment, indicated at diagnosis in essentially every patient, with the exception of very mild disease or stable patients awaiting lymph node or bone marrow biopsy to exclude malignancy. Typically, treatment is initiated via daily IV "pulse" therapy of methylprednisolone 30 mg/kg (maximum 1,000 mg), often for three days, followed by dosing of 1 to 2 mg/kg per day IV divided.[13] The duration of glucocorticoid therapy is variable but typically extends with taper to 4 to 8 weeks. The HLH-94 and HLH-2004 regimens for primary HLH employ dexamethasone instead of methylprednisolone.[56]

Anakinra. Use of this short-acting recombinant IL-1 receptor antagonist for MAS has not been studied in a randomized controlled trial but is supported by extensive observational

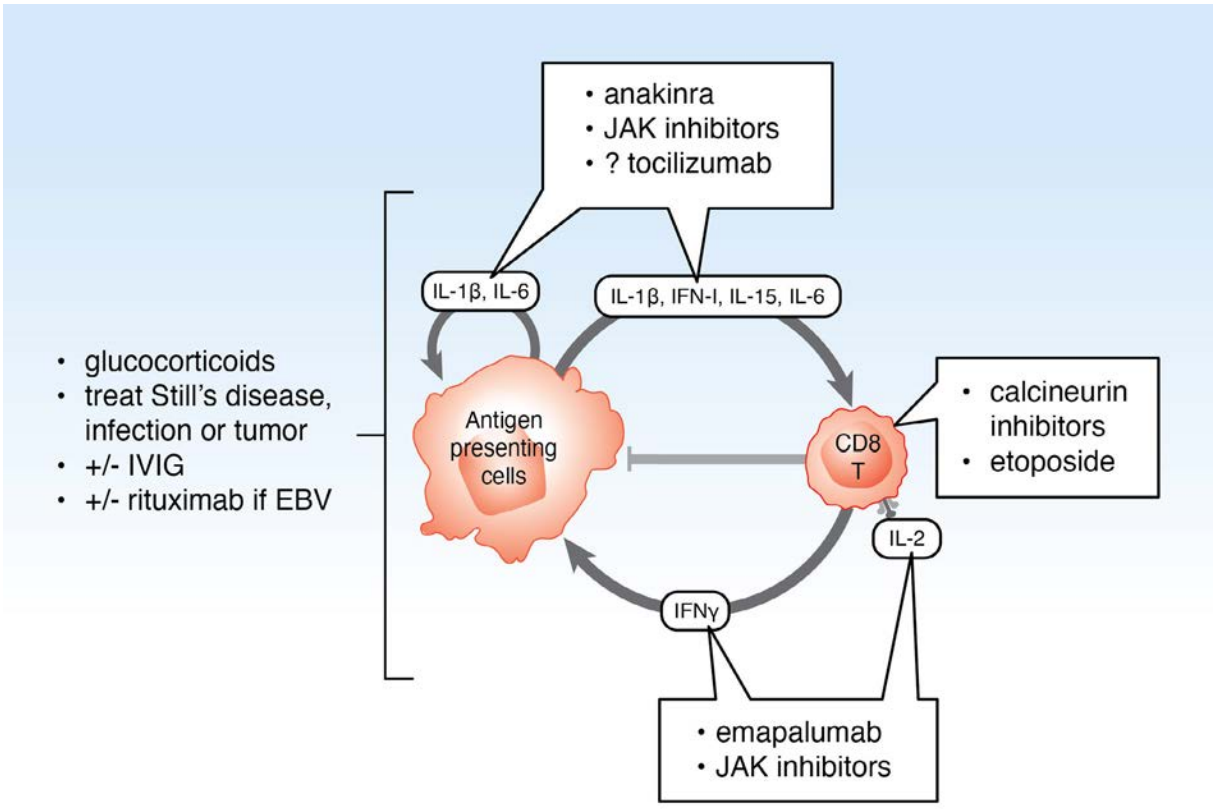

Figure 3. Blocking the vicious cycle in macrophage activation syndrome (MAS). Glucocorticoids are broadly immunosuppressive and form the foundation of treatment. Anakinra, JAK inhibitors, and tocilizumab block cytokines derived from antigen-presenting cells that stimulate T cells, including IL-1β, type I IFNs, and IL-6; JAK inhibitors also block IL-2, IL-12, and IL-15. Calcineurin inhibitors (tacrolimus and cyclosporine) reduce T cell activation and thereby production of IFNγ and IL-2, and etoposide depletes cycling lymphocytes, especially activated $CD8^+$ T cells. Emapalumab blocks IFNγ, as do JAK inhibitors. Treatment of Still's disease activity, infection, or malignancy reduces overall immunostimulation. Rituximab reduces antigen load in EBV-associated MAS. IVIG may control infectious triggers while blocking other immunostimulants or dampening inflammation via inhibitory Fc receptors, although is generally considered a weak agent against MAS. Not shown are investigational targets including IL-18, IL-33, and mechanistic target of rapamycin complex 1. EBV, Epstein-Barr virus; IFN, interferon; IL, interleukin; IVIG, intravenous immunoglobulin. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43052/abstract.

experience.[13,57] It is useful especially in MAS associated with Still's disease, given the pivotal contribution of IL-1 to systemic inflammation in this condition. Therapy of MAS typically requires elevated dosing, 5 to 10 mg/kg per day or higher, divided two to four times a day SC or (more typically) IV.[58] Although first-line anakinra monotherapy is appropriate for many cases of new-onset Still's disease, it is generally insufficient for Still's accompanied by MAS, in which combination therapy with glucocorticoids should be employed.[59] Canakinumab (monoclonal anti–IL-1β) is less useful for MAS treatment because of its long half-life, limited dose-escalation options, poorer central nervous system (CNS) penetration, and cost, although accelerated dosing has been effective in some patients with Still's disease with MAS.[60]

JAK inhibitors. The Janus kinases mediate signals from multiple pathways relevant to MAS, including IFNγ (JAK1, JAK2), IFN-I (JAK1), IL-2 (JAK1, JAK3), IL-12 (JAK2), and IL-15 (JAK1, JAK3). Correspondingly, JAK inhibitors have proved useful in MAS.[13] In mice, JAK1 inhibition was sufficient to control HLH driven by repeat injection of CpG, but viral-induced disease required both JAK1 and JAK2 blockade.[61] A study of ruxolitinib (JAK1/2 selective) as first biologic in 54 children with HLH found an overall response rate approaching 70%, with 42% achieving remission; patients with EBV-associated HLH (in the absence of EBV-induced lymphoproliferative disease) exhibited the best overall response, at approximately 80%.[62]

Calcineurin inhibitors. Tacrolimus and cyclosporine target activated T cells to inhibit proliferation and production of cytokines including IL-2 and IFNγ. These calcineurin inhibitors are often paired with glucocorticoids and anakinra. Studies in HLH found no clear benefit to adding cyclosporine to etoposide,

Table 2. Therapeutic options in MAS*

Medication	Dose	Level of evidence	Notes
Glucocorticoids	Methylprednisolone 30 mg/kg IV (max 1,000 mg) daily × 1–3 d; dexamethasone 10 mg/m^2/d or 0.3 mg/kg/d (usual max 10 mg/d) IV or by mouth; maintenance dosing: methylprednisolone, prednisolone, or prednisone 1–2 mg/kg/d IV or by mouth, typically divided every 12 h (usual max 60 mg/d)	2B	"Pulse" steroids often used at diagnosis to secure rapid control, followed by lower-dose steroids to maintain control. Dexamethasone may be advantageous for CNS disease. Taper gradually over 1–2 mo. Consider PJP and gastrointestinal prophylaxis if therapy is prolonged.
Anakinra	5–10 mg/kg/d divided every 6–12 h IV or SC; usual max 100 mg/dose	3	IL-1 antagonist. Half-life 4–6 h favors divided dosing. Canakinumab (anti–IL-1β) is an alternative but typically not preferred for MAS because of longer half-life and less flexibility in dosing.
Tacrolimus	0.1 mg/kg/d by mouth divided every 12 h	3	Calcineurin inhibitor. Tough level goal before third dose <20 ng/mL. Monitor blood pressure, electrolytes.
Cyclosporine (Sandimmune for IV; Neoral or Gengraf by mouth)	3–7 mg/kg/d by mouth divided every 12 h or 3–5 mg/kg/d IV divided ever 12 h	3	Calcineurin inhibitor. Preparations are not interchangeable. Trough level goal before third dose 50–100 ng/mL. Monitor electrolytes, magnesium, blood pressure; consider daily drug levels in setting of evolving organ dysfunction. Consider discontinuation if concern for PRES.
Ruxolitinib	5–50 mg/d or 50 mg/m^2/d (max 25 mg/dose) divided every 12 h. Typical starting regimen: ≤10kg: 2.5 mg by mouth every 12 h; 10–20 kg: 5 mg by mouth twice daily; >20 kg: 10 mg by mouth twice daily	2B	JAK inhibitor. If ruxolitinib is not available, can substitute another JAK inhibitor. Reduce dose for liver or renal impairment or if coadministered with itraconazole, voriconazole, or posaconazole.
IVIG	1 g/kg/d IV × 2 d	3	Immunomodulatory mechanism undefined; use as part of combination therapy with other agents. Monitor for fluid overload and hemolysis.
Emapalumab	6 mg/kg day 1, then 3 mg/kg every 3–4 d	2B	IFNγ antagonist. Dose and frequency may be increased up to 10 mg/kg per 3 d for inadequate response. Monitor CXCL9 to confirm functional IFNγ blockade. Tested in combination with cyclosporine and anakinra, but not other biologics. Consider antiviral (valacyclovir), antifungal (fluconazole), and PJP (eg, TMP/SMX) prophylaxis; test for tuberculosis before therapy is initiated (need not delay start unless high risk); baseline plus periodic monitoring for EBV, CMV, and adenovirus by qPCR.
Etoposide	50–150 mg/m^2 per dose 1–2 times per wk (wk 1–2) then weekly (wk 3–8)	2B	Deletes cycling lymphocytes. Should be administered with hematology/oncology supervision. Secondary malignancy risk.
Rituximab	375 mg/m^2 per dose (frequency targeted to EBV titer)	3	Delete B cells as source for EBV; for use as adjuvant therapy in EBV-triggered MAS. Follow CD19 count and EBV PCR.

* Dosing and recommendations adapted from references Halyabar et al[13] and Shakoory et al,[5] and from the Boston Children's Hospital Evidence-Based Guideline for HLH and MAS, version 31.[85] Level of evidence assigned by author as per van der Heijde et al[86]: 1A, meta-analysis of randomized controlled trials; 1B, at least one randomized controlled trial; 2A, at least one controlled study without randomization; 2B, at least one type of quasiexperimental study; 3, descriptive studies, such as comparative studies, correlation studies or case–control studies; 4, expert committee reports or opinions and/or clinical experience of respected authorities. CMV, cytomegalovirus; CNS, central nervous system; EBV, Epstein-Barr virus; IFN, interferon; IL, interleukin; IV, intravenous; IVIG, intravenous immunoglobulin; PJP, *Pneumocystis jirovecii* pneumonia; MAS, macrophage activation syndrome; PCR, polymerase chain reaction; PJP; PRES, posterior reversible encephalopathy syndrome; qPCR, quantitative PCR; TMP/SMX, trimethoprim/sulfamethoxazole.

whereas some patients experienced hypertension and related complications, such that this combination is generally avoided.[56,63]

Emapalumab. This monoclonal antibody neutralizes IFNγ. Emapalumab was effective for primary HLH refractory to conventional therapy in most patients including etoposide and dexamethasone, with a response rate of approximately 65%, leading to US Food and Drug Administration approval for this indication.[38] In Still's disease–associated MAS refractory to high-dose glucocorticoids with or without anakinra and cyclosporine, emapalumab controlled disease in 13 of 14 patients enrolled in a single-arm clinical trial.[39] Comparable efficacy was reported in real-world observational data.[64] Interestingly, in patients taking anakinra in whom this therapy was withdrawn upon initiation of emapalumab, Still's disease tended to flare; we therefore generally continue both agents together. Given the key role of IFNγ in immune defense, patients treated with emapalumab receive concomitant antiviral, antifungal, and anti–*Pneumocystis jirovecii* pneumonia prophylaxis. Emapalumab is cleared much more rapidly when levels of IFNγ are high (half-life 3 days vs 24 days with low levels), such that more frequent dosing may be required in some patients, especially early in MAS.[29,39] Cost remains an important limiting factor for the use of this agent; at Boston Children's Hospital, the current hospital charge for a 28-day course of emapalumab for a 20-kg child is approximately $1.5 million.

Etoposide. Etoposide is a cytotoxic agent that binds DNA topoisomerase II to induce double-strand breaks, triggering apoptosis during mitosis. In murine HLH, etoposide selectively depletes activated T cells, likely explaining its utility.[65] Etoposide has long been standard of care in primary HLH, contributing to the marked improvement in survival in this previously almost uniformly fatal condition.[56] In MAS associated with rheumatic diseases, etoposide is typically reserved for rescue therapy, principally because of concern over secondary malignancies, although this rate is fortunately low (~0.5% at five years).[56]

Other agents can be useful for MAS under some circumstances. MAS associated with EBV infection may benefit from rituximab to deplete EBV-infected B cells and thereby reduce circulating viremia.[66] The efficacy of intravenous immunoglobulin (IVIG) is supported by small uncontrolled series, although its mechanism of action in MAS is unclear, and experience suggests that it is unlikely to be particularly potent.[67–69] IL-18 blockade is not commercially available but can be considered on a compassionate-use basis (ClinicalTrials.gov identifier: NCT04641442, testing the bispecific IL-1β/IL-18 monoclonal antibody MAS825 in patients with autoinflammation due to mutations in *NLRC4* or *XIAP*).[70,71]

The role of IL-6 blockade in MAS treatment is not defined. Although effective in Still's disease without MAS, tocilizumab does not clearly reduce the incidence of MAS (as is the case also for canakinumab).[72,73] Levels of IL-6 in the blood do not distinguish active Still's disease from Still's–associated MAS, and indeed high IL-6 levels often correlate with a course of Still's disease with more arthritis and less MAS.[31,74] Yet there are clear reasons to expect a role for IL-6 in MAS, including improvement in NK cell cytotoxic function with IL-6 blockade and a murine model of IFNγ-dependent MAS that arises through IL-6 excess.[53,75] Tocilizumab is effective in inflammation associated with CAR-T cell therapy or SARS-CoV-2 infection, although neither of these states closely resembles Still's disease–associated MAS.[76] Correspondingly, tocilizumab has been used for MAS.[69,77] We do not typically employ tocilizumab for this indication, given limited evidentiary support, interference with interpretation of CRP and ferritin, and concerns over compromised antibacterial defense; yet no direct evidence underlie this reluctance.[13,51]

Just as control of Still's disease activity contributes to treatment of MAS, so treatment of underlying infection and/or malignancy is critical in patients with MAS associated with these conditions, presumably to reduce antigenic load. Successful treatment of MAS without effective therapy of the underlying disease is uncommon, likely accounting for the high mortality of MAS associated with cancer.[16,17]

Choosing treatment and monitoring response

Treatment of MAS is not standardized. Intensity of therapy is generally proportionate to illness severity. The vicious cycle depicted in Figure 2 confers upon MAS the propensity to intensify rapidly. It is important for the clinician not to be reassured if the patient appears relatively well, or to mistake "normal" platelet count or falling ESR as evidence of improvement. Primary HLH is typically managed by hematology/oncology specialists using etoposide, but this agent is generally not indicated for MAS related to Still's disease unless other options have failed.

An algorithm for treatment of MAS in suspected Still's disease is provided in Figure 4, with drug dosing and monitoring detailed in Table 2. These guidelines incorporate input from MAS experts at Boston Children's Hospital and beyond[5,13] but reflect the author's current approach rather than consensus recommendations and are not intended to define standard of care. We begin with pulse-dose methylprednisolone together with IV anakinra. Anakinra may be used alone in some patients with mild MAS under close monitoring, especially if glucocorticoid use would complicate ongoing evaluation for hematologic malignancy. We add ruxolitinib as a third agent if the response is not brisk. If control remains incomplete but the patient is stable, we add tacrolimus, monitoring blood pressure and drug levels carefully; cyclosporine is an alternative, although we tend to choose tacrolimus based on side effect profile. IVIG may be added at any time as adjuvant therapy, with its principal advantage being lack of immunosuppression, although experience suggests IVIG contributes only marginally to response. Rituximab may contribute to

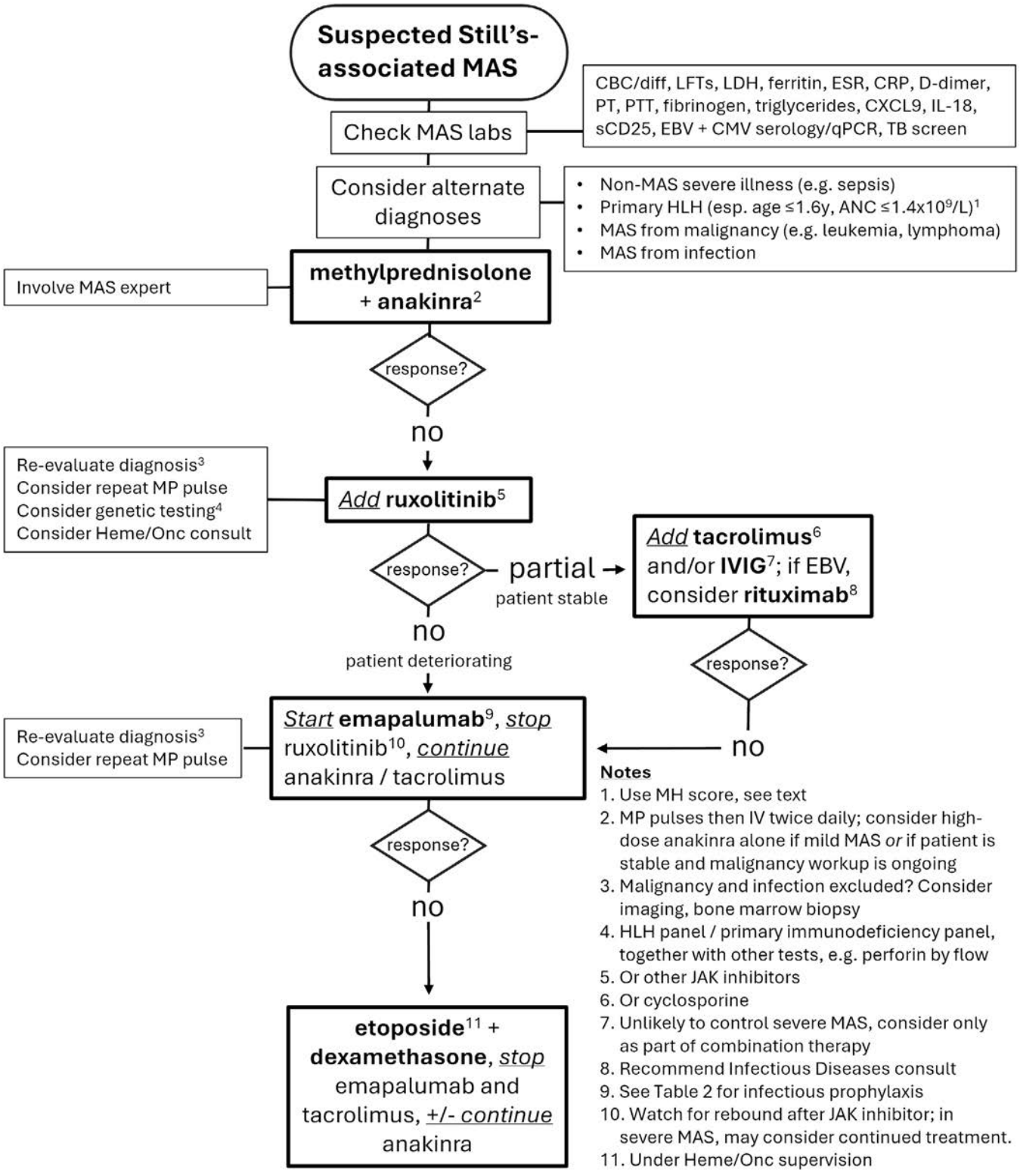

Figure 4. Treatment algorithm for suspected Still's-associated MAS. Supporting data for this order of treatment are very limited; the algorithm thus reflects expert opinion not standard of care. The duration of each step will vary with the patient's clinical condition. Biomarkers should be followed closely throughout. See Table 2 for dosing and toxicity monitoring, and see text for rationale, recommended laboratory monitoring schedule, and evidence base. ANC, absolute neutrophil count; CBC, complete blood cell count; CMV, cytomegalovirus; CRP, C-reactive protein; EBV, Epstein-Barr virus; ESR, erythrocyte sedimentation rate; Heme/Onc, hematology/oncology; HLH, hemophagocytic lymphohistiocytosis; IL, interleukin; IV, intravenous; IVIG, IV immunoglobulin; LDH, lactate dehydrogenase; LFT, liver function test; MAS, macrophage activation syndrome; MP, methylprednisolone; PT, prothrombin time; PTT, partial thromboplastin time; qPCR, quantitative polymerase chain reaction; sCD25, soluble CD25; TB, tuberculosis.

treatment of EBV-triggered MAS by reducing antigen load. If disease continues to worsen, we proceed to emapalumab, adding this therapy to ongoing high-dose IV steroids, anakinra, and/or calcineurin inhibitor. Combination therapy with emapalumab and JAK inhibition has not been reported in humans, and its safety is unknown, although this strategy is effective in mice and could be considered for very severe disease.[78] If emapalumab is not available or ineffective, we proceed with etoposide, typically switching from methylprednisolone to dexamethasone in conformity with the HLH-2004 regimen and for possible benefit with respect to CNS penetration.[56] For continued refractory disease, allogenic bone marrow transplantation may be considered, especially for patients in whom genetic testing shows an associated HLH variant.[64] Glucocorticoids are continued throughout, and repeat pulses may be helpful if deterioration continues. Importantly, the order of treatments proposed is based on experience rather than data. Choices are often driven by drug availability and cost; for example, emapalumab is better supported by efficacy data than some other options and could arguably be considered earlier in the algorithm but for its prohibitive cost profile. If anakinra were not available, we would employ pulse glucocorticoids and ruxolitinib as initial treatment. Infectious diseases consultation can help guide antimicrobial prophylaxis, especially in patients taking emapalumab or taking combination immunosuppressants, with attention to drug–drug interactions (eg, ruxolitinib with azole antifungals).

Therapeutic response monitoring is critical. As MAS improves, fever and hypotension abate, ferritin and D-dimer levels fall, platelets and fibrinogen rise, and transaminases normalize. These variables should be tracked daily at the start of treatment, adjusting therapy as needed to ensure continued improvement; specialized testing (CXCL9, IL-18, sCD25) is commonly obtained twice weekly. Although initial evidence of response is typically evident within days of initiating effective therapy, for severe MAS, improvement may lag by several weeks. Ferritin, transaminases, and platelet count respond most rapidly, whereas CXCL9 and sCD25 normalize more slowly.[39,62] IL-18 improves even more slowly and commonly remains elevated in patients with Still's disease without MAS.[28]

MAS of the CNS

A particularly difficult diagnostic challenge is MAS affecting the CNS. In a series of 193 patients with primary HLH, 37% of patients had seizures, meningitis, or mental status changes; 52% had abnormal cerebrospinal fluid (CSF) findings; and 15% of survivors had neurologic sequelae including development delay and epilepsy.[79] CNS involvement can occur even in the absence of systemic HLH, posing a major diagnostic challenge.[56] Such cases typically are diagnosed by a combination of clinical features, imaging abnormalities, testing for genetic causes of HLH, and/or biopsy, where findings include meningeal infiltration, perivascular lymphocytic infiltrates, and often (but not invariably) hemophagocytes.

In Still's disease–associated MAS, one-third of patients exhibit CNS manifestations, with seizures in 9%.[11] The CSF may reveal evidence of macrophage activation; often measured in this context is neopterin, a metabolite released by macrophages exposed to IFNγ and elevated also in the peripheral blood of patients with MAS, although with less discriminative value than other markers.[74] CNS MAS is treated similarly to peripheral MAS; dexamethasone may be favored over other glucocorticoids because of superior CNS penetration, although at the high doses of methylprednisolone employed in MAS, any difference is likely small.[80] In contrast to primary HLH, in Still's disease–associated MAS, intrathecal chemotherapy is rarely (if ever) required, and isolated CNS MAS has not been reported. CNS MAS must be distinguished from thrombotic or hemorrhagic stroke from DIC, venous sinus thrombosis, infection, malignancy, and posterior reversible encephalopathy syndrome (PRES), a failure of intracerebral vasoregulation leading to intracerebral edema and tissue compromise that—despite the name—is neither invariably posterior nor always fully reversible. Risk factors for PRES include hypertension and renal dysfunction; calcineurin inhibitors have been implicated as possible triggers, although the relationship remains uncertain; concern over PRES as well as efficacy underlie our preference for ruxolitinib over calcineurin inhibitors for patients who do not respond briskly to glucocorticoids and anakinra.[63]

Still's disease–associated lung disease

Recurrent MAS and high levels of the MAS-associated cytokine IL-18 are risk factors for the development of a chronic inflammatory and fibrotic lung disease termed Still's disease–associated lung disease.[81] Disease onset before age two is a further risk factor, although adults may also be affected. Concern over a causal role for biologics has led some physicians and patients to withdraw therapy, but this strategy can precipitate MAS and is not generally recommended.[4] Awareness of Still's disease–associated lung disease is important because pre-existing undiagnosed disease could complicate MAS management through reduced pulmonary reserve and pulmonary hypertension. Patients with Still's disease who have had MAS should be monitored for associated lung disease, especially for patients in whom Still's disease began early in childhood.[82,83]

Prognosis

MAS is a dangerous condition, with appreciable mortality. In the largest series of MAS associated with pediatric Still's disease, one-third of patients required intensive care unit admission, and 28 of 347 (8%) died.[11] MAS associated with malignancy typically has a much poorer prognosis, with all-cause mortality often exceeding 50%.[16,17,57] Early recognition and treatment of MAS

benefits from a coordinated approach among hospitalists and consulting services, leading to improved outcomes and reduced mortality.[84] Patients who have had one episode of MAS are at risk for further episodes, such that vigilant monitoring is essential, especially during medication changes and infectious illness. Note that we continue anakinra therapy through stressors such as infection or surgery, both because anakinra typically does not increase infection risk and because abrupt discontinuation can precipitate MAS, especially during stress; discontinuation of JAK inhibitors can similarly risk MAS rebound, requiring case-by-case decision-making in the face of infection or surgery.

In conclusion, MAS is a clinical syndrome associated with Still's disease but also SLE, Kawasaki disease, infection, malignancy, and inborn errors of immunity. Considerable advances have been made in understanding its pathogenesis, facilitating prompt recognition and improved management, although treating children and adults with MAS remains a major clinical challenge.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Nigrovic confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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EDITORIAL

Understanding Late-Onset Interstitial Lung Disease in Systemic Sclerosis: Implications for Clinical Practice and Trial Design

Elizabeth R. Volkmann

As a leading cause of death in systemic sclerosis (SSc), interstitial lung disease (ILD) affects the majority of patients with SSc.[1] Historical studies demonstrated that ILD presents early in the course of SSc and progresses most rapidly during the first 2 years of the illness.[2] Later observational studies found that among patients with SSc without ILD detected on initial high-resolution computed tomography (HRCT) of the chest, the risk of developing ILD during a mean follow-up of 3.1 years was 0%.[3] Based on these and other studies, many health care providers routinely counsel patients that they need not "worry" about ILD if their initial ILD screening tests are negative or if their ILD does not progress (ie, worsen) significantly within the first 5 years of their illness.

However, our understanding of SSc-ILD onset and progression has evolved in recent years. In this issue of *Arthritis and Rheumatology*, Hoa and colleagues present compelling evidence that ILD can present later in the SSc disease course (ie, after 7 years from the onset of the first non-Raynaud symptom).[4] Through examining nearly 1,000 patients with SSc enrolled in the Canadian Scleroderma Research Group from 2004 to 2020 *without* prevalent ILD, the authors demonstrated that 21% of patients developed incident ILD over a median follow-up of only 2.4 years. They also demonstrated that risk factors for later-onset ILD were consistent with risk factors for earlier-onset ILD (eg, male sex, non-White race, diffuse cutaneous disease, arthritis, myositis, antitopoisomerase I autoantibodies, and higher C-reactive protein levels). Taken together, the findings of this multicenter study, which included 14 sites in Canada and 1 site in Mexico, suggest that surveillance for ILD should continue regardless of SSc disease duration.

One important caveat of this study is that HRCT of the chest was not performed in all patients at the time of SSc diagnosis.[4] During the study period, HRCTs were most often ordered in the presence of risk factors for ILD, symptoms, radiologic abnormalities on chest x-ray, or physiologic abnormalities on pulmonary function tests (PFTs). Studies have demonstrated that neither chest x-rays[5] nor PFTs[6] are sensitive ILD screening methods, particularly early in the SSc-ILD disease course. Moreover, many patients with early SSc-ILD report no respiratory symptoms, and studies have demonstrated that asymptomatic patients experience ILD progression at the same rate as those patients who report respiratory symptoms.[7] Therefore, it is possible that a proportion of the later-onset ILD cases represented missed ILD diagnoses because of lack of uniform HRCT screening at the time of SSc diagnosis. Indeed, when sensitivity analyses were performed, excluding patients who did not have prior HRCT assessment indicating the absence of ILD, the incidence of later-onset ILD was lower.[4]

However, because of access/cost barriers and/or concerns about radiation exposure, HRCT of the chest is not always routinely performed on patients with SSc to screen for ILD. In the 2023 American College of Rheumatology/American College of Chest Physicians Guideline for screening and monitoring of ILD in people with systemic autoimmune rheumatic diseases (SARDs), the authors conditionally recommended screening with HRCT chest over history and physical examination or PFTs alone among patients with SARDs at increased risk of developing ILD.[8] The guideline did not make screening recommendations for patients with SARDs who were not at increased risk of developing ILD. Thus, the clinical practice standards of the present study were in line with the recently published guidelines, and the study findings may be most generalizable to populations in which HRCT of the chest is only performed when risk factors for ILD are present.

Another striking discovery of this study was that ILD progression rates were similar for patients with later-onset and earlier-onset ILD (adjusted hazards ratio of 1.11; 95% confidence

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interval 0.58–2.10).[4] Nearly half of all patients in the later-onset and earlier-onset ILD groups experienced ILD progression over a median follow-up duration of 3.1 years. In this study, ILD progression was defined as a ≥10% relative decline in percentage-predicted forced vital capacity (FVC), or a ≥5% to <10% relative decline in percentage-predicted FVC combined with a ≥15% relative decline in percentage predicted the diffuse capacity for carbon monoxide (DLco).

The aforementioned findings not only have implications for clinical practice, but they also have consequences for clinical trial design. Most clinical trials for SSc-ILD restrict enrollment to individuals with a disease duration of SSc less than 5 to 7 years from the onset of the first non-Raynaud symptom of SSc.[9] This study calls into question this commonly used entry criterion, which is severely limiting the diversity of our clinical trial populations.[10] These findings are also consistent with recent reports demonstrating similar ILD progression rates (using the same physiologic definition of ILD progression as the present study) among patients with SSc-ILD with a disease duration less than or equal to 3 years, between 3 and 7 years, between 7 and 15 years, and those with a disease duration of more than 15 years.[11] This emerging research should inspire efforts to reconsider inclusion criteria for SSc-ILD trials to ensure that enrichment strategies are grounded in solid science and not based solely on "expert opinion."

In an exploratory analysis on immunosuppressive drug exposure, Hoa and colleagues found that a minority (9%) of person visits were exposed to immunosuppressive drugs.[4] Acknowledging that a subset of patients with SSc-ILD experience progressive pulmonary fibrosis without treatment (ie, a recent study demonstrated that 40% of untreated SSc-ILD experienced ILD progression in a 3-year follow-up period[12]), this finding is somewhat surprising given the accumulating evidence that the therapies of interest in this study (eg, mycophenolate mofetil, cyclophosphamide, tocilizumab, rituximab) have each been found to favorably modify the course of FVC in SSc.[13] Exposed person visits to immunosuppressive drugs occurred more commonly in patients with earlier-onset ILD compared with later-onset ILD (35% vs 61%, respectively), even though patients in each subgroup had a similar FVC and DLco at the time of their ILD diagnosis.[4] Because of the relatively small proportion of patients who received treatment with immunosuppressive drugs in this cohort, there was inadequate power to detect significant differences in treatment-related lung disease progression between the earlier-onset and later-onset ILD groups. To fully understand the efficacy of the currently available and emerging therapies for SSc-ILD, future clinical trials are needed that include patients with longer SSc disease duration.

What can we learn from Hoa and colleagues' study? Routine surveillance for ILD should occur even after the initial screening for ILD is negative. Surveillance for ILD should also continue for patients living with SSc for more than 7 years. In a recent post-hoc analysis of the European Scleroderma Trials and Research group cohort, the annual incidence of new-onset ILD

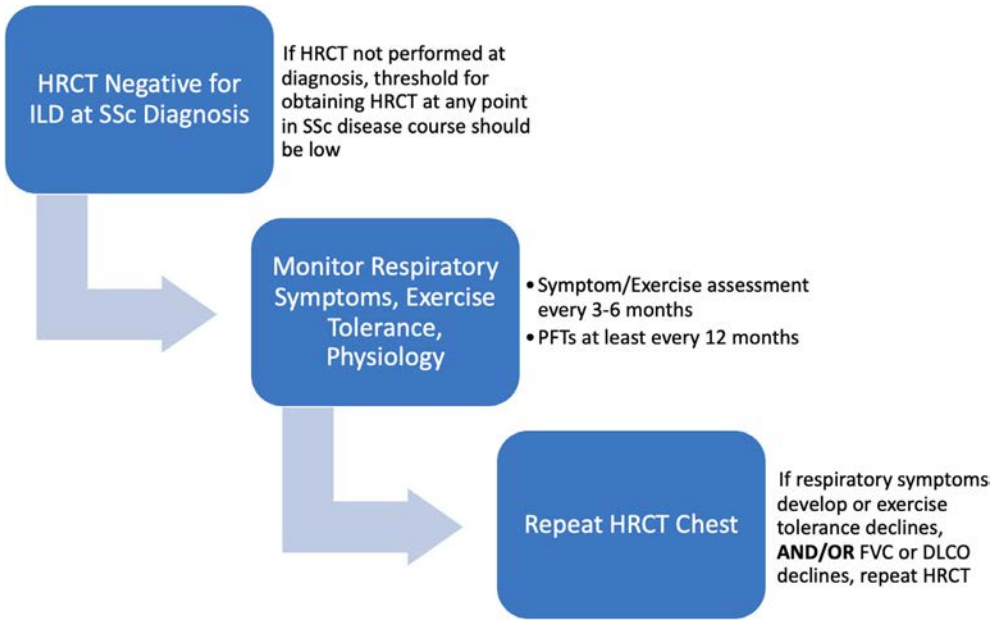

Figure 1. Proposed algorithm for screening for ILD in patients with SSc who initially had no evidence of ILD on HRCT at the time of SSc diagnosis. Although no valid exercise assessment measures exist in SSc-ILD, one may consider tracking average daily step count, as many patients with early SSc-ILD unconsciously modify their lifestyle to avoid provoking symptoms that arise with activity (personal opinion). Interval of PFT assessments should be tailored to the individual; if risk factors for ILD are present (eg, diffuse cutaneous disease, anti-Scl-70 antibody positivity), PFTs should be performed more frequently. DLco, diffuse capacity for carbon monoxide; FVC, forced vital capacity; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; PFT, pulmonary function test; SSc, systemic sclerosis.

was similar for patients with a disease duration less than or equal to 5 years and those with a disease duration between 5 and 10 years.[14] Figure 1 describes a proposed algorithm for ILD surveillance in patients with SSc with no evidence of ILD on HRCT at the time of SSc diagnosis. Timely diagnosis of ILD ensures early therapeutic intervention, prompt referral to other specialists (eg, pulmonologists), as well as closer monitoring for ILD progression. These three factors may improve survival for patients with SSc-ILD, particularly because multiple studies have demonstrated that early progression of SSc-ILD is associated diminished long-term survival.[15,16]

The work of Hoa and colleagues has enlightened our understanding of later-onset ILD in patients with SSc. We owe it to our patients who live with this condition to apply the lessons learned from this research to improve how we care for patients and how we design studies to investigate novel therapies for SSc-ILD.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Volkmann confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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NOTES FROM THE FIELD

Recommendations for Aligned Nomenclature of Peripheral Nervous System Disorders Across Rheumatology and Neurology

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Introduction

Effective multidisciplinary care for patients with complex conditions such as Sjögren's disease (SjD) is often hindered by inconsistent nomenclature across medical specialties. The Sjögren's Foundation guidelines development panel encountered this challenge when rheumatologists and neurologists came together to formulate guidelines for the care of patients with peripheral nervous system (PNS) disorders with SjD. To address this barrier, a standardized nomenclature was defined to improve communication across specialties for patient care and collaboration in implementing evidence-based medicine.

Evidence-based clinical practice guidelines

Since its emergence in the 1990s, evidence-based medicine has integrated the latest research findings into clinical practice by combining the best available evidence with clinicians' expertise and patients' needs and preferences, making evidence-based guidelines essential resources for informed clinical decisions, especially in areas in which data may be lacking or insufficient.[1,2] Central to the development of evidence-based clinical practice guidelines (CPGs) is the concept of implementability, which emphasizes creating guidelines that are practical and straightforward to implement.[3] Factors influencing a guideline's uptake include its intrinsic implementability, defined by characteristics that predict and promote its use in health care systems.[4,5] Language and nomenclature play a significant role; vague and unclear CPGs can hinder implementation, whereas unambiguous ones can enhance it.[5] A guideline's intrinsic implementability is particularly challenging when recommendations require alignment of language across multiple medical specialties. Mutual understanding across specialties is crucial for providing care to patients with complex conditions involving comorbidities or multisystem manifestations. SjD exemplifies this challenge.

PNS manifestations of SjD

SjD is a systemic autoimmune disease in which almost all body organs can be affected, including the PNS. In addition, SjD is characterized by exocrine gland dysfunction, resulting in dry eyes and dry mouth.[6] PNS involvement in SjD significantly impacts patient quality of life,[7] and a coordinated approach

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among different subspecialties is essential in the care of these patients.[8]

PNS involvement in SjD frequently poses significant diagnostic challenges to clinicians: it is often the first manifestation of the disease, preceding SjD diagnosis.[9–11] Certain patterns of PNS involvement, such as small fiber neuropathy (SFN), are more commonly observed in patients who are seronegative (ie, those who are negative for anti-SSA),[12] in whom the diagnosis cannot be established without a positive minor salivary gland biopsy (MSGB). This can be a diagnostic challenge in practices in which an MSGB is not routinely performed as part of the clinical evaluation.

In addition to the challenge of establishing the diagnosis of SjD, there is the challenge of characterizing the nature of PNS dysfunction. Neuropathies can be classified based on a variety of features: symptomology (ataxic or nonataxic, painful or nonpainful), anatomic pattern of involvement (polyneuropathy vs mononeuropathy vs multiple mononeuropathies, length dependent vs non–length dependent), anatomic localization (peripheral nerve, nerve root, dorsal root ganglia), nerve fiber type affected (large fiber vs small fiber, sensory and/or motor, autonomic), electrophysiology (axonal vs demyelinating), or pathophysiology/etiology (vasculitic, toxic, immune mediated). This has resulted in heterogeneous classification systems and nonuniform nomenclature that interfere with communication in the clinical care of patients and in apprising the literature, as outlined in Table 1.

Regardless of the classification method used, a basic knowledge of anatomy and pathophysiology is essential to understand the spectrum of clinical presentations of neuropathies. Damage to or dysfunction of large nerve fibers (myelinated Aβ axons mediating the sensations of proprioception, vibration, and touch) typically leads to paresthesia and ataxia. Motor nerve involvement leads to clinical or subclinical muscle weakness. Damage to the small nerve fibers (ie, thinly myelinated Aδ and unmyelinated C fibers) leads to SFN, which affects pain and temperature sensations. Therefore, SFN causes sensory symptoms, usually pain, numbness, and tingling but not muscle weakness or ataxia.

Table 1. Examples of disparate neuropathy classification in Sjögren's Disease

Study	Neuropathy patterns identified	Comments

Delalande 2004: 51 patients with SjD with peripheral nervous system involvement (more than one pattern observed in some patients)[11]	• Distal axonal sensorimotor neuropathy (19 patients)
	• Pure sensory neuropathy (nine patients)
	  ○ Symmetric axonal sensory neuropathy without motor involvement (five patients)
	  ○ Ganglionopathy with severe ataxia (four patients)
	• Multiple mononeuropathy (seven patients)
	• Chronic polyradiculoneuropathy (myeloradiculitis; one patient)
	• Cranial neuropathy (16 patients)
		The first group with 19 patients had predominantly sensory symptoms (ie, the "motor" component was observed in electromyographic studies but was not clinically apparent).

Terrier 2007: 40 patients with SjD with neuropathy who underwent muscle and nerve biopsies[13]	Classification based on types of neuropathy: • Polyneuropathy (25 patients) • Multiple mononeuropathy (11 patients) • Ganglionopathy (five patients) • Trigeminal neuropathy (one patient) Classification based on symptoms: • Pure superficial sensory • Profound with or without superficial sensory • Sensorimotor patterns Classification based on electromyographic patterns: • Sensorimotor involvement • Axonal impairment • Axonal and demyelinating impairment	Different strategies to classify these patients were used based on anatomy, symptoms, and electromyographic findings.
Sireesha 2019: 21 patients with SjD with peripheral nervous system involvement[9]	• Mononeuritis multiplex (mononeuropathy multiplex; seven patients) • Ganglionopathy with sensory ataxia (four patients) • Length-dependent sensorimotor neuropathy (two patients) • Painful small fiber neuropathy (one patient) • Autonomic neuropathy (two patients) • Trigeminal neuropathy (two patients) • Cranial neuropathy (two patients)	• Authors described different "phenotypic patterns of neuropathy." • Unexpectedly, mononeuritis multiplex was the most common phenotype observed.
Mori 2005: 92 patients with SjD with associated neuropathy[10]	• Sensory ataxic neuropathy (36 patients) • Painful sensory neuropathy without sensory ataxia (18 patients) • Multiple mononeuropathy (11 patients) • Radiculoneuropathy (four patients) • Autonomic neuropathy (three patients) • Trigeminal neuropathy (15 patients) • Multiple cranial neuropathy (five patients)	• The term "sensorimotor polyneuropathy" was not used in this series despite common usage in related literature. • The term ganglionopathy was not used in classification but was discussed in detail in the Discussion section.

Table 2. Peripheral neuropathies in Sjögren's disease*

Neuropathy type	Description
Mononeuropathy	
Other nomenclature	Neuropathy; focal neuropathy
Definition	Mononeuropathy refers to dysfunction or disorder of a single nerve. This is in contrast to the more diffuse dysfunction seen in polyneuropathy.
Symmetry	Involvement of only one nerve would result in an asymmetric presentation.
Presentation	Patients will present with sensory and/or motor symptoms and signs in the distribution of a single nerve. Sensory symptoms can include negative (numbness) or positive (tingling paresthesias or pins and needles) symptoms or neuropathic pain. Motor symptoms would be weakness or loss of muscle bulk. On examination, sensory loss should be restricted to the cutaneous distribution of the single nerve, and weakness or atrophy should be found in muscles innervated by the affected nerve. If the nerve mediates a deep tendon reflex, that reflex may be reduced or absent. A Tinel sign may be elicited by tapping on the affected nerve, which would result in electric or pins and needles paresthesias in the cutaneous distribution of the nerve. Signs and symptoms can affect the face if a cranial nerve, such as the facial or trigeminal, is involved.
Etiologies/differential diagnosis	Mononeuropathy can be mechanical due to compression or entrapment, such as in median neuropathy at the wrist seen in carpal tunnel syndrome, which is the most common mononeuropathy.[14] Infection, inflammation, trauma, ischemia, or vasculitis are other etiologies.
Pathophysiology	This varies based on the etiology and can be axonal or demyelinating.
Evaluation	Electrodiagnostic studies (nerve conduction studies [NCS] and electromyography [EMG]) can confirm the clinical suspicion of a mononeuropathy and help to localize the lesion along the course of the nerve. Imaging, with ultrasound or magnetic resonance imaging (MRI), may be helpful to evaluate for a structural cause.
Large fiber (axonal) neuropathy	
Other nomenclature	Sensory polyneuropathy (pure sensory axonal neuropathy of the distal nerves); sensory motor polyneuropathy (axonal sensorimotor polyneuropathy)
Definition	Large fiber neuropathy is a peripheral neuropathy primarily affecting sensory nerves. Motor nerves, which are also large fiber, can be involved, resulting in a sensory and motor (or sensorimotor) polyneuropathy. In large fiber neuropathy, there is dysfunction of Aβ fibers, which are myelinated fibers involved in proprioception, vibration, and touch sensations. Motor neuropathies or motor neuronopathies can rarely be seen in Sjögren's disease.
Symmetry	Usually symmetric[15]
Presentation	Large fiber neuropathy results from the dysfunction or damage of Aβ fibers, which mediate the sensations of proprioception, vibration, and touch. Abnormal proprioception may result in problems with balance and an ataxic gait (wide-based, unsteady).
Etiologies/differential diagnosis	Large fiber neuropathy may occur idiopathically or due to immune-mediated, metabolic, hereditary, infectious, or toxic etiologies.
Pathophysiology	Damage to Aβ axons
Evaluation	Electrodiagnostic studies (NCSs and EMG) should be performed. Consider the following: • Fat pad biopsy can assess for an amyloid or other infiltrative process. • Nerve biopsy can assess for vasculitis, neoplasm, amyloid, or other infiltrative process if the index of suspicion is high for these processes. • Lumbar puncture should be reserved for assessing suspected cases of inflammatory demyelinating polyradiculoneuropathy (including AIDP and CIDP), ganglionopathy, neoplastic diseases, or infection.
Small fiber neuropathy[16]	
Other nomenclature	Small fiber polyneuropathy; small fiber sensory neuropathy
Definition	Small fiber neuropathy is a peripheral neuropathy affecting small nerve fibers—thinly myelinated Aδ and unmyelinated C nerve fibers.
Symmetry	Small fiber neuropathy is usually symmetric and length dependent but can present in a patchy or asymmetric manner.[15]
Presentation	Small fiber neuropathy typically presents with pain, burning, numbness, and tingling in a stocking-glove distribution. Symptoms usually begin starting in the feet and can ascend. Examination will show diminished pain and temperature sensations in the distal limbs. Less frequently, there can be early proximal or patchy evolution. Because large fibers that mediate proprioceptive (balance) and motor functions are not involved, these patients should not have ataxia or muscle weakness.[17] Fibers of the peripheral autonomic nervous system are also small caliber fibers, and small fiber neuropathy can affect these autonomic fibers, leading to autonomic dysfunction. This will result in autonomic symptoms that otherwise can be difficult to localize.[18]
Etiologies/differential diagnosis	A clear etiologic explanation is commonly not identified even after extensive laboratory testing. However, testing is aimed at uncovering potential immune-mediated, metabolic, hereditary, infectious, or toxic etiologies.
Pathophysiology	Small fiber neuropathy is due to either dysfunction or loss of small Aδ and C fibers. These fibers convey pain and temperature sensations. Injury thus results in either sensory loss/impairment or

(Continued)

Table 2. (*Cont'd*)

Neuropathy type	Description
	abnormal sensation. Mechanisms resulting in painless sensory loss vs painful abnormal sensation are an area of ongoing investigation. Distal axonal loss (length dependent) or proximal dorsal root ganglion degeneration (non–length dependent) can both lead to small fiber neuropathy symptoms.
Evaluation	The case definition or diagnostic criteria of small fiber neuropathy is not settled. Electrodiagnostic studies (NCSs and EMG) are helpful to exclude subclinical large fiber involvement. Skin biopsy is helpful to demonstrate a reduction in intradermal nerve fiber density compared to reference populations.[19] The test result provides a statistical diagnosis but does not assess small fiber nerve function. There are a number of other neurophysiologic test modalities, such as quantitative sensory testing, quantitative sudomotor axon reflex test (QSART), and sympathetic skin response, which are used to assess small fiber function, each with advantages and disadvantages.
Demyelinating polyradiculoneuropathy[20]	
Other nomenclature	Demyelinating polyneuropathy (including chronic inflammatory demyelinating polyradiculoneuropathy [CIDP])
Definition	Demyelinating polyradiculoneuropathy is a polyneuropathy that occurs with damage to the myelin sheath. A polyradiculoneuropathy indicates impairment at the level of sensory and motor roots as well as their distal peripheral nerve segments.
Symmetry	Demyelinating polyradiculoneuropathy may be symmetric but can be focal or multifocal.[15]
Presentation	The presentation can be progressive or relapsing and remitting. Sensory symptoms can include numbness, burning pain, throbbing, or dysesthesias. Motor complaints are weakness and loss of muscle bulk. Characteristic features of the examination include motor findings of weakness and muscle atrophy and sensory deficits in vibration and proprioception with loss of reflexes. Patients with demyelinating polyradiculoneuropathy will most commonly have a combination of proximal and distal findings on neurologic examination and electrodiagnostic testing. This proximal involvement often distinguishes demyelinating polyradiculoneuropathy from the more common length-dependent polyneuropathies that have only distal involvement initially.
Etiologies/differential diagnosis	CIDP is immune-mediated.
Pathophysiology	By definition, demyelinating neuropathies affect the large myelinated motor (Aα) and sensory (Aβ) and small myelinated (Aδ) fibers of nerves. Whether the neuropathy is acute or chronic, the demyelinating neuropathies are usually accompanied by damage to the axon. The extent of this axonal loss usually correlates with the degree of fixed clinical deficit. Thus, demyelinating neuropathies are often mixed disorders.
Evaluation	Electrodiagnostic studies (NCSs and EMG) play a crucial role in making this determination. It should be recognized that electrodiagnostic testing performed in the first weeks of presentation may not show all characteristics but can provide useful information to guide treatment early on. Findings of significant slowing indicate a primarily demyelinating process, but the electrodiagnostic picture can sometimes be difficult to interpret. Lumbar puncture is often performed to demonstrate increased protein in the cerebrospinal fluid in CIDP.
Ganglionopathy	
Other nomenclature	Sensory neuronopathy of the dorsal root ganglion
Definition	Ganglionopathy is a pure sensory neuronopathy that is caused by dorsal root ganglia injury or irritation.
Symmetry	This disorder most often presents in a non–length-dependent manner. Ganglionopathies can be symmetric or asymmetric.[15,21]
Presentation	The dorsal root ganglion is a pure sensory structure that contains the cell bodies of large and small fiber sensory neurons. The precise nature of the symptoms is related to which sensory modality-specific nerve cell bodies have been affected. Ataxia or severe incoordination is reflective of large fiber sensory nerve cell body injury. Injury to the cell bodies of small fiber nerves results in impaired pain and temperature sensation. Patients may complain of weakness, but by definition, strength is preserved. The weakness symptom results from proprioceptive deficits. There may be autonomic dysfunction associated because conditions that cause sensory ganglionopathy can also affect the autonomic nervous system.
Etiologies/differential diagnosis	Causes of ganglionopathy are varied; however, the likelihood of identifying an immune or paraneoplastic disorder is higher in ganglionopathies than with most length-dependent neuropathies. Drug-related, nutraceutical toxicity and infectious agents should all be considered in the right clinical context.[21,22]
Pathophysiology	The dorsal root ganglia contain cell bodies and have a fenestrated blood supply, resulting in a relatively leakier blood–nerve barrier, thereby making these cells more susceptible to injury. The exact injury to the cell bodies depends on the etiology, and much remains to be learned about the exact pathomechanisms.[22]
Evaluation	Electrodiagnostic studies (NCSs and EMG) should be performed. Motor NCSs and needle EMG should be normal. Sensory nerve responses in both the upper and lower extremities are typically absent. MRI of the spinal cord may show hyperintense T2-weighted lesions of the posterior

(Continued)

Table 2. (*Cont'd*)

Neuropathy type	Description
	columns due to the degeneration of the dorsal root ganglia's central projections in the gracile and cuneate fasciculi. Excisional biopsy of dorsal root ganglion with histologic analysis is rarely performed. Sural nerve biopsy may show axon loss, but this finding is not specific to ganglionopathy and typically does not clarify etiology. Lumbar puncture could be considered.
Vasculitic neuropathy	
Other nomenclature	Multiple mononeuropathy; mononeuritis multiplex
Definition	Vasculitic neuropathies refer to neuropathies associated with histologic evidence of a vasculitic process involving the peripheral nerves, leading to immune-mediated injury of nerve blood vessels and subsequent ischemic injury, and are associated with several clinical and electrophysiologic presentations. These neuropathies can occur in isolation as well as part of systemic vasculitis. Multiple mononeuropathy is the pattern most closely associated with vasculitic neuropathy. Vasculitic neuropathy is a peripheral neuropathy that affects large and small fibers in sensory and motor nerves.
Symmetry	Typically, vasculitic neuropathies are asymmetric at the onset, though with time, patients who do not receive treatment can become confluent, giving the impression of a symmetric polyneuropathy.
Presentation	Vasculitic neuropathy is typically acute or subacute and usually painful. Asymmetric foot or wrist drop is a common initial presentation.
Etiologies/differential diagnosis	By definition, vasculitic neuropathy implies an immune-mediated disorder. Other causes of asymmetric neuropathies not due to immune causes should be excluded.
Pathophysiology	Immune-mediated inflammation involving the vasa nervorum leads to ischemic injury of the peripheral nerves and occurs as part of a systemic inflammatory process or as an isolated process confined to the nerves.
Evaluation	Electrodiagnostic testing of right and left motor and sensory nerves should demonstrate markedly asymmetric responses. Nerve and muscle biopsy should be obtained to provide pathologic evidence of blood vessel inflammation.[13,23] Serologic testing can be used to support evidence of systemic involvement.
Autonomic nervous system neuropathy	
Other nomenclature	Autonomic neuropathy; autonomic ganglionopathy
Definition	Autonomic nervous system neuropathy is a form of polyneuropathy that affects the autonomic nervous system, and its regulation of functions is mediated by the parasympathetic and sympathetic nervous systems.
Symmetry	N/A
Presentation	Organ systems involved include the cardiovascular, gastrointestinal, genitourinary, thermoregulatory, and secretory. Symptoms can include hypotension, tachycardia, constipation, bloating, early satiety, nocturnal diarrhea, sexual dysfunction, bladder dysfunction, photosensitivity, impaired vision, anhidrosis, and sicca symptoms.[24,25]
Etiologies/differential diagnosis	Acute etiologies include autoimmune disorders (Sjögren's disease, celiac disease), paraneoplastic syndromes, autoimmune autonomic ganglionopathy, Guillain–Barré syndrome, infection, toxins, and medications/chemotherapy.[26–28] Chronic etiologies include diabetes, amyloidosis, and being hereditary.
Pathophysiology	The pathophysiology remains unclear; some cases appear to be due to an autonomic ganglionopathy, others from peripheral autonomic nerve damage, and there is a report of a T cell–mediated process.
Evaluation	Screening questions and questionnaires should be performed, and the following should be considered: • Tilt table test • The QSART • Measures of heart rate variability • Assessment of blood pressure changes, including the Valsalva maneuver • Gastrointestinal motility testing • Urodynamic testing • Skin biopsy to assess sweat gland nerve fiber density • Laboratory testing, such as vitamin B12, hemoglobin A1c, serum immunofixation, dysautonomia autoantibody panel (ganglionic acetylcholine receptor and paraneoplastic autoantibodies), to exclude other potential causes.

* Peripheral nervous system involvement in Sjögren's disease is common, occurs in several forms, and may be underdiagnosed.[29] In a series of 92 patients with Sjögren's disease–related neuropathies, 39% had sensory neuropathy, 20% had SFN, 16% had trigeminal neuropathy, 12% had multiple mononeuropathies, 5% had multiple cranial neuropathies, 4% had polyradiculoneuropathies, and 3% had autonomic neuropathies.[10,30] Some authors estimate that among all patients with Sjögren's disease, 5% have sensory neuropathy and 5% to 10% have an SFN.[30] Although less frequent than other forms of peripheral neuropathies, sensory ganglionopathy tends to be fulminant, resulting in greater disability early on. Patients with Sjögren's disease can develop more than one type of peripheral neuropathy, and peripheral neuropathies stemming from other etiologies that are common in the general population can also occur. Therefore, involvement of a neurologist in the care of patients with Sjögren's disease early when neuropathy is suspected is ideal. N/A, not applicable; SFN, small fiber neuropathy. AIDP. Acute inflammatory demyelinating polyradiculoneuropathy. CIDP. Chronic inflammatory demyelinating polyradiculoneuropathy. *Source*: Reprinted with permission from the Sjögren's Foundation.

Electrodiagnostic studies are invaluable tools in characterizing neuropathy, but one must understand their utility and limitations. Nerve conduction studies and electromyography can reveal patterns of neuropathy and nerve fiber type involvement as well as demonstrate axonal or demyelinating pathophysiology, but electrophysiologic abnormalities may not entirely correspond to clinical findings, which can be confusing to practitioners. For example, most patients with SjD with electrophysiologic evidence of sensorimotor polyneuropathy have predominantly sensory symptoms with minimal or no clinical muscle weakness.[11] Another point of confusion is that routine nerve conduction studies do not detect SFN, and a skin biopsy or specialized neurophysiologic testing may be necessary to confirm the diagnosis. Similarly, specialized autonomic nervous system testing is needed to demonstrate autonomic neuropathy but may not be available in all locations. Despite the challenges, electrophysiologic studies complement the clinical examination to better assess and classify PNS disorders.

Disparate definitions of neuropathies

The Sjögren's Foundation convened an interdisciplinary panel of experts to develop evidence-based CPGs for PNS manifestations of SjD. This panel, including neurologists and rheumatologists, followed an evidence-based process during which it became clear that the medical literature contains disparate definitions of types of neuropathies. Examples are provided in Table 1.

Which type is the most common in SjD? As shown in Table 1, different studies suggested different frequencies, in part due to the small sample size in most studies. Together, heterogeneity of data and definitions often leads to difficulty in understanding and reconciling the data.

Aligned nomenclature of the PNS neuropathies

This stark lack of standardized nomenclature highlights a broader issue impacting effective communication across medical specialties. To address this need, the Sjögren's Foundation PNS guideline panel undertook an effort to create shared definitions of the types of neuropathies that occur in SjD. The project aimed to define specific PNS manifestations that could be understood across disciplines and interdisciplinary teams. We initially identified differences in terminology and definitions used by both specialties as well as areas of overlap. We then developed an aligned nomenclature and defined the terminology used to describe specific peripheral neurologic manifestations of SjD to ensure agreement among rheumatologists, neurologists, and other medical specialties (Table 2). Terms describing seven PNS categories were delineated, with descriptions of clinical presentations, possible etiologies, diagnoses, pathologies, and evaluation. This included commonly used synonymous terms for clarification.

Conclusions

Clear communication among specialists involved in the care of patients with complex conditions, such as SjD, is essential. The communication gap identified by the Sjögren's Foundation PNS CPGs panel highlights a challenge for multispecialty collaboration in clinical management and clinical research. The development of shared definitions for PNS manifestations bridges this gap by harmonizing the terminology used by rheumatologists and neurologists. This alignment of nomenclature is an attempt to enhance communication across different medical specialties with the goal of improving the multidisciplinary management of autoimmune-mediated peripheral neuropathies, ultimately leading to better patient outcomes and a higher quality of care.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Ms. Hammitt confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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EDITORIAL

Target Trial Emulations of Sodium-Glucose Cotransporter 2 Inhibitors in Systemic Lupus Erythematosus

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In this issue of *Arthritis & Rheumatology*, Ma et al[1] have published an emulated clinical trial of sodium-glucose cotransporter 2 inhibitors (SGLT2is; such as canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin) for prevention of renal and cardiovascular outcomes in patients with systemic lupus erythematosus (SLE) and type 2 diabetes mellitus (T2DM), using data from an American insurance–based population. They studied 2,165 patients starting an SGLT2i and the same number of propensity score–matched patients who were starting a dipeptidyl peptidase 4 inhibitor (DPP4i; including sitagliptin, saxagliptin, linagliptin, and alogliptin), a different class of oral diabetes medications, which, unlike SGLT2is, has not been shown to have cardio- or nephroprotective properties.[2] They found that patients with SLE and comorbid T2DM who were treated with SGLT2is had reduced rates of incident renal outcomes including acute renal injury, chronic renal disease, and end-stage renal failure, with risk reductions of approximately 40% to 60%. Heart failure was also significantly reduced, whereas myocardial infarctions, hospitalizations, number of patients with new lupus nephritis (LN), and mortality rates were numerically, but not significantly, reduced versus in DDP4i users with SLE.

The strengths of the study included using validated algorithms for the diagnosis of SLE, considering only incident users of each drug class and following them, considering a three-month washout for attributing events to a study drug after it is discontinued, matching for potential known confounders (other diabetic drugs, comorbidities, age, sex, body mass index, renal function, and gylcated hemoglobin), including a negative control outcome, and excluding patients who already had LN. Surprisingly, less than one-third of patients received hydroxychloroquine; other immune suppressant use was very low, and >60% of patients had been prescribed glucocorticoids over the previous year. This could affect the generalizability of the findings to patients with SLE in a rheumatology practice. The use of DPP4is is far lower now than it was during the study's enrollment period (2016–2020) because evidence has continued to build supporting the cardiovascular, renal, and mortality rate benefits of both SGLT2is and glucagon-like peptide-1 receptor agonists (GLP-1-RAs) in T2DM.[2] The renal protective benefits of SGLT2is in T2DM were known before the onset of the study period,[3,4] so the use of DPP4is at a higher rate (approximately two times more users) before propensity score matching is surprising. Other factors such as drug access or socioeconomic status may have differed between the groups. There were more genital infections within the group with SGLT2i, who has been previously observed.[5] Perhaps other confounders may have affected the choice of T2DM and SLE medications, such as subspecialty care (endocrinologist, rheumatologist, nephrologist, or a general internist), severity of comorbidities, and access to optimal care.

Jorge et al[6] also used a target trial emulation framework to study more than 95,000 patients from a US multicenter electronic health records database (TriNetX) with SLE, with or without LN. They identified those with comorbid T2DM who were new users of SGLT2is (n = 426) or DPP4is (n = 865). They observed risk reductions of approximately 30% for both renal progression and major adverse cardiovascular events (MACEs) with SGLT2i versus DPP4i exposure. This corresponded to numbers needed to treat of 22 and 25 for renal progression and MACEs, respectively. Subgroup analyses in patients with LN revealed significantly lower risk of MACEs with SGLT2i use but not renal progression.[6] A recently published retrospective cohort study of patients with SLE and T2DM, also using data from the TriNetX platform, yielded similar results upon evaluating 1,775 propensity score–matched pairs of SGLT2i users versus nonusers.[7] SGLT2i users had significantly lower risk of developing LN, starting on dialysis, undergoing renal transplant, developing heart failure, and dying of any cause versus nonusers. Unlike the previously discussed studies, this was not a target trial emulation and did not use an active

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comparator in the primary analyses. This could introduce bias. The authors did, however, perform a sensitivity analysis with matched DPP4i users as active comparators and found that differences in rates of LN, dialysis, renal transplant, and heart failure were no longer significant.

Studies with experimental designs assessing SGLT2is in SLE are limited. One phase I/II single-arm, open-label trial of 38 patients demonstrated safety, but not efficacy, with six months of dapagliflozin use added to standard care.[8] Recently shared preliminary results from a crossover randomized trial of dapagliflozin plus standard of care versus standard of care in 24 patients with inactive LN and residual proteinuria showed reduced proteinuria with the addition of dapagliflozin.[9] As more data emerge, understanding which patients with SLE may benefit from using SGLT2is may become clearer.

In patients with autoimmune rheumatic diseases, other treatments for T2DM have shown superiority over using DDP4is such as GLP-1-RAs when studying mortality rates and cardiovascular events. A population-based cohort study that used British Columbia administrative data and compared new users of GLP-1-RAs with new users of DPP4is after propensity score overlap weighting found that GLP-1-RAs were associated with reduced mortality rates and MACEs in patients with T2DM and immune-mediated inflammatory diseases compared to using DPP4is.[10] GLP-1-RAs, like SGLT2is, are cardio- and nephroprotective and facilitate weight loss in those with excess adiposity.[2,11] With the increasing popularity of GLP-1-RAs in the management of diabetes and obesity, evaluating this drug class in SLE, either alongside or compared to SGLT2is, presents another opportunity for study given the importance of addressing cardiometabolic comorbidities in lupus.

The target trial emulation framework involves designing a hypothetical randomized controlled trial (RCT; ie, the target trial) and then emulating the protocol components using large observational datasets.[12] Like an RCT, emulated trials explicitly define eligibility criteria, treatment strategies, assignment procedures, follow-up period, outcome(s), causal contrast(s), and analysis plan. Random allocation is emulated by adjusting for all (measurable) confounders required to ensure the treatment groups are comparable at baseline (eg, via propensity score matching). This framework can reduce biases of observational data such as timing of treatment and the observation period.[12,13] Because these are not RCTs but have post hoc inclusion and exclusion criteria, comparison group(s), and observations over the same time for incident users who are matched for several confounders that are available in the database, biases are reduced but not necessarily eliminated. Large RCTs should have both known and unknown confounders balanced between the groups. Of course, the cost of emulated trials is far lower, but only factors collected in the database can be adjusted for. As an example, smoking and SLE activity would not be known in an administrative database but may be in electronic medical records. What is reassuring about the current study[1] is that the infection risk and benefits (cardiovascular and renal) observed were similar to those seen in RCTs in patients with T2DM treated with SGLT2is, adding face validity to the study results. We searched PubMed to October 2024 and American College of Rheumatology and EULAR meetings from 2023 and 2024 to display instances in which emulation trials have been used in SLE or LN. The results are shown in Table 1. The studies compared SLE with DM treated with diabetic drugs or compared outcomes in patients with SLE with various treatments. The hazard ratios may be more impressive than actual randomized trials due to channeling bias, especially if one group within the emulation has a far smaller sample size than a comparison group. Other biases could be severity of disease, access to medications, use of glucocorticoids, etc. Other emulated trials in

Table 1. Emulation trials in SLE

Characteristic	HR (95% CI)[a]
SLE and DM US Insurance Database[b]	
Kidney injury	0.49 (0.39–0.63)
CKD	0.61 (0.50–0.76)
ESKD	0.40 (0.20–0.80)
CHF	0.72 (0.56–0.92)
ER visits	0.90 (0.82–0.99)
All-cause death[c]	0.89 (0.65–1.21)
Lupus nephritis[c]	0.67 (0.38–1.15)
MI[c]	0.81 (0.54–1.23)
CVA[c]	1.03 (0.74–1.44)
Hospitalizations[c]	0.76 (0.51–1.12)
SLE but not lupus nephritis[d]	
Serious infection	
Belimumab vs AZA	0.82 (0.72–0.92)
Belimumab vs MMF	0.69 (0.61–0.78)
Hospitalization for infection	
Belimumab vs AZA	0.73 (0.57–0.94)
Belimumab vs MMF	0.56 (0.43–0.71)
Infection in lower limb, belimumab vs MTX	0.86 (0.76–0.97)
SLE and DM US EHR Database[e]	
SLE	
MACE	0.69 (0.48–0.99)
Renal progression	0.71(0.51–0.98)
Lupus nephritis and DM MACE[e]	0.58 (0.34–0.99)

* AZA, azathioprine; CHF, congestive heart failure; CI, confidence interval; CKD, chronic kidney disease; CVA, cerebral vascular accident; DM, diabetes mellitus; DPP4i, dipeptidyl peptidase 4 inhibitor; EHR, electronic health records; ER, emergency room; ESKD, end-stage kidney disease; HR, hazard ratio; MACE, major adverse cardiovascular event; MI, myocardial infarction; MMF, mycophenolate mofetil; MTX, methotrexate; SGLT2i, sodium-glucose cotransporter 2 inhibitor; SLE, systemic lupus erythematosus.

[a] Values were adjusted when provided.

[b] This trial compared SLE outcomes of SGLT2i users versus DPP4i users in a US insurance database. Included were 2,165 SGLT2i users with 2,165 matched patients using DPP4i.[1]

[c] Value was not statistically significant.

[d] This trial featured treatment with belimumab, AZA, MMF, and MTX in patients with SLE but not lupus nephritis. Data were from a US multicenter electronic health record database. Included were 21,481 patients with SLE, with 2,841 belimumab users versus 6,343 AZA users, 2,813 belimumab users versus 8,407 MMF users, and 2,642 belimumab users versus 8,242 MTX users.[14]

[e] This trial compared outcomes of SGLT2i versus DPP4i in 96,511 patients in a large multisite US electronic health records database. Included were 426 SGLT2i users and 865 DPP4i users.[6]

rheumatology have helped to understand associations in rheumatic diseases. For instance, in a large population from multiple Veterans Administration sites, an emulated trial comparing matched patients with rheumatoid arthritis and interstitial lung disease, initiating either a tumor necrosis factor inhibitor or another advanced therapy found no difference in risk of respiratory hospitalization or death between groups.[15]

Do we need to study the benefits of SGLT2is in each population of patients with T2DM and specific comorbidities, or can we generalize the protective effects of SGLT2is to any patient with T2DM using this class of drugs? It seems that we do not need to look at subsets of patients with T2DM because the data show the protective effects in people with T2DM.[2–4] Possibly more important is to study patients without diabetes who will be prescribed SGLT2is for other reasons such as autoimmune diseases with renal involvement such as patients with SLE, granulomatosis with polyangiitis, and other diseases. Already known are the benefits of SGLT2is in patients with chronic kidney disease (CKD) who are not diabetic.[16] So, are the data sufficient to consider routine use of SGLT2is in all patients with SLE without diabetes (given they are at elevated risk for cardiovascular and renal events) or just those with LN or CKD? It is unlikely that a large RCT will be performed due to costs, the widespread use of the SGLT2i by nephrologists, and ethical concerns of withholding effective treatment, so large databases of patients with SLE without diabetes will need to be explored to answer these questions.

We conclude that emulated trials give insights into observations in rheumatic diseases and are more efficient and far less expensive than RCTs. However, there still can be biases because prescribing certain medications is not usually random, there can be other confounding in large databases, and databases may not capture certain relevant confounders. Another approach may be performing pragmatic RCTs in clinical care when there is equipoise for treatments to learn about effectiveness, safety, and other important insights beyond the phase III RCTs. The current emulated trial concludes that the benefits of SGLT2is are also present in patients with T2DM who have concomitant SLE and are helping to improve our standard of care for these patients and likely patients with SLE and renal involvement who do not have DM.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Pope confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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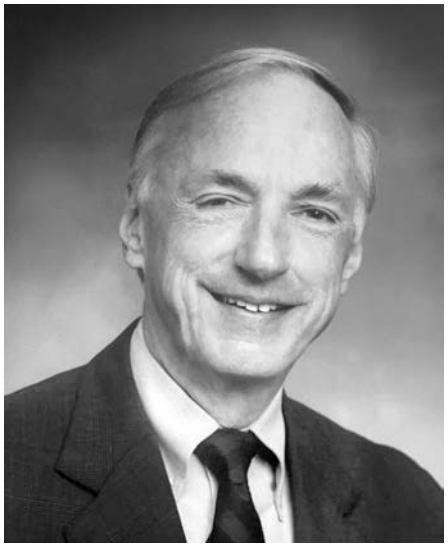
IN MEMORIAM

Joseph D. Croft Jr, MD, 1936-2024



Dr. Joseph D. Croft Jr. died on September 27, 2024, at the age of 88. He grew up in Evanston, Illinois and received his undergraduate degree from Princeton University and his medical degree from Cornell University School of Medicine. He completed his residency and rheumatology fellowship at Strong Memorial Hospital in Rochester, New York, where he was chief resident in medicine.

Following his fellowship Joe spent two years as a clinical associate at the NIH's National Cancer Institute, subsequently remaining in the Washington, DC area for the rest of his long and illustrious career in rheumatology. He opened a private practice in 1969 and in that same year, became affiliated with Georgetown University School of Medicine, where he later became a clinical professor. Georgetown honored him for his teaching and mentoring contributions with its Outstanding Visit Award for Excellence in Teaching Medical House Officers in the Art and Science of Medicine. He additionally was a recipient of the American College of Physicians Preceptorship of the Year, which recognizes exceptional community-based teaching.

Joe's contributions to the American College of Rheumatology (ACR) as a dedicated volunteer spanned multiple decades. He became a member of the Audiovisual Aids Subcommittee in 1974 and chaired the subcommittee from 1975–1982. Over subsequent years he served on the Education Committee, the Committee on Rheumatologic Care, and as Chair of the Legislative Affairs Subcommittee, Ethics Committee, and Nominating Committee. In 1994 he led the ACR's first Ad Hoc Planning and Organizational Review Committee, and in 1999 he chaired the Ad Hoc Committee to Review Governance, whose work resulted in policy and procedural innovations to enhance the function of the organization's Board of Directors and committees and increase members' access to volunteer and leadership roles. He served on the Board of Directors for a number of years and after holding the offices of Secretary and President Elect, was the organization's President from 1999–2000. After his presidency, Joe continued to volunteer with the ACR, serving on the Leadership Council of the Foundation's Within Our Reach program and on various ad hoc task forces.

In recognition of his extraordinary contributions to patient care, the discipline of rheumatology, and the organization, the ACR presented Joe with two of its prestigious Awards of Distinction. In 1990 he received the Paulding Phelps Award, given to a clinical rheumatologist for outstanding service to patients, community, and the practice of medicine. In 2014 he received organization's highest award: the Presidential Gold Medal, awarded in recognition of achievements of significant and lasting benefit to the field of rheumatology.

As a long-time Washington, DC rheumatologist colleague of Joe's, I (DB) had the frequent pleasure of witnessing the numerous qualities that endeared him to many of the individuals he met, both locally and far from home. Among these, he was a great raconteur, mentor, and advocate. At many a meeting, where individuals were hesitant to stand up and make comments, Joe was the first to get to his feet. The participants were relieved because they knew that the ensuing story would be insightful and humorous. He never seemed at a loss for words. He knew so many of his colleagues so well and had personal anecdotes about all of them. It might take time to get to the punchline, but it was always worth the wait.

Joe was generous with his time and knowledge. He was extremely dedicated to his patients and was highly regarded by them, both for his compassion and for his medical expertise. He served the DC medical community as President of the DC Rheumatism Society and as Chair of the DC Medical Society's Medical Economics Committee. After retiring from private practice in

2007, he spent five years in a consulting position with Mercy Clinic, a community-based nonprofit primary care center serving uninsured patients in Gaithersburg, Maryland. He also continued to participate regularly in teaching activities at Georgetown. He was a long-term member of the Washington Academy of Medicine.

Joe was a mentor to countless Georgetown medical students, house staff, and rheumatology fellows. His consideration of patients and his example of professionalism were a gold standard for rheumatologists in DC and the national ACR community. At numerous luncheons I attended, including one just before his death, he was detailing how the changes in the practice of medicine would impact the field of rheumatology.

In his rare spare time (because even after he was retired, old and new patients were trying to convince him to return to practice), Joe and his wife Jane loved to travel. They had a flat in London, where they would go to experience stage shows, concerts, museums, or the local urban and nearby rural scenery. They also rented a villa in Tuscany, where the family would meet in the summer to enjoy the countryside of Italy, as well as many tasty meals.

I (MA) first met Joe in 1989 (two years into my tenure as ACR Executive Vice President) at a meeting of the Committee on Rheumatologic Care, where he gave a presentation on his participation in a Physician Payment Reform Committee meeting at which he represented the ACR. From that moment on, I had a dear friend and a (com)passionate volunteer who would take on any task for the ACR. Any time we were embarking on an important project, Joe's name was at the top of the list of those who could be trusted to return a thoughtful set of recommendations.

Being a resident of the Washington, DC area, Joe was interested in the areas of government affairs and physician reimbursement. He was always willing to attend a Congressional hearing or regulatory hearing if needed. He felt strongly that the voice of rheumatology should be heard, and that those in decision-making roles should understand our positions. Not only did he participate in these areas but he recruited numerous practicing rheumatologists to follow in his footsteps. A number of these recruits eventually served in major leadership roles for the College.

Outside of the ACR, Joe had an eclectic set of interests. He enjoyed collecting and learning about antique oriental rugs and educating others about the different types of rugs, their country of origin, and the significance of the various patterns. He also enjoyed standard poodles. He had a few of them over the years and talked fondly of them.

Joe is survived by his wife of 64 years, his high school sweetheart Jane Grubb Croft. He is also survived by his son Joseph D. Croft III of Charleston, South Carolina and Joseph's wife Rebecca; his daughter Julia E. Croft of Bethesda, Maryland; four grandchildren (Nicole E. Croft, Spencer E. Croft, Campbell H. Peterson, and Peyton C. Peterson); and his sister Mary Anne Osborne of Dedham, Massachusetts. His family has requested that those who wish to make a donation in his memory donate to the Rheumatology Research Foundation.

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Neutrophil Activation Markers and Rheumatoid Arthritis Treatment Response to the JAK1/2 Inhibitor Baricitinib

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Objective. Neutrophils play an important role in regulating immune and inflammatory responses in patients with rheumatoid arthritis (RA). We assessed whether baricitinib, a JAK1/JAK2 inhibitor, could reduce neutrophil activation and whether a neutrophil activation score could predict treatment response.

Methods. Markers of neutrophil activation, calprotectin, and neutrophil extracellular traps (NETs) were analyzed using enzyme-linked immunosorbent assay in plasma from patients with RA (n = 271) and healthy controls (n = 39). For patients with RA, neutrophil activation markers were measured at baseline, 12 weeks, and 24 weeks after receiving placebo and 2 and 4 mg baricitinib. Whole-blood RNA analyses from multiple randomized baricitinib RA trials were performed to study neutrophil-related transcripts (n = 1,651).

Results. Baseline levels of plasma neutrophil markers were elevated in patients with RA compared to healthy controls ($P < 0.001$). Receiving baricitinib reduced levels of soluble calprotectin at 12 and 24 weeks, especially in patients with RA responding to treatment, as determined by American College of Rheumatology 20% improvement criteria. Whole-blood RNA analysis revealed similar changes in the predominant neutrophil markers calprotectin and Fcα receptor I upon reception of baricitinib in three randomized clinical trials involving patients with at various stages of disease-modifying therapy. Clustering analysis of plasma activation markers showed elevated levels of calprotectin and NETs (eg, a neutrophil activation score, at baseline, could predict treatment response to baricitinib). In contrast, C-reactive protein levels could not distinguish between responders and nonresponders.

Conclusion. Neutrophil activation markers may add clinical value in predicting treatment response to baricitinib and other drugs targeting RA. This study supports personalized medicine in treating patients with RA, not only based on symptoms but also based on immunophenotyping.

INTRODUCTION

Neutrophils are essential cells of innate immunity with well-known host-defense functions such as phagocytosis, generation of reactive oxygen species, and release of antimicrobial peptides.[1] Neutrophils can also undergo formation of neutrophil extracellular traps (NETs), a cell death process resulting in formation of extracellular networks of chromatin and granular components, which can trap and eliminate extracellular pathogens.[2–4] Though important in host defense, excessive neutrophil activation is associated with inflammation and autoimmune diseases,[1,5–7] including rheumatoid arthritis (RA).[8,9] In patients with RA, neutrophils are commonly found in the synovial fluid, contributing to local inflammation, tissue damage, and erosion.[10] Neutrophils, through peptidyl-arginine deiminase activation, may also contribute to generation of citrullinated proteins, the main target of anti–citrullinated protein antibodies (ACPAs), a hallmark of RA.[11–13]

In a recent study, we found that patients with RA have markedly elevated levels of NETs and calprotectin (S100A8/A9 complex) in the circulation, both of which track with markers of disease activity. These neutrophil-derived markers performed better compared to current markers of inflammation of disease activity, including C-reactive protein (CRP), in identifying patients with active disease. Of note, using a longitudinal inception cohort,

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patients with a neutrophil activation score (NAS) at baseline had a much more severe disease course and were predicted to develop erosive disease, joint space narrowing, and extra-articular disease within eight years of follow-up.[14] Hence, neutrophil activation is an early event in RA pathogenesis and is associated with disease progression, emphasizing the clinical value of these novel markers in better disease monitoring and prognosis for patients with RA.

Given the importance of neutrophils in RA pathogenesis, targeting neutrophil effector functions, such as NET formation, should ameliorate disease, as has been shown in mouse models.[15] Additionally, depletion of neutrophils significantly reduces the severity of experimental arthritis, further implying the essential role of neutrophils in RA pathogenesis.[16] In human RA, neutrophil activation is induced through several pathways, among others through inflammatory cytokines acting on the JAK–STAT pathway, an important regulator of neutrophil effector functions.[17] This signaling pathway is activated in patients with RA, resulting in immune-mediated pathology driving chronic inflammation that leads to systemic illness and joint destruction.[17–20] Therefore, the JAK–STAT pathway is crucial in maintaining immune homeostasis and provides the rationale for targeting it with therapeutics to treat autoimmune and inflammatory diseases.[20]

Baricitinib, an oral JAK1/JAK2 inhibitor, has the capacity to block several of the key cytokine receptors involved in neutrophil activation, including the receptors for interleukin-6, type I interferons, granulocyte colony-stimulating factor (CSF) and granulocyte macrophage CSF, thus reducing downstream immune cell function.[21] As such, we hypothesize that baricitinib treatment will decrease markers of cytokine-mediated neutrophil activation and cell death (eg, NET formation) in patients with RA, subsequently alleviating disease. To investigate this hypothesis, we assessed levels of neutrophil activation and NET formation in patients with RA who received baricitinib. In brief, our findings demonstrate that patients with RA have highly elevated levels of neutrophil markers, many of which were reduced upon reception of baricitinib, both on the messenger RNA (mRNA) and protein levels. Of note, presence of an NAS, consisting of calprotectin and NETs, but not levels of CRP at baseline, could predict treatment response with baricitinib. In all, assessing neutrophil markers in patients with RA, including calprotectin and NETs, may have clinical utility in monitoring and predicting treatment response to some drugs targeting RA.

PATIENTS AND METHODS

Patient cohort and ethical statement. Patients with RA (n = 271) were recruited to participate in RA-BEACON by Eli Lilly.[22] These were patients advanced in the treatment algorithm, with an inadequate response or intolerance to one or more prior tumor necrosis factor (TNF) inhibitors. Among the 271 patients with RA, 51 patients received placebo, 106 patients received 2 mg of baricitinib, and 114 patients received 4 mg of baricitinib. Plasma samples from patients enrolled in these studies were collected at baseline and weeks 12 and 24 after receiving baricitinib. Plasma from healthy controls (HCs; n = 39) was obtained through the University of Washington, Division of Rheumatology Biorepository. Patient characteristics are summarized in Table 1. The studies were approved by the regional ethics board at the University of Washington (Institutional Review Board 3100), and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Neither patients nor the public were involved in the design or analysis of the current study.

Whole-blood transcriptome. Analysis of whole-blood RNA transcripts was conducted using samples from three large randomized, controlled clinical trials in patients with moderate to severe RA: (1) in RA-BEGIN (NCT01711359),[23] patients were disease-modifying antirheumatic drug (DMARD) naive and received methotrexate (MTX), 4 mg baricitinib monotherapy, or 4 mg baricitinib plus MTX; (2) in RA-BEAM (NCT01710358),[24] patients were MTX-inadequate responders and received placebo plus MTX, adalimumab plus MTX, or 4 mg baricitinib plus MTX; and (3) in RA-BEACON (NCT01721044),[22] patients with an inadequate response or intolerance to one or more prior TNF inhibitors received 2 mg baricitinib plus conventional synthetic DMARDs (csDMARDs), 4 mg baricitinib plus csDMARDs, or placebo plus csDMARDs. A randomized set of patients from the three phase III trials was selected for mRNA analysis. Final numbers of patients were as follows: 172 from RA-BEGIN, 1,043 from RA-BEAM, and 436 from RA-BEACON. Total RNA was extracted from whole blood drawn from baseline and at weeks 4 and

Table 1. Patient characteristics

Treatment group	Placebo	2 mg baricitinib	4 mg baricitinib	HC
Total patients	51	106	114	39
Female, n (%)	46 (90)	78 (74)	94 (82)	33 (85)
Age at diagnosis, median (range), y	56 (31–74)	55 (26–80)	59 (24–82)	49 (18–81)
CRP, median (range), mg/dL	9.7 (1.0–155.2)	12.1 (0.2–108.2)	11.0 (1–172.5)	N/A
ACR20 response, n (%)	2 (4)	63 (59)	61 (54)	N/A
ACR50 response, n (%)	2 (4)	34 (33)	43 (38)	N/A

* ACR20, American College of Rheumatology 20% improvement criteria; ACR50, American College of Rheumatology 50% improvement criteria; CRP, C-reactive protein; HC, healthy control; N/A, not applicable.

12 and was analyzed using the GeneChip Human Transcriptome Array 2.0 (Affymetrix).

Neutrophil activation and cell death marker assays. Levels of plasma calprotectin were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instructions (R&D Systems). Levels of NETs in plasma were quantified by neutrophil elastase (NE)–DNA ELISA, as described previously.[14,25] Briefly, medium binding (Corning) and high-binding, 96-well ELISA microplates (Fischer Scientific) were coated with rabbit anti-human NE (4 μg/mL; Calbiochem) in phosphate-buffered saline (PBS), respectively, and incubated overnight at 4°C. Plates were blocked with 1% bovine serum albumin (BSA) in PBS for two hours at room temperature (RT). Plasma samples were diluted to 1:10 in reagent diluent (1% BSA in PBS with 2 mM EDTA) and added to the plates followed by overnight incubation at 4°C. Anti-DNA horseradish peroxidase from Cell Death Detection ELISA kit (clone MCA-33; Roche) was added at 1:100 dilution as detection antibody and incubated for an additional two hours at RT. The reaction was developed with 3,3′,5,5′ tetramethylbenzidine (BD Biosciences) and ended by the addition of 2N sulfuric acid. Known concentrations of NE–DNA complexes (purified human NE, Innovative Research; calf thymus DNA, Trevigon) were used to construct a standard curve. The complexes were prepared by addition of NE (100 nM) along with double-stranded DNA (1 μM) in PBS and incubated overnight at 4°C. The complexes were aliquoted and stored at –20°C until use. For all the ELISAs, absorbance was measured by a plate reader at 450 nm (Synergy 2, BioTek).

Statistics. To investigate changes in the levels of circulating calprotectin, CRP, and NE–DNA in the plasma samples under different treatment conditions over a 12- and 24-week period, we employed a mixed-model repeated measures (MMRM) approach using categorical time effects on log2-transformed data.[26] The model accounts for the correlation of observations collected from the same patients over time. MMRM incorporates both fixed and random effects to address within-patient correlation and between-patient variability. Fixed effects represent population-level effects that remain constant across all patients, whereas random effects capture patient-specific variability and the correlation among repeated measures within the same patient. The MMRM model fitting was performed in R using the mmrm library. For visualization purposes, log-transformed data were back-transformed in the figures.

For other analyses of plasma samples, the Mann–Whitney U test and Spearman's correlation test were used as applicable. Logistic regression analyses were employed for prediction analyses. For neutrophil markers, the cutoff for positivity was determined by the 95th percentile of HCs. The NAS was defined on a scale of 0 to 2, with 0 representing low levels of both calprotectin and NE–DNA, 1 representing elevated levels of either calprotectin or NE–DNA, and 2 representing elevated levels of both calprotectin and NE–DNA.

High levels of CRP were defined as >10 mg/L. GraphPad Prism and SPSS software were used for the analyses. Odds ratios (ORs) were calculated using logistic regression analysis. All analyses were considered statistically significant at $P < 0.05$. Hierarchical clustering was performed using the R version 4.0.2 pheatmap version 1.0.12 package (https://www.r-pkg.org/pkg/pheatmap). For whole-blood mRNA studies, changes were calculated in R using a mixed-effects model on the log2-transformed response, as done previously.[27] For some analyses, gene expression changes were controlled for changes in cell counts, including neutrophils, as covariates to correct for any observed gene changes due to variations in cell counts.

RESULTS

Neutrophil activation marker elevation in patients with RA and association with markers of inflammation. To investigate whether neutrophil activation occurred in patients with RA, baseline levels of calprotectin (S100A8/A9 complex) and NETs (NE–DNA complexes) were analyzed in plasma samples from a large cohort of patients with RA, independent of treatment response or dose of drug received (n = 271; Table 1) and compared with levels found in HCs (n = 39). Calprotectin, an acute-phase protein, and NETs are known to be elevated in several inflammatory conditions including RA.[2,28] Consistent with prior findings,[14] levels of all neutrophil activation markers were elevated in the cohorts with RA compared to HCs (calprotectin, $P < 0.001$; and NE–DNA complexes, $P < 0.001$; Supplementary Figures 1A and B). Levels of calprotectin correlated with levels of NE–DNA (r = 0.23, $P < 0.0001$). These data indicate neutrophils undergoing marked activation and cell death in patients with RA. We then evaluated if neutrophil markers had any clinical significance by assessing correlations with a current established marker of disease activity, CRP. The correlation analysis was performed at baseline in all patients with RA (n = 271), independent of treatment response or dose of drug received. Consistent with prior findings,[14] levels of calprotectin strongly correlated with CRP levels (r = 0.58, $P < 0.0001$; Supplementary Figure 1C). In contrast, a direct correlation between levels of NETs and CRP was not found in the cohort with RA (r = 0.17, $P = 0.07$; data not shown).

Neutrophil marker reduction upon reception of baricitinib. Reception of baricitinib, an oral JAK1/JAK2 inhibitor, has been previously shown to improve signs and symptoms of RA.[22–24,29] In the current study, we evaluated the treatment response of baricitinib in patients with RA with regard to neutrophil activation markers. In patients who received 2 mg (the clinically approved dose by the Food and Drug Administration

[FDA]), with respect to placebo, reduction of calprotectin levels was observed only at 12 weeks of treatment. Comparatively, for patients who received 4 mg baricitinib (the clinical approved dose by, for example, the European Medicines Agency), marked reduction of calprotectin levels were observed already at 12 weeks of treatment and maintained at 24 weeks with respect to placebo (Figure 1A). In contrast, levels of NE–DNA complexes did not change upon baricitinib reception in patients with RA (Figure 1B). Thus, baricitinib treatment is associated with a decrease in calprotectin levels but no decrease in levels of NETs.

Levels of calprotectin reduction in patients who respond to receiving baricitinib. To determine whether the reduction in neutrophil activation markers was associated with treatment response, patients were stratified based on their American College of Rheumatology 20% improvement criteria (ACR20) response[30] at week 24. Consistent with our hypothesis, patients achieving ACR20 response (eg, responded to treatment) had lower levels of calprotectin after 12 and 24 weeks of baricitinib reception for both the group who received 2 mg and the group who received 4 mg (Figure 2A). In contrast, no reduction in calprotectin levels was observed in the nonresponder group who received 2 mg baricitinib (Figure 2B). For unknown reasons, receiving 4 mg of baricitinib reduced calprotectin levels in otherwise clinical nonresponders, though the reduction was modest (about 500 ng/mL) and only statistically significant at 12 weeks (Figure 2B). Consistent with the ACR20 response data, levels of calprotectin were reduced also in American College of Rheumatology 50% improvement criteria (ACR50) responders, but not in nonresponders, for those who received the 2-mg dose of baricitinib (Supplementary Figure 2). Unlike calprotectin, NET levels did not differ over time between responders and nonresponders (data not shown). Thus, levels of calprotectin are reduced upon reception of baricitinib, particularly within the responders. Similarly, levels of CRP were reduced primarily in responders (Figure 2C and D), both at low and high doses of baricitinib.

Neutrophil-related transcript reduction in whole blood upon treatment with baricitinib. To validate the role of baricitinib in reducing neutrophil activation across a broad set of clinical trials and clinical presentation, RNA analysis of predominantly neutrophil-related transcripts—S100A9, S100A8 (part of the calprotectin complex), and Fcα receptor I (FcαRI)—was performed on select samples from three distinct clinical trials: RA-BEGIN, RA-BEACON, and RA-BEAM. The pharmacodynamic changes in these transcripts upon introduction of either 2 or 4 mg baricitinib in samples from participants of RA-BEGIN suggests that patients who are early in their progression of RA have a robust effect on these markers (Figure 3A), even adjusting for changes in cell count (Figure 3B). In contrast, patients from RA-BEACON, who represent having a refractory form of RA, had limited or no effect on the neutrophil transcripts with either 2 or 4 mg baricitinib. Notably, in the RA-BEAM trial, for patients who were MTX-inadequate responders, there was an early reduction in these whole-blood markers at week 4 that became less evident as the treatment time period progressed to week 12. It is important to note that in the patients of RA-BEAM, there was a baricitinib-mediated reduction in neutrophil levels that persisted until week 24.

Prediction of treatment response using baseline levels of neutrophil markers. To determine the prognostic capacity of plasma neutrophil markers, we compared baseline levels of individual neutrophil activation markers in patients with RA in the groups who received placebo (n = 51), 2 mg baricitinib (n = 106), and 4 mg baricitinib (n = 114) and classified them as responders and nonresponders as per clinical outcome at 24 weeks on the basis of ACR20. For neutrophil activation markers, the cutoff for positivity at baseline was determined by the 95th percentile of the HCs. Levels of calprotectin, as well as

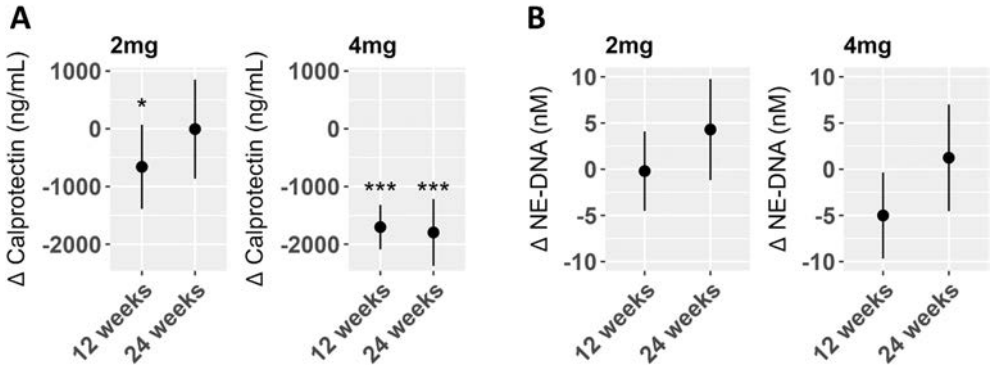

Figure 1. Reduced neutrophil activation in response to reception of baricitinib in patients with rheumatoid arthritis. (A and B) The mean changes, accompanied by the SE, in the levels of plasma neutrophil activation markers (calprotectin and NE–DNA complexes) at 12 and 24 weeks for patients who received 2 mg baricitinib (n = 106) and those who received 4 mg baricitinib (n = 114) were determined with respect to baseline values using the mixed-model repeated measures model on log2-transformed data. *$P < 0.05$, ***$P < 0.001$. NE, neutrophil elastase.

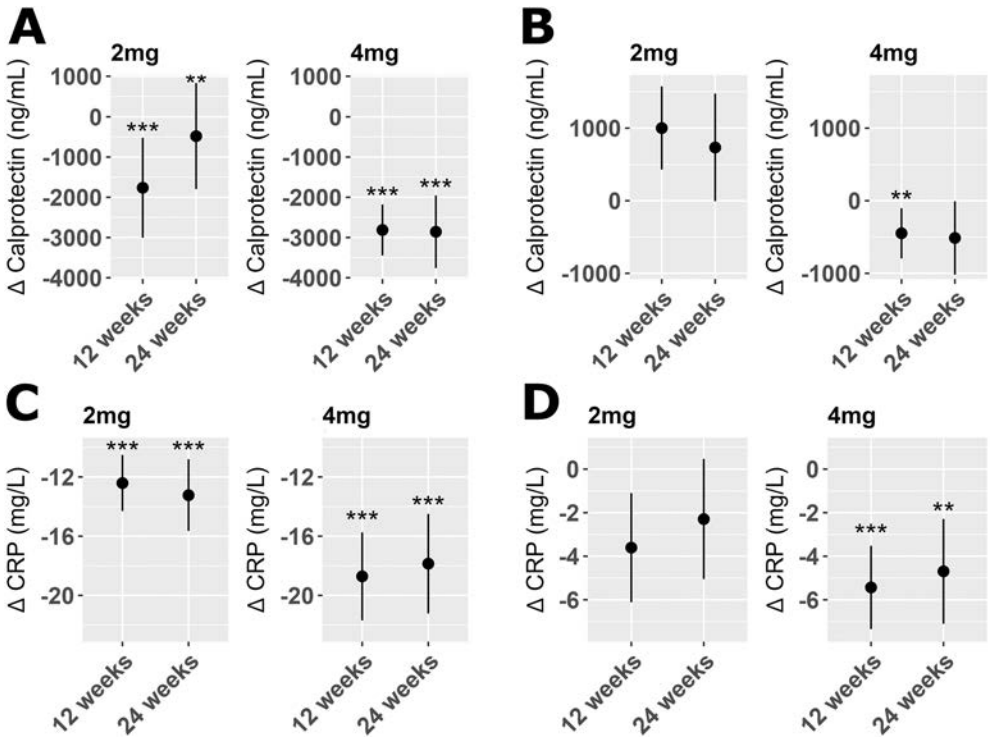

Figure 2. Comparison of changes in the levels of calprotectin and CRP between responders and nonresponders following reception of baricitinib. (A and B) Depicted is the mean difference and SEs of the changes in the levels of plasma neutrophil activation marker calprotectin and (C and D) the marker of inflammation CRP at 12 and 24 weeks, relative to baseline, in patients with rheumatoid arthritis within individual subgroups classified according to clinical response after baricitinib reception (2 mg baricitinib, n = 106; and 4 mg baricitinib, n = 114). (A and C) Represented is the difference in levels of calprotectin and CRP measured in patients with a clinical response after baricitinib reception, as determined by ACR20 (responders). (B and D) Shown are calprotectin and CRP levels measured in patients with no clinical response after baricitinib reception, as determined by ACR20 (nonresponders). The statistics were determined using the mixed-model repeated measures model on log2-transformed data. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ACR20, American College of Rheumatology 20% improvement criteria; CRP, C-reactive protein.

NE–DNA, were elevated at baseline in patients with RA responding to treatment as compared to nonresponders, though not reaching statistical significance for the group who received 4 mg baricitinib for calprotectin levels (Figure 4A and B). Levels of CRP did not differ between responders and nonresponders at baseline (Figure 4C). Using logistic regression analysis, elevated levels of

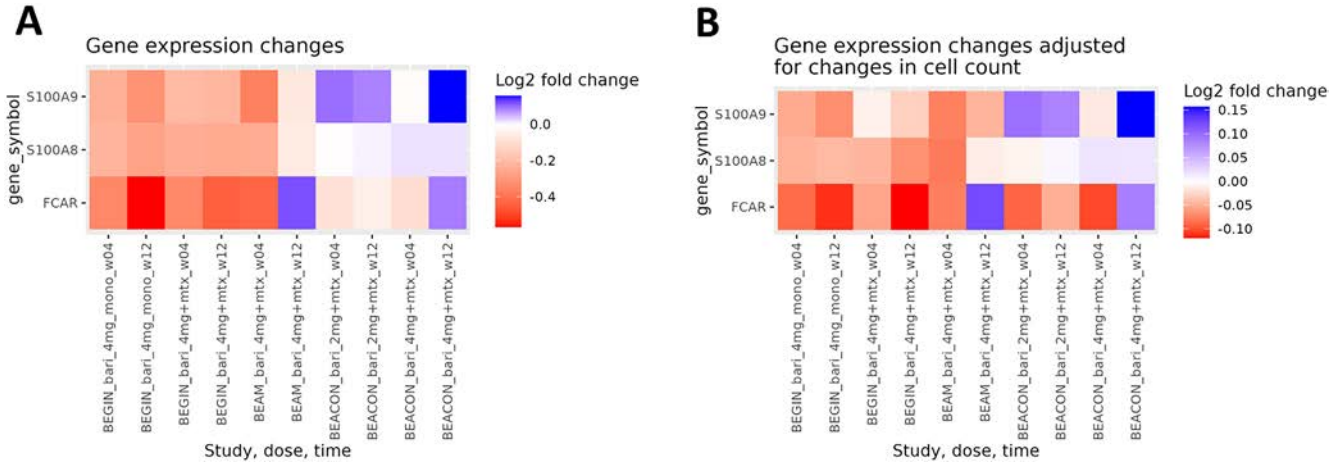

Figure 3. Gene expression changes after 4 or 12 weeks following reception of baricitinib. Marker gene changes in whole blood are shown either (A) unadjusted or (B) adjusted for cell count, across three phase III clinical trials with baricitinib in patients with RA (RA-BEGIN, mtx naive; RA-BEAM, mtx iR; and RA-BEACON, tumor necrosis factor iR). All baricitinib doses are 4 mg unless otherwise specified. Heat maps represent the fold change over time. iR, inhibitor receptor; mtx, methotrexate; RA, rheumatoid arthritis; w, week.

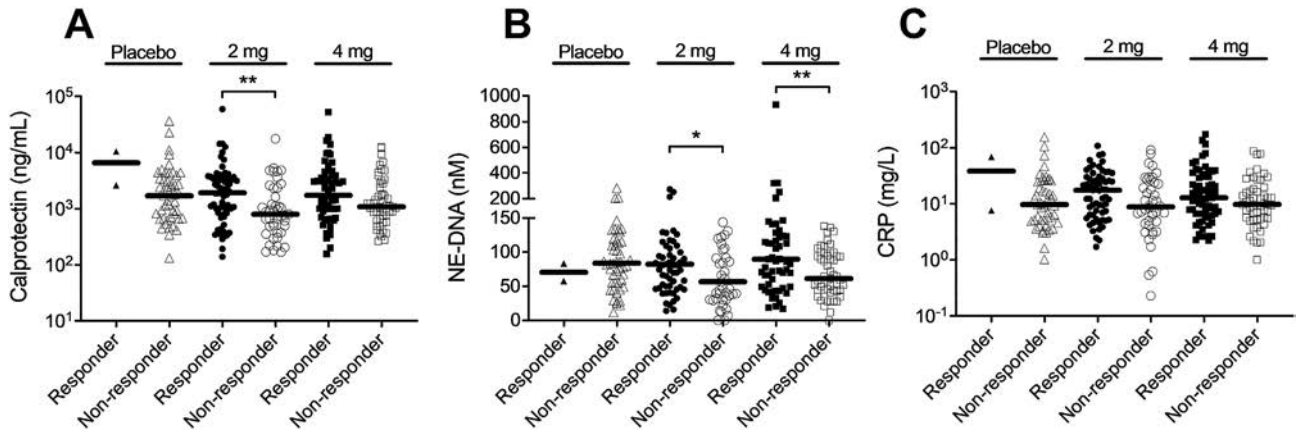

Figure 4. Levels of calprotectin and NE–DNA complexes are elevated at baseline in patients with RA responding to baricitinib reception. (A and B) Levels of plasma neutrophil activation markers (calprotectin and NE–DNA complexes) and (C) marker of inflammation (CRP) were compared at baseline according to clinical response at 24 weeks after baricitinib reception in patients with RA, as determined by American College of Rheumatology 20% improvement criteria response. Patients with RA with or without clinical response to baricitinib were categorized as responders and nonresponders, respectively. Each symbol represents a single patient. Statistics were determined by Mann–Whitney U test. *$P < 0.05$, **$P < 0.01$. CRP, C-reactive protein; NE, neutrophil elastase; RA, rheumatoid arthritis.

calprotectin at baseline predicted treatment response in the group who received 2 mg baricitinib (OR 4.57, [95% CI 1.14–18.37], $P = 0.03$). Further, elevated levels of NE–DNA complexes at baseline predicted treatment response in both the group who received 2 mg baricitinib and the group who received 4 mg baricitinib (OR 2.30 [95% CI 1.04–5.10], $P = 0.04$, and OR 2.90 [95% CI 1.33–6.31] $P = 0.007$, respectively). Similar results were observed also for ACR50 response, with baseline levels of NE–DNA complexes being elevated in responders for both the group who received 2 mg baricitinib and the group who received 4 mg baricitinib ($P = 0.007$ and $P = 0.04$, respectively; data not shown), with OR 2.02 (95% CI 0.84–4.85), $P = 0.12$, and OR 3.12 (95% CI 1.33–7.28), $P < 0.01$, to predict treatment response in the group who received 2 mg baricitinib and the group who received 4 mg baricitinib, respectively. Sensitivity and specificity of the neutrophil assays to predict treatment response are summarized in Supplementary Table 1.

Given the ability of both calprotectin and NE–DNA levels to predict treatment response, we next asked whether a combined NAS could better identify patients predicted to respond to reception of baricitinib. Among patients with low levels of NE–DNA complexes, only 47.7% and 37.8% achieved an ACR20 response for the group who received 2 mg baricitinib and the group who received 4 mg baricitinib, whereas patients with high levels of NE–DNA complexes achieved 67.7% ($P < 0.05$) and 63.8% ($P < 0.01$) ACR20 responses (OR 2.30 [1.04–5.10], $P = 0.04$, and OR 2.90 [1.33–6.31], $P = 0.007$, respectively; Figure 5A). For the NAS model, assessing levels of calprotectin and NE–DNA complexes, there was a clear increase in ACR20 response, with higher NAS in the patients from both treatment groups; 16.7%, 51.2%, and 70.2% for the group who received 2 mg baricitinib and 40.0%, 39.0%, and 65.2% for the group who received 4 mg baricitinib with NAS 0, 1, and 2, respectively (Figure 5B). Of note, the clinically relevant dose, 2 mg, showed better discrimination between NAS and ACR20 outcomes as compared to the 4-mg dose. In contrast, levels of CRP did not predict ACR20 response (Figure 5C), further highlighting the effectiveness of neutrophil activation markers, in particular calprotectin and NE–DNA complexes, to predict treatment effect of baricitinib in patients with RA.

Because calprotectin and NE–DNA complexes were primarily associated with treatment response to baricitinib, we further performed clustering analysis to assess associations of these neutrophil activation markers with ACR20 and ACR50 responses. For these analyses, CRP, a clinical diagnostic marker of inflammation in patients with RA, was also included. The three markers of neutrophil activation and inflammation had a fair overlap, suggesting that in many—but not all—patients, neutrophil activation will be concomitant with inflammation (CPR; Figures 5D–F). Of note, a substantial number of patients had NE–DNA as the main neutrophil marker elevated at baseline, without immediate overlap with calprotectin. Consistent with Figure 5A and B, presence of NE–DNA at baseline was associated with both ACR20 and ACR50 response in patients from both the group who received 2 mg baricitinib and the group who received 4 mg baricitinib (Figure 5E and F) but not for those who received placebo (Figure 5D). This demonstrates that NE–DNA complexes by themselves are not a marker of disease improvement but only upon reception of baricitinib.

DISCUSSION

Neutrophils play a key role in initiation and perpetuation of aberrant immune responses and inflammation in patients with

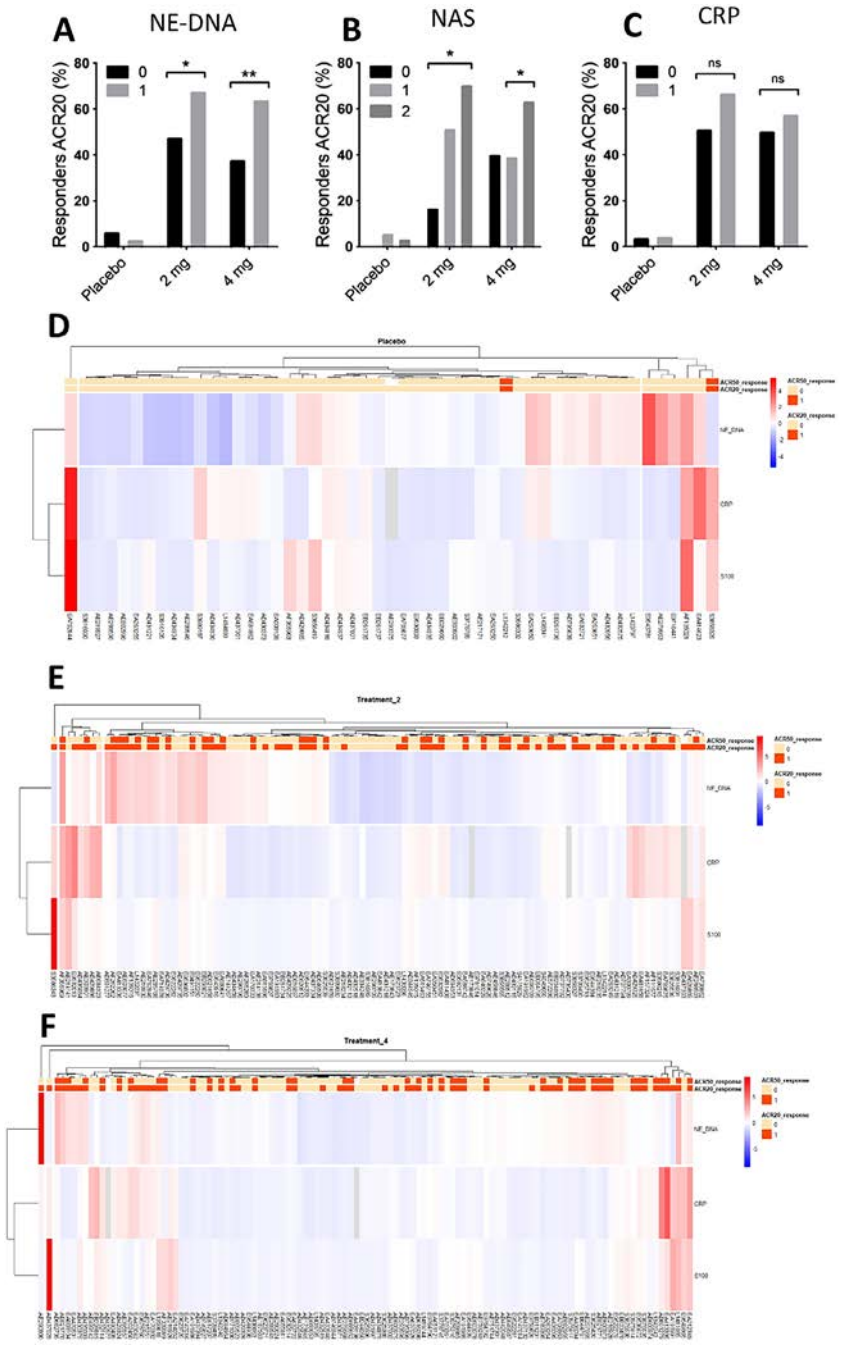

Figure 5. Prediction of treatment response in patients with RA who received baricitinib. (A) Patients with RA were stratified as having either low (0) or high (1) levels of NE–DNA at baseline and analyzed for treatment response in different treatment groups (as per ACR20 response). (B) Patients with RA were stratified as having either no (0), one (1), or two (2) neutrophil activation markers (calprotectin and NE–DNA complexes; defined as neutrophil activation score [NAS]) that were elevated at baseline and analyzed for treatment response in the different treatment arms (as per ACR20 response). *$P < 0.05$, **$P < 0.01$. (C) Patients with RA were stratified as having either low (0) or high (1) levels of CRP at baseline and analyzed for treatment response in different treatment groups (as per ACR20 response). (A–C) Statistics were performed by Fisher's exact test. (D–F) Heat maps showing hierarchical clustering of calprotectin (S100), NE–DNA, and CRP with treatment response (ACR20 and ACR50 responses) for patients with RA who received (D) placebo, (E) 2 mg baricitinib, and (F) 4 mg baricitinib. Neutrophil activation markers and CRP data measured at baseline and ACR20 response at week 24 were used for the analysis. Rows and columns represent markers and patients, respectively. Color key represents Z-scores. ACR20, American College of Rheumatology 20% improvement criteria; ACR50, American College of Rheumatology 50% improvement criteria; CRP, C-reactive protein; NE, neutrophil elastase; ns, nonsignificant; RA, rheumatoid arthritis.

RA.[9,31,32] Although neutrophil markers are elevated in patients with RA, these markers are rarely used in the clinical setting, compared to that of current gold-standard serologic markers of inflammation of disease activity, such as CRP, ACPAs, and elevated erythrocyte sedimentation rate.[14,33] The JAK1/JAK2 inhibitor baricitinib has been highly effective and relatively safe with respect to treatment in patients with RA.[17] Baricitinib monotherapy has been shown to be superior to MTX monotherapy in treatment of patients with RA.[23] In addition, combination therapies including MTX with baricitinib, compared to adalimumab, showed sustained improvements in RA signs and symptoms and disease activity and physical function and slow progression of structural joint damage.[24,34] However, a potential drawback of baricitinib and other treatments is that not all patients respond to the same extent to these interventions. Hence, diagnostic markers with clinical value in predicting treatment responses would be useful in identifying patients with RA responding to baricitinib. Our investigation is the first to demonstrate baseline levels of circulating neutrophil activation markers as a reliable marker for predicting baricitinib treatment response in patients with RA. Among the neutrophil activation markers, elevated levels of NE–DNA complexes and calprotectin were important in predicting who would respond to reception of baricitinib.

The neutrophil activation marker calprotectin is a valuable candidate for assessing inflammation-associated diseases because elevated levels of circulating calprotectin have been observed in several inflammatory rheumatic diseases, including systemic lupus erythematosus (SLE),[34] systemic sclerosis,[35] gout,[36] vasculitis,[37] and primary Sjögren disease.[38] Although not exclusively restricted to neutrophils, calprotectin represents 60% of the cytosolic protein of neutrophils. Further, levels of calprotectin have been shown to correspond to treatment response with immunosuppressive treatment in patients with lupus nephritis.[39] Similarly, as demonstrated in the current study, circulating calprotectin levels are also elevated in patients with RA, and receiving baricitinib significantly decreased these levels. These data suggest a direct effect on calprotectin release from neutrophils by baricitinib through suppression of JAK/STAT signaling. These observations are consistent with a previous study in which restraining of JAK2/STAT3 signaling in colonic neutrophils limited calprotectin expression and established a protective gut commensal microbial community.[40] Among all neutrophil activation markers, only calprotectin strongly correlated with markers of disease activity (CRP), an observation consistent with the strong clustering of calprotectin and CRP. Despite the strong link between calprotectin and CRP, only levels of calprotectin, and not CRP, were reduced in patients responding to baricitinib treatment, indicating the superiority of calprotectin to CRP in identifying patients with RA who specifically respond to reception of baricitinib. In addition, NAS comprised of baseline levels of calprotectin in combination with NETs, but not CRP, improved the ability of predicting treatment response with baricitinib. Moreover, calprotectin has been also shown as a better marker than CRP in assessing disease activity,[14] further strengthening the clinical potential of this neutrophil-derived marker than the clinically used marker of inflammation, CRP.

The role of NETs in the pathogenesis of RA is well established. NETs released from the neutrophils of patients with RA contain citrullinated proteins that enhance the inflammatory response in fibroblasts in joints and subsequent induction of synovial inflammation.[9,41] Although aberrant formation of NETs is a key feature in patients with RA and has been suggested to be implicated in response to TNF inhibitors,[42] the diagnostic potential of NETs has not been extensively investigated across all RA therapies. More importantly, this study was among the first to demonstrate association of elevated circulating NET levels with disease activity in patients with RA, emphasizing the clinical value of NETs.[14] Consistent with our prior work,[14] we also found elevated levels of NETs in plasma samples of patients with RA compared to HCs. Although NET levels were elevated in patients with RA, these levels did not change upon reception of baricitinib. Additionally, NET levels did not differ after 24 weeks of baricitinib reception between the responders and nonresponders. However, baricitinib reception did reduce the NASs of patients with RA in our study, likely driven by the reduction in calprotectin levels. The presence of an elevated level of NETs in responders who received baricitinib is in line with observations from our previous study in which elevated NET levels were present in patients with RA in clinical remission.[14] The reason why elevated NET levels are present in patients with RA is not clearly understood, but previous studies in patients with SLE and RA have suggested impaired NET degradation as a possible cause. In the sera of patients with SLE, the presence of DNase-I inhibitor or the presence of high titers of anti-NET antibodies that could shield NETs from DNase-I were the main mechanisms that caused impaired NET degradation.[43,44] In patients with RA, a similar mechanism of autoantibody-mediated blockade of DNase-I as observed in patients with SLE could account for impaired NET clearance. Apart from NET clearance, the activated phenotype of neutrophils from patients with RA characterized by excessive release of NETs could also account for elevated NET levels.[14,45] Based on those cumulative data, it thus seems that neutrophil release of calprotectin is JAK/STAT dependent, whereas NET formation and/or its degradation is JAK/STAT independent. If validated, additional targeting of NETs, such as by DNases, should be considered in patients with RA and related diseases, including lupus.

Though our findings suggest a reduced neutrophil activation upon baricitinib reception, the reduction in S100A8 and S100A9 mRNA levels, even upon adjusting for cell counts, would indicate a potential transcriptional regulation of neutrophil inflammatory potential by baricitinib. Support for such JAK/STAT-mediated direct transcriptional activation for S100A8/A9 regulation has been previously reported in the activated neutrophils of the colon mucosa in animal models with irritable bowel disease.[40] However, it is noteworthy to recognize that these pharmacodynamic changes with baricitinib are more evident in patients with early

RA and are not a prerequisite for association with baricitinib responsiveness in patients with RA. It is possible that enhanced neutrophil function represents a form of immune dysregulation in patients with RA, which portends greater inflammatory burden in target tissues typically seen in patients with progressive RA.

In conclusion, exaggerated neutrophil activation is one of the key processes in RA pathogenesis that could be leveraged diagnostically in clinical settings. Our prior data showed the efficient use of neutrophil activation markers in monitoring disease activity and predicting disease severity in patients with RA.[14] In the current study, we have shown that the neutrophil marker calprotectin is decreased by baricitinib reception in patients with RA, both at the plasma antigenic level and at the mRNA level in whole blood. Consistent with this observation, baricitinib decreased the NAS in plasma and the neutrophil markers S100A8 and FcαRI at the whole-blood mRNA level. Importantly, our current study demonstrates the utility of an NAS in predicting treatment response to baricitinib, not seen by other clinical markers, such as CRP. Thus, neutrophil activation markers can be instrumental in adding clinical value in monitoring and prognosis of patients with RA as well as early identification of patients likely to respond to baricitinib. Further studies are warranted to validate the current findings to explore whether an NAS similarly can predict treatment response to baricitinib in other rheumatic conditions, as well as the role of the NAS in monitoring treatment response to other interventions.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Lood confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/ Declaration of Helsinki requirements.

ROLE OF THE STUDY SPONSOR

Eli Lilly had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication was contingent upon approval by Eli Lilly.

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Characterizing Nonarticular Pain at Early Rheumatoid Arthritis Diagnosis: Evolution Over the First Year of Treatment and Impact on Remission in a Prospective Real-World Early Rheumatoid Arthritis Cohort

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Objective. Our objective was to characterize nonarticular pain (NAP) at early rheumatoid arthritis (RA) diagnosis, the evolution over the first year of treatment, associations with active RA inflammation, and the impact on remission.

Methods. This real-world, longitudinal multicenter cohort study observed participants with active early RA (symptoms <1 year and Clinical Disease Activity Index [CDAI] >2.8) enrolled between January 2017 and January 2022 who completed a body pain diagram over 1 year. Participants were grouped by prespecified definitions of NAP: (1) none, (2) regional, or (3) widespread. Rheumatologists performed joint counts. Descriptive statistics summarized the frequency and evolution of NAP patterns over 1 year. Chi-square tests compared the proportions of tender and/or swollen joints by the presence of pain in each NAP section. Multiadjusted generalized estimating equations regression models estimated associations of NAP patterns with remission outcomes.

Results. Participants (N = 392) were 70% female, with a mean ± SD age of 56 ± 14 years and mean ± SD symptoms duration of 5.1 ± 2.7 months. More than half reported NAP at baseline, with most (73%) presenting with regional NAP. Common patterns of regional NAP were axial (40%) and pain in upper quadrants (17%). A total of 43% of those with regional NAP persisted or worsened over 1 year, whereas 73% of those with widespread NAP resolved or improved. Joint inflammation was more frequently reported in areas with NAP versus areas without NAP. Regional and widespread NAP were associated with lower odds of reaching CDAI remission (adjusted odds ratio 0.42, 95% confidence interval 0.26–0.70 and adjusted odds ratio 0.30, 95% confidence interval 0.12–0.74), respectively.

Conclusion. Regional NAP is common and persistent in early RA and impacts remission. RA activity may contribute to NAP. More attention to NAP in RA care is warranted.

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[Correction added on 17 February 2025, after first online publication: The author's name "Kuriya Bindee" was corrected to "Bindee Kuriya" in this version.]

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INTRODUCTION

Pain is one of the most important symptoms experienced by patients with rheumatoid arthritis (RA). However, underlying causes can vary and are not necessarily accurately identified. RA disease activity, RA-related joint damage, mechanical etiologies, and/or abnormal pain processing may all contribute to the experience of pain in patients with RA. Pain may sometimes occur outside the joints as nonarticular pain (NAP).[1,2] Both articular pain and NAP may contribute to the overall pain reported in RA,[1,3] and this could potentially affect how disease activity is classified by clinicians and perceived by patients. Accurately identifying and treating specific pain patterns, including NAP, in patients with RA is often challenging but essential for treatment. For example, intensifying disease-modifying antirheumatic drugs (DMARDs) is unlikely to improve NAP caused by mechanical etiologies and may increase adverse effects. Undertreated regional NAP may contribute to deconditioning, sarcopenia, further injuries, and central sensitization. From the perspective of patients with RA, having their pain symptoms addressed is important, whether the pain is in the joint or from other anatomic structures.

A better understanding of NAP in patients with RA has been a longstanding unmet need for patients and rheumatologists. The relationship between active RA joint inflammation and specific regional NAP disorders is not well understood. Regional causes of NAP include localized musculoskeletal conditions: tendonitis, bursitis, enthesitis, and referred pain.[4,5] These regional conditions also exist in patients with RA although there is limited published literature on this topic.[6–8] Further, active RA may lead to regional NAP through a cycle of inactivity, deconditioning, and soft tissue injury.[9]

NAP may also be widespread.[10] Widespread pain is often associated with fibromyalgia, although not all patients with widespread pain meet the classification criteria for fibromyalgia. Because the prevalence of fibromyalgia in RA studies varies widely (ie, from 4.9% to 52.4%[2,11]) and sometimes the diagnosis fluctuates over time, these figures should be interpreted carefully.[12] Importantly, the criteria for fibromyalgia have not been validated for use in patients with inflammatory arthritis.[12,13] It is possible that regional NAP might be misclassified as widespread pain or fibromyalgia, leading to missed opportunities to provide appropriate treatment.

Our objectives were to describe (1) the prevalence of specific NAP types (regional vs widespread), (2) their evolution over the first year after RA diagnosis, (3) the frequency of contiguous RA-related joint activity in NAP, and (4) the impact of NAP on RA remission rates in a prospective, protocolized study of patients with early RA seen in usual care settings. We hypothesized that (1) regional NAP would be more common than widespread NAP, (2) NAP would improve over the first year of treatment, (3) active RA joints would be more commonly associated with NAP, and (4) NAP would negatively impact RA remission rates.

PATIENTS AND METHODS

Participants and setting. The analytic sample included participants enrolled in the Canadian Early Arthritis Cohort (CATCH), a multicenter prospective cohort study of patients with early RA seen across 18 sites (rural, suburban, and urban) in Canada.[14] Participants had to be at least age 18 years, have had joint symptoms for ≥6 weeks and ≤12 months, have at least two swollen joints or one swollen joint at the metacarpophalangeal or proximal interphalangeal joint and one of the following: morning stiffness lasting >45 minutes, response to nonsteroidal anti-inflammatory drugs, painful joints assessed by the metatarsophalangeal squeeze test, rheumatoid factor >20 IU, or anticitrullinated protein antibody positivity. Patients were excluded or withdrawn from the cohort if an alternate diagnosis, such as crystal-induced arthritis, infection-induced arthritis, psoriatic arthritis, or connective tissue disease, was made. Standardized assessments were performed at baseline and every 3 months for the first year. Treatment decisions were at the discretion of the rheumatologist guided by treat-to-target guidelines[15]; strong adherence to system-level performance benchmarks has been previously documented in the CATCH cohort.[16] Additional details regarding this cohort have been published elsewhere.[14]

The analytic sample included participants enrolled between January 2017 and February 2022 who had completed a body pain diagram (BPD) (Figure 1)[17,18] at baseline and at least one follow-up visit at 6 or 12 months. Other eligibility criteria included active RA disease as defined by a Clinical Disease Activity Index (CDAI) score of ≥2.8 at baseline and using treatment with DMARDs including conventional synthetic, biologic, or targeted-synthetic DMARDs within 3 months after study enrollment. Those lost to follow-up did not contribute data beyond their last study visit.

Written informed consent was obtained from all study participants. Research ethics boards representing all study sites approved the study; this was renewed at every site on an annual basis. The trial registration is Pro00016064.

Measures. Participants completed a BPD (Figure 1) at baseline and at least one of the 6- or 12-month visits. They were given explicit instructions to record nonjoint pain on the BPD (eg, "Do you have pain in areas other than your joints?"). If so, they were instructed to mark on the BPD where they had pain. Patients were grouped by prespecified patterns of NAP (Figure 1) reported in four quadrants (right upper, right lower, left upper, and left lower) and axial sections as follows: (1) none, (2) regional NAP if one to three painful sections (three sections if limited to one half of the body (upper half, lower half, right half, or left half), and (3) widespread NAP if three to four painful sections (three sections if bilateral plus above and below the waist[19]). Prior work has defined widespread pain as having a minimum of three or four painful sections on the BPD.[10,20] Based on our criteria, the maximum number of sections possible was four (Figure 1).

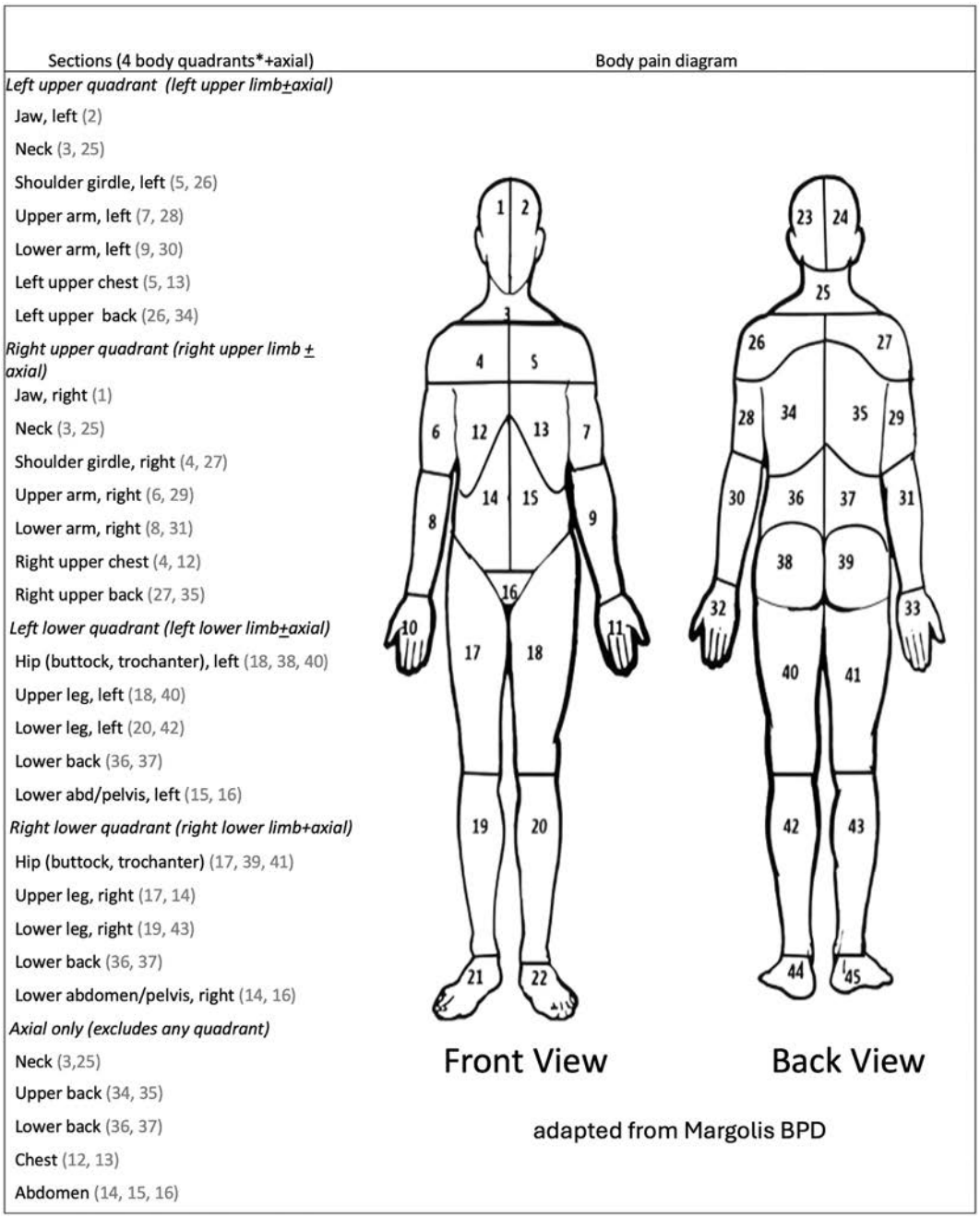


Figure 1. Classification of NAP according to patient-reported BPD. Each numbered area checked by the patient on BPD corresponds to an area listed under each section. Because it is not possible to have all five sections, the maximum number of sections is four. The criteria for NAP are (1) no sections if it is no NAP, (2) one to three sections if it is regional NAP (three sections if limited to one half of body [upper half or lower half or right half or left half]), and (3) three to four sections if it is widespread NAP (three sections if bilateral plus above and below the waist). *If it is neck- or back-only areas, use the axial section, and if it is neck or back plus another area in the quadrant use the quadrant section. BPD, body pain diagram; NAP, nonarticular pain.

Hands and feet were excluded from these definitions. We have found that patients with three painful sections had clinical features that tracked more closely with those with four sections than those with one to two sections (Supplementary Table 1), so we included those with three sections in our widespread NAP definition. To ensure more accurate classification, we split patients who had three sections into two groups: (1) we assigned participants whose NAP was more extensive as defined above into the widespread NAP group and (2) we assigned participants who did not meet this more extensive criteria to the regional NAP group.

We calculated the frequencies and evolution of different NAP patterns at baseline and over the 12 months of follow-up. Recurring NAP was defined as NAP reported at baseline, not at 6 months, and again at 12 months. Persisting NAP was defined

as NAP occurring at all available timepoints of baseline and 6 and 12 months. We compared the frequency of regional and widespread NAP recurring or persisting in the same sections. Regional NAP could evolve to widespread NAP over time, and, conversely, widespread NAP could evolve to regional NAP. We assessed the proportions of tender and/or swollen large proximal joints (shoulders, elbows, hips, and knees) by the presence of pain in each corresponding NAP section reported on the BPD. Small joints in hands and feet were not included because they were not included in our definitions of NAP.

We compared sociodemographic (race was self-reported from categories and open-ended options) and RA clinical (seropositivity for rheumatoid factor and/or anticitrullinated peptide, tender-swollen joint differences [tender joint count minus swollen joint count], patient global assessments, and patient-reported outcome measures [Multidimensional Health Assessment Questionnaire range 0–10]) factors across pain groups at baseline. We also compared associations between pain patterns and rates of remission and low disease activity (LDA) across NAP groups. Remission was defined as CDAI remission (commonly used) and as Boolean remission (more stringent definition).[21] We used the recently revised version Boolean 2.0.[22]

Statistical analysis. *Descriptive univariate analysis.* Summary statistics (mean [SD], median [interquartile range], or frequency [%]) were calculated to describe the baseline characteristics of the whole patient sample and to compare patients with no NAP, regional NAP, and widespread NAP. Descriptive statistics were used to summarize the frequency and evolution of different NAP patterns at baseline and over 12 months of follow-up. We compared the proportions of tender and/or swollen large proximal joints (shoulders, elbows, hips, and knees) by the presence of corresponding pain in each NAP section reported on the BPD at baseline and at 6 and 12 months. These frequencies were compared using chi-square tests.

Adjusted multivariable analysis. Longitudinal associations between NAP and remission were estimated by fitting separate crude and multivariable generalized estimating equation (GEE) regression models for each disease outcome (Boolean remission, CDAI remission, CDAI LDA, or remission) appropriately considering within-person clustering of repeated measures over the first year of follow-up. Multivariable GEE regression models were adjusted for age, sex, Rheumatic Disease Comorbidity Index, symptom duration, seropositivity, methotrexate use in the first 3 months, and oral steroid use in the first 3 months.

We fit separate GEE regression models for binary outcomes using 6- and 12-month disease status as the outcome (Boolean remission, CDAI remission, and CDAI LDA/remission), presenting odds ratios (95% confidence intervals) for time. Models were adjusted for the potential covariates/confounders identified above.

RESULTS

Data were available from 392 patients with early RA who met the eligibility criteria after excluding those with missing data (Supplementary Figure 1). Our study cohort was mostly female (70%) and White (80%), with a mean ± SD age of 56 ± 14 years, mean ± SD symptoms duration of 5.1 ± 2.7 months, and mean ± SD CDAI of 26.6 ± 13.4 (Table 1). Most (79%) were treated with a methotrexate-inclusive regimen.

Patterns around RA diagnosis. At baseline, more than half of the patients (n = 201, 51%) reported NAP, with most (n = 146, 73%) presenting with regional NAP (Table 1). Compared with those without NAP, participants with NAP were more frequently White women who finished high school and had osteoarthritis and/or back pain and a diagnosis of fibromyalgia. Compared with those without NAP, participants with baseline NAP reported higher pain and perceived stress scores. They also exhibited higher tender-swollen joint count differences and greater discrepancies between patient global and medical doctor global assessments compared with those without NAP at baseline. These differences were often greatest in those with widespread NAP. Those with baseline NAP were less frequently seropositive. Participants with baseline widespread NAP had higher C-reactive protein levels compared with those of the other groups (Table 1).

Patterns over 1 year. The point prevalence of regional NAP decreased from 37% at baseline to 27% at 12 months; widespread NAP decreased from 14% to 7% (Figure 2). Of those with regional NAP at baseline, 43% experienced recurrence, persistence, or evolution to widespread NAP over 1 year (Figure 3). Of those with widespread NAP at baseline, 73% resolved or evolved to regional NAP. Only 2 (5%) of 38 participants who developed persistent regional NAP had pain in the same sections over 1 year, whereas five (56%) of nine with persistent widespread NAP recurred in the same sections. Incident NAP developed in 64 (16.3%) of 392 patients over follow-up, of which the majority (92.2%) was regional NAP (Figure 3).

The most frequent patterns of regional NAP over time were axial (40.3%), pain in both upper quadrants (17.0%), and pain in both lower quadrants (9.6%). The most frequent areas of widespread NAP over time were both upper and lower quadrants (42.3%), axial plus both upper quadrants (15.5%), and axial plus both lower quadrants (12.4%). Joint inflammation was more frequently reported in corresponding locations with NAP versus locations without NAP (Figure 4). This relationship persisted over time in some areas but was most notable earlier in the disease course.

Remission. NAP, both regional and widespread, was an independent predictor of a reduced odds of CDAI remission at 12 months (Table 2). Widespread, but not regional, NAP independently predicted not reaching Boolean remission and CDAI

Table 1. Baseline characteristics across NAP pain patterns by patients with early RA*

	Total sample (N = 392)	No NAP (n = 191)	Regional (n = 146)	Widespread (n = 55)
Age, mean (SD), years	56 (14)	57 (14)	55 (14)	57 (14)
Female, n (%)	276 (70)	126 (66)	106 (73)	44 (80)
White, n (%)[a]	314 (80)	146 (76)	122 (84)	46 (84)
BMI, mean (SD),[b] kg/m^2	28.4 (6.9)	28.0 (6.8)	28.1 (6.6)	30.4 (7.8)
Obese, n (%)[b]	117 (31)	55 (30)	38 (28)	24 (46)
Postsecondary education, n (%)	245 (63)	110 (58)	98 (67)	37 (67)
Current smoker, n (%)	55 (14)	23 (12)	21 (14)	11 (20)
Comorbidities RDCI (0–9), mean (SD)	1.3 (1.4)	1.2 (1.3)	1.3 (1.3)	1.9 (1.7)
Osteoarthritis/back pain,[c] n (%)	116 (30)	39 (20)	51 (35)	26 (47)
Fibromyalgia, n (%)	8 (2)	0 (0)	3 (2)	5 (9)
Perceived stress scale, mean (SD)	5.9 (3.3)	5.3 (3.2)	6.1 (3.0)	7.7 (3.5)
RA disease characteristics				
Disease duration, mean (SD), months	5.1 (2.7)	5.0 (2.7)	5.3 (2.7)	5.0 (2.5)
Seropositivity (RF/CCP),[d] n (%)	282 (75)	144 (78)	100 (71)	38 (72)
TJC28, median (IQR)	8 (4–12)	7 (4– 12)	8 (4–12)	9 (4–13)
SJC28, median (IQR)	7 (3–11)	7 (3–11)	7 (3–11)	6 (4–10)
TSJD28 (TJC−SJC), mean (SD)	1.1 (4.8)	0.8 (4.8)	1.0 (4.5)	1.9 (5.2)
Pain intensity (0–10) (NRS), mean (SD)	5.8 (2.8)	5.2 (3.0)	6.1 (2.5)	7.2 (2.2)
MDGA (0–10), mean (SD)	5.4 (2.5)	5.5 (2.6)	5.3 (2.3)	5.5 (2.2)
PTGA (0–10), mean (SD)	5.0 (2.8)	4.4 (2.9)	5.3 (2.5)	6.2 (2.5)
Difference PTGA−MDGA, mean (SD)	−0.4 (3.4)	−1.1 (3.6)	0.0 (3.2)	0.7 (3.0)
CRP (mg/L), median (IQR)	6.2 (2.9–18.4)	6.3 (2.9–22.6)	5.1 (2.4–12.8)	10.1 (2.9–24.7)
CDAI, mean (SD)	26.6 (13.4)	26.1 (14.0)	26.3 (12.7)	28.7 (13.1)
Boolean remission version 2,[e] n (%)	3 (1)	1 (0)	1 (1)	1 (2)
MDHAQ 10 items (0–3), mean (SD)	0.8 (0.6)	0.7 (0.6)	0.9 (0.6)	1.2 (0.6)
Treatment at baseline unless otherwise indicated				
Oral steroids, n (%)	112 (29)	59 (31)	39 (27)	14 (25)
MTX, n (%)	309 (79)	154 (81)	110 (75)	45 (82)
Non-MTX DMARDs, n (%)	226 (58)	116 (61)	82 (56)	28 (51)
Advanced therapy in first 9 months, n (%)	47 (12)	23 (12)	13 (9)	11 (20)
TNF inhibitors[f]	20 (5)	10 (5)	7 (5)	3 (5)
Biosimilars	14 (4)	6 (3)	4 (3)	4 (7)
Non–TNF inhibitors biologic DMARDs[f]	4 (1)	1 (1)	0 (0)	3 (5)
JAK inhibitors	12 (3)	7 (4)	3 (2)	2 (4)
MTX in first 3 months	320 (82)	156 (82)	117 (80)	47 (85)
Oral steroids in first 3 months	124 (32)	62 (32)	44 (30)	18 (33)

* BMI, body mass index; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; IQR, interquartile range; MDGA, medical doctor global assessment score; MDHAQ, Multidimensional Health Assessment Questionnaire; MTX, methotrexate; NAP, nonarticular pain; NRS, Numerical Rating Scale; PTGA, Patient Global Assessment Score; RA, rheumatoid arthritis; RDCI, Rheumatic Disease Comorbidity Index; RF, rheumatoid factor; SJC, swollen joint count; TJC tender joint count; TNF, tumor necrosis factor; TSJD, tender-swollen joint difference.
[a] Includes 1.3% who identify as White and another race; breakdown of racial and ethnic minority categories: 5.6% South Asian, 2.0% Hispanic or Latino, 1.5% South East Asian, 1.5% Native, 0.8% Black, and 2.3% missing.
[b] First reported BMI, percentage of nonmissing.
[c] Designates a category that included all forms of osteoarthritis and back pain.
[d] In the first year, percentage of nonmissing.
[e] Version 2 of Boolean remission uses a PTGA threshold of 2.
[f] Includes both bio-originators and biosimilars.

remission/LDA. Symptom duration and the use of oral steroids were independent predictors of a reduced odds of remission at 12 months (Supplementary Table 1). Symptom duration also independently predicted a reduced odds of CDAI remission/LDA; there was a trend for oral steroids to predict a lower odds of remission/LDA, but this did not reach statistical significance (Supplementary Table 1).

Missing data. We excluded 334 patients because of missing data. The excluded participants, compared with those in our sample, were less frequently White (59% vs 80%), less likely to have postsecondary education (45% vs 63%), and less likely to report osteoarthritis or back pain (21% vs 30%) or to have been treated with advanced therapy in the first 9 months (5% vs 12%).

DISCUSSION

This large real-world, prospective, protocolized study is the first to carefully characterize the prevalence, evolution, and patterns of both regional and widespread NAP in the first year of RA diagnosis. Regional NAP is common (37%) and often persists in the first year of RA. Frequent patterns of regional NAP include axial and both upper quadrants, suggesting concomitant

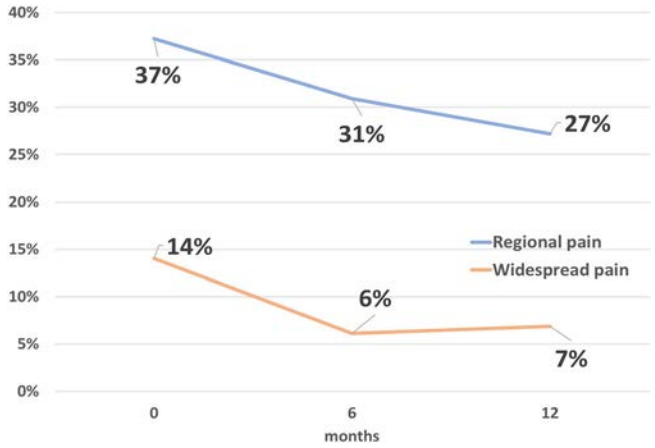


Figure 2. Point prevalence of regional and widespread nonarticular pain over 1-year follow-up in the early rheumatoid arthritis cohort. There were 392 patients at baseline, 343 at 6 months, and 305 at 12 months.

mechanical conditions. The higher frequency of active joints within areas of NAP and the improvement of both over time suggests that RA activity may contribute to NAP. Finally, both regional and widespread NAP independently predicted lower odds of remission.

Efforts to study NAP have been hampered, in part, by varying definitions. For example, prior published definitions of widespread pain, similar to the one used in this study, have included pain in three sites that involved bilaterality plus above and below the waist distribution.[20] Another study of 724 patients with early RA by Schelin et al reported an 8% prevalence of widespread nonjoint pain at 3 years of follow-up.[10] However, they defined widespread nonjoint pain as having pain in at least one nonjoint area in all four body quadrants. We also found a low prevalence of widespread NAP of 7% at 1-year follow-up. A strength of our study is that our classification of NAP was data driven (see the Methods section). Our study thus supports and extends the findings of Schelin et al by including both an evaluation of regional NAP and a data-driven classification of NAP.

More than half (51%) of the patients in our sample had NAP around diagnosis, and the prevalence of NAP decreased over time to 34%, likely reflecting the effect of RA treatment. This was especially apparent in the group with widespread NAP, in which 73% resolved or improved (evolved into regional NAP) over the year. This may indicate that inflammation is driving NAP, particularly in the first few months of RA diagnosis and more so in those with widespread NAP. The group with widespread NAP also had the highest levels of inflammation (ie, median C-reactive protein levels) around diagnosis. In contrast, among those with regional NAP at baseline, NAP persisted in nearly half of the patients during the year, suggesting that other etiologies besides RA inflammation may have been contributory. Interestingly, when we looked to see how often NAP recurred in the same areas, we found that the location of NAP often fluctuated. This phenomenon also warrants further study. Importantly, the presence of regional or widespread NAP in patients with RA reduced their odds of reaching remission, identifying these patients as a high-risk group. These patients may benefit from earlier and targeted interventions, such as physical therapy and pain management, in addition to their DMARD treatments.

Active joints were often identified in areas of NAP over time, especially earlier in the disease course, which may have contributed to our finding that both RA and NAP improved with treatment and over time. However, tender joints may not always indicate RA inflammation,[23–25] nor may tender joints indicate a problem restricted to the joint.[5] NAP may have different or multiple underlying mechanisms, including RA inflammation and mechanical and nociplastic causes. More data are needed to

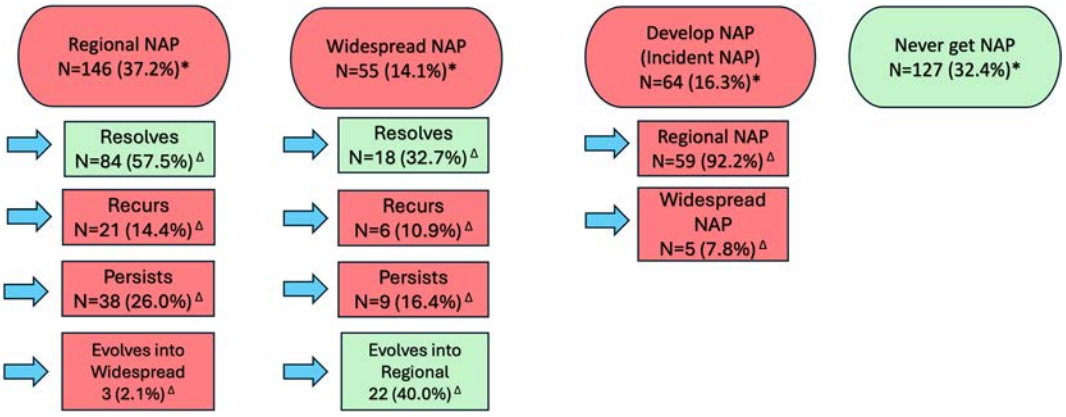


Figure 3. Evolution of NAP patterns during the first year following early rheumatoid arthritis diagnosis (N = 392). *Percentages in oval shapes are calculated from the entire sample (N = 392). ΔPercentages in boxes are calculated from the denominators indicated in the oval shapes. Red shading indicates the presence of prevalent, incident, recurring, or persistent NAP or the evolution of regional to widespread NAP. Green shading indicates the absence or resolution of NAP or the evolution of widespread to regional NAP. NAP, nonarticular pain.

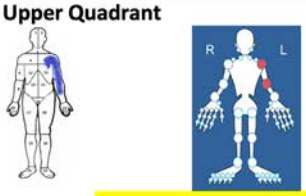
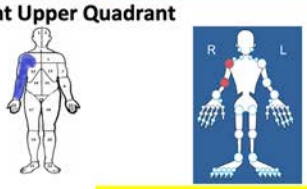
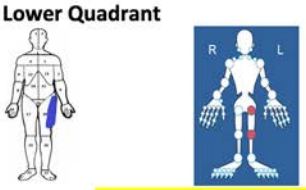
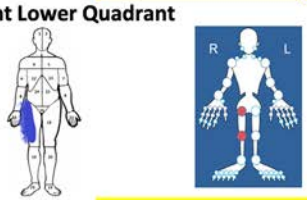
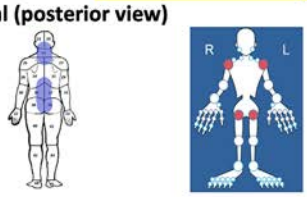
NAP section[Δ]	Tender and/or swollen joints in NAP section*	Baseline (n=392) NAP in section	Baseline (n=392) No NAP	6 months (n=343) NAP in section	6 months (n=343) No NAP	12 months (n=305) NAP in section	12 months (n=305) No NAP
Left Upper Quadrant		86	306	38	305	37	268
		45%	33%	34%	11%	19%	9%
	p-values[Φ]	0.03		<0.0001		0.08	
Right Upper Quadrant		93	299	38	305	43	262
		51%	34%	34%	12%	21%	10%
	p-values[Φ]	0.004		0.0003		0.04	
Left Lower Quadrant		74	318	40	303	29	276
		43%	30%	20%	7%	10%	7%
	p-values[Φ]	0.02		0.01		0.47	
Right Lower Quadrant		62	330	32	311	30	275
		47%	33%	16%	10%	13%	7%
	p-values[Φ]	0.03		0.36		0.26	
Axial (posterior view)		80	312	63	280	43	262
		29%	39%	21%	17%	9%	13%
	p-values[Φ]	0.09		0.51		0.62	

Figure 4. Frequency of active rheumatoid arthritis joints in corresponding areas of NAP compared to no NAP in early rheumatoid arthritis. [Δ]Shaded areas on the body pain diagram are illustrative examples; all views are anterior except the axial (adapted from Margolis et al).[18] The joint homunculus was adapted from an image in the University of Alberta's Disease Activity Score Calculator (https://www.epicore.ualberta.ca/demo/joints). *Joints assessed for tenderness and/or swelling within each NAP section; the frequency of active joints in each section for patients with NAP and those without NAP is indicated in red. [Φ]These *P* values compare the frequency of active joints in each section for patients with NAP and those without NAP; a chi-square test was used for categorical variables. NAP, nonarticular pain.

better understand the mechanisms underlying these different types of pain in RA. These study limitations underscore the need for the improved reporting of both articular pain and NAP in patients with RA.

Regional NAP was most commonly axial, followed by upper body and lower body. These areas of NAP could be due to degenerative disc disease, tendinopathies, and other diagnoses of musculoskeletal pain. Wolfe et al conducted a mail survey among patients with rheumatic diseases, including RA, with a goal to validate a preliminary regional pain scale.[26] They found that, in RA, the most common areas of pain were back, neck, and both shoulders. Our results using a BPD in a well-characterized cohort with early RA seen in usual care settings support and update these findings.

Table 2. GEE analysis showing NAP association with odds of achieving remission and remission/LDA in early rheumatoid arthritis*

	GEE analysis using 6- and 12-month visits[a]					
	2023 Boolean remission		CDAI remission		CDAI remission/LDA	
Analysis	Adjusted for NAP	Fully adjusted[b]	Adjusted for NAP	Fully adjusted[b]	Adjusted for NAP	Fully adjusted[b]
N	495	476	611	580	611	580
Adjusted for type of NAP, OR (95% CI)						
Regional NAP vs no NAP	0.58 (0.36–0.93)	0.62 (0.37–1.02)	0.42 (0.27–0.67)	0.42 (0.26–0.70)	0.92 (0.63–1.35)	1.01 (0.67–1.51)
Widespread NAP vs no NAP	0.29 (0.12–0.70)	0.30 (0.12–0.76)	0.27 (0.11–0.66)	0.30 (0.12–0.74)	0.35 (0.19–0.64)	0.40 (0.21–0.76)
Adjusted for visit, OR (95% CI)						
12 months vs 6 months	1.53 (1.11–2.10)	1.48 (1.06–2.07)	1.52 (1.12–2.06)	1.44 (1.04–1.99)	1.96 (1.44–2.68)	1.89 (1.37–2.62)

* CDAI, Clinical Disease Activity Index; CI, confidence interval; GEE, generalized estimating equation; LDA, low disease activity; NAP, nonarticular pain; OR, odds ratio.
[a] GEE logit models using NAP at 6 and 12 months predicting Boolean version 2 remission and CDAI remission and CDAI remission/LDA at 6- and 12-month time-adjusted models and fully adjusted using baseline variables.
[b] Adjusted for age, sex, Rheumatic Disease Comorbidity Index, symptom duration, seropositivity in the first year, methotrexate use in the first 3 months, and oral steroid use in the first 3 months.

Finally, the BPD is a commonly accepted measure of pain location and distribution in patients with musculoskeletal pain.[19,27–30] Although the BPD used in our study has shown good test-retest reliability in patients without RA,[18] it is not known if patients with RA can readily distinguish between joint pain and NAP. Little has been published on the use of BPD in patients with RA,[10,31] and our study adds to this sparse literature. For now, the use of a BPD to screen for NAP in patients with RA should be followed by a more specific evaluation. Rheumatologists would benefit from a simple pragmatic tool to quickly identify NAP and to help tailor person-centered RA care.

The strengths of our study include the use of a large, multisite, well-characterized cohort of participants with early RA who were observed longitudinally. Our participants were seen in usual care settings where the practice follows treat-to-target guidelines. This CATCH cohort of patients was diagnosed and treated early, many with methotrexate-inclusive regimens.

There are also study limitations. Our study is observational, and there is the possibility of unmeasured confounding. For instance, we do not know which participants may have been prescribed other interventions, such as physical therapy and pain medications, during the year of follow-up. There may be practice variation between the sites. The CATCH consortium has been shown to meet high performance benchmarks[16] and meets annually to review their practices.

More than 2 years of the study overlapped with the COVID-19 pandemic. As a result, in-person rheumatology assessments for collecting joint counts for disease activity measurements were limited across Canada. This coincided with the introduction of the BPD to CATCH, and some patients were not able to complete this (had not reached the follow-up endpoint). However, all CATCH clinics faced these constraints, and it is likely that the missing data was missing completely at random. Although this may have reduced the precision of the study estimates, it would be less likely to have biased them. Most of our participants were White, which may limit the external validity of our findings. However, the large sample size and the multisite nature of our cohort also enhanced the generalizability of our results. Finally, our longitudinal GEE analysis enabled the analysis of all patients, including those with missing data. We have not yet corroborated the diagnosis of NAP with more specific diagnoses; this study is underway.

In conclusion, NAP, particularly regional, is common in early RA and negatively impacts remission at 1 year. Regional NAP persisted in nearly half of patients by 1 year of follow-up. Widespread NAP was less common at diagnosis, and it evolved into regional NAP or resolved in most of our early RA cohort by 1 year. Our findings raise the possibility of an RA-related mechanism for NAP that warrants further study. This study should alert clinicians to look for NAP in their patients with RA, which can potentially help tailor treatment and identify those at higher risk of not achieving treatment targets in the initial year of RA.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Meng confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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Efficacy and Safety of Sodium–Glucose Cotransporter 2 Inhibitors for the Primary Prevention of Cardiovascular, Renal Events, and Safety Outcomes in Patients With Systemic Lupus Erythematosus and Comorbid Type 2 Diabetes: A Population-Based Target Trial Emulation

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Objective. Patients with systemic lupus erythematosus (SLE) were excluded from sodium–glucose cotransporter 2 inhibitors (SGLT2i) clinical trials. It is unknown whether the cardiorenal benefits of SGLT2i extend to patients with SLE and comorbid type 2 diabetes (T2D).

Methods. We performed an emulated clinical trial in an insurance-based cohort in the United States, evaluating SGLT2i versus dipeptidyl peptidase-4 inhibitors (DPP4i) for primary prevention of cardiovascular, renal, and other clinical outcomes among patients with both SLE and comorbid T2D. SGLT2i initiators were matched to DPP4i initiators using propensity scores (PSs) based on clinical and demographic factors. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated using Cox models.

Results. Outcomes among 2,165 patients starting SGLT2i and 2,165 PS-matched patients starting DPP4i were compared. Over 753.1 (±479.2) mean days, SGLT2i recipients had significantly lower risks of incident acute kidney injury (HR 0.49, 95% CI 0.39–0.63), chronic kidney disease (HR 0.61, 95% CI 0.50–0.76), end-stage renal disease (HR 0.40, 95% CI 0.20–0.80), heart failure (HR 0.72, 95% CI 0.56–0.92), emergency department visits (HR 0.90, 0.82–0.99), and severe sepsis (HR 0.61, 95% CI 0.39–0.94). Risks of all-cause mortality (HR 0.89, 95% CI 0.65–1.21), lupus nephritis (HR 0.67, 95% CI 0.38–1.15), myocardial infarction (HR 0.81, 95% CI 0.54–1.23), stroke (HR 1.03, 95% CI 0.74–1.44), and hospitalizations (HR 0.76, 95% CI 0.51–1.12) did not differ. Genital infection risk (HR 1.31, 95% CI 1.07–1.61) was increased, but urinary tract infection risk (HR 0.90, 95% CI 0.79–1.03) did not differ. No significant difference was observed for diabetic ketoacidosis risk (HR 1.07, 95% CI 0.53–2.14) and fractures (HR 0.95, 95% CI 0.66–1.36).

Conclusion. In this emulated clinical trial, treatment with SGLT2i, compared to DPP4i therapy, was associated with significantly reduced risks of several cardiorenal complications among patients with both SLE and T2D.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a severe multisystem autoimmune disease with a high risk for renal and cardiovascular disease (CVD). Patients with SLE are at least twice as likely to develop stroke, myocardial infarction, and hypertension compared to the general population,[1] and up to half of patients with SLE experience renal involvement, most often lupus nephritis, which can progress to end-stage renal disease (ESRD) in up to a quarter of patients.[2–4] The coexistence of both SLE and type 2 diabetes (T2D) amplifies the risk of these complications, with studies indicating that the risk of ESRD is three

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times higher in patients with both conditions compared to those with SLE only.[5,6]

Sodium–glucose cotransporter 2 inhibitors (SGLT2i) and dipeptidyl peptidase-4 inhibitors (DPP4i) are two classes of glucose-lowering medications commonly used in the management of T2D. SGLT2i were initially designed as oral hypoglycemics because they induce glucosuria by blocking glucose reabsorption in the proximal tubule.[7] However, in trials focusing on CVD outcomes, SGLT2i were found to confer significant cardiovascular and renal benefits.[8,9] In patients with both T2D and established or high-risk atherosclerotic CVD, SGLT2i have been associated with decreased risks of CVD events and slower progression of kidney disease.[8,9]

In contrast, DPP4i, another class of oral hypoglycemic agents, work by preventing the degradation of glucagon-like peptide 1 (GLP1), which stimulates glucose-dependent insulin secretion and suppresses glucagon production,[10] resulting in a modest reduction in hemoglobin A1C (HbA1c). Although large CVD outcome trials have shown that DPP4i are safe regarding CVD outcomes compared to placebo, DPP4i did not appear to provide additional cardiovascular or renal benefits,[11,12] nor to affect mortality rates.[13]

The outcomes of patients with SLE following SGLT2i remain unclear. Large trials of SGLT2i have excluded patients with SLE and other autoimmune diseases due to hypothetical concerns about increased infectious risk from immunosuppression and difficulty discerning the effects of SGLT2i on renal outcomes from those of immunosuppression.[9,14] The potential therapeutic value of SGLT2i for patients with lupus nephritis has recently been proposed. A small pilot study of five patients with lupus nephritis showed significant improvement in renal outcomes after eight weeks of empagliflozin.[15] However, risks associated with SGLT2i treatment in patients with SLE have also been reported. An open-label six-month trial of dapagliflozin in 38 patients with SLE reported that half of the patients experienced adverse events, including SLE flares (18% of patients) and infections (10% of patients), leading to a 21% drug discontinuation rate.[16] No changes in proteinuria or estimated glomerular filtration rate (eGFR) were observed in the subgroup of 17 patients with lupus nephritis. Thus, the risk-benefit ratio of SGLT2i treatment for patients with SLE has not been established. While SGLT2i may offer favorable benefits on eGFR, proteinuria, heart failure, and CVD, these benefits could be potentially offset by an increased risk of serious infections in patients who are immunosuppressed, particularly genitourinary infections, such as Fournier gangrene.[9] We aimed to test the efficacy and safety of SGLT2i versus DPP4i for the primary prevention of cardiovascular and renal events, as well as risks for infections and other safety and mortality outcomes, among patients with both SLE and T2D who were newly treated with SGLT2i in an emulated target trial design.

PATIENTS AND METHODS

Study population. We used a large, insurance-based cohort with electronic health record data from 92 health care organizations across the United States (Diamond Network, TriNetX, LLC).[17–19] We identified patients ≥18 years old with both SLE and T2D by the *International Classification of Diseases, Tenth Revision* (ICD-10) codes (≥2 codes for M32 on separate days for SLE and ≥1 code of E11 for T2D) before starting SGLT2i (canagliflozin, dapagliflozin, empagliflozin, or ertugliflozin) or DPP4i (alogliptin, saxagliptin, linagliptin, or sitagliptin) between January 2016 and December 2020. Codes for identifying patients with SLE have been validated in prior studies.[20,21] The study emulated a target trial using an active comparator new user study design,[17,22,23] with a three-month washout period. The index date was the first prescription record of SGLT2i or DPP4i, and baseline period referred to the two years before the index date. Patients with existing lupus nephritis, type 1 diabetes, or aged <18 years at the index date were excluded (Supplementary Figure 1). To control for potential confounding by indication, propensity score (PS) matching was used to match two groups based on baseline covariates, including demographics, comorbidities, medications (antidiabetic agents), and laboratory data (Table 1).

Outcomes and follow-up. The outcomes of interest included primary renal outcomes, primary CVD outcomes, infectious outcomes, safety outcomes, and all-cause mortality. Renal outcomes included acute kidney injury (AKI), chronic kidney disease (CKD), ESRD, and glomerular disease in SLE, also defined as lupus nephritis. CVD outcomes included heart failure, myocardial infarction, and stroke. Infectious outcomes included urinary tract infection (UTI), genital infections, severe sepsis, and herpes zoster infection. Safety outcomes included emergency department visits, hospitalizations, diabetic ketoacidosis, new immunomodulator prescriptions as surrogates for lupus flare (e.g., mycophenolate mofetil and rituximab), and all-cause mortality, and fractures as a negative control. For both CVD and renal outcomes, those with evidence of these conditions during the baseline period before the index date were excluded separately in order to assess these agents for the primary prevention of each cardiovascular and renal disease. Various coding systems, including ICD-10, RxNorm, Healthcare Common Procedure Coding System (HCPCS), Current Procedure Terminology (CPT), and self-defined codes within TriNetX, were used to identify medical conditions and outcomes. Specifically, ICD-10 codes identified diagnoses, RxNorm and HCPCS codes determined medications, and CPT codes were used for procedures and medical services. The codes for identifying the outcomes are listed in Supplementary Table 1.

Statistical analyses. Baseline characteristics of the SGLT2i and DPP4i initiating groups were compared using mean (± standard deviation [SD]) for continuous variables and counts

Table 1. Baseline characteristics in patients with SLE with T2D initiating either SGLT2i or DPP4i*

	Before propensity score matching			After propensity score matching		
	SGLT2i (n = 2,464)	DPP4i (n = 4,761)	SMD	SGLT2i (n = 2,165)	DPP4i (n = 2,165)	SMD
Age at index, mean ± SD	57.6 ± 10.6	62.2 ± 11.8	0.409	58.2 ± 10.6	58.3 ± 11.5	0.002
Demographic, n (%)						
Female	2,210 (89.7)	4,231 (88.9)	0.027	1,937 (89.5)	1,952 (90.2)	0.023
Male	254 (10.3)	528 (11.1)	0.025	228 (10.5)	213 (9.8)	0.023
White	843 (34.2)	1,353 (28.4)	0.125	708 (32.7)	694 (32.1)	0.014
Black or African American	233 (9.5)	542 (11.4)	0.063	206 (9.5)	218 (10.1)	0.019
Asian	12 (0.5)	21 (0.4)	0.007	≤10 (0.5)	≤10 (0.5)	<0.001
Hispanic or Latino	169 (6.9)	328 (6.9)	0.001	153 (7.1)	149 (6.9)	0.007
Comorbidities, n (%)						
Cardiovascular diseases						
Acute myocardial infarction	66 (2.7)	165 (3.5)	0.046	58 (2.7)	56 (2.6)	0.006
Atrial fibrillation	114 (4.6)	361 (7.6)	0.124	99 (4.6)	113 (5.2)	0.030
Cerebrovascular diseases	244 (9.9)	689 (14.5)	0.140	218 (10.1)	208 (9.6)	0.016
Heart failure	257 (10.4)	798 (16.8)	0.186	228 (10.5)	217 (10.0)	0.017
Hypertension	1,829 (74.2)	3,805 (79.9)	0.136	1,612 (74.5)	1,624 (75.0)	0.013
Ischemic heart diseases	511 (20.7)	1,291 (27.1)	0.150	463 (21.4)	462 (21.3)	0.001
Cerebral infarction	244 (9.9)	689 (14.5)	0.140	218 (10.1)	208 (9.6)	0.016
Endocrine diseases						
T2D with diabetic kidney complications	79 (3.2)	282 (5.9)	0.130	72 (3.3)	60 (2.8)	0.032
T2D with diabetic neuropathy	394 (16.0)	795 (16.7)	0.019	339 (15.7)	311 (14.4)	0.036
T2D with ophthalmic complications	151 (6.1)	327 (6.9)	0.030	125 (5.8)	109 (5.0)	0.033
Renal diseases						
Acute kidney failure	128 (5.2)	622 (13.1)	0.276	122 (5.6)	111 (5.1)	0.023
CKD	276 (11.2)	1,214 (25.5)	0.376	255 (11.8)	227 (10.5)	0.041
Pulmonary diseases						
Chronic obstructive pulmonary disease	354 (14.4)	943 (19.8)	0.145	328 (15.2)	346 (16.0)	0.023
Pneumonia	202 (8.2)	544 (11.4)	0.109	183 (8.5)	175 (8.1)	0.013
Rheumatologic diseases						
Rheumatoid arthritis	116 (4.7)	250 (5.3)	0.025	101 (4.7)	101 (4.7)	<0.001
Systemic connective tissue disease	339 (13.8)	686 (14.4)	0.019	296 (13.7)	313 (14.5)	0.023
Sjogren's syndrome	185 (7.5)	355 (7.5)	0.002	159 (7.3)	166 (7.7)	0.012
Antiphospholipid syndrome	46 (1.9)	123 (2.6)	0.049	42 (1.9)	53 (2.4)	0.035
Systemic sclerosis	29 (1.2)	74 (1.6)	0.033	25 (1.2)	29 (1.3)	0.017
Autoimmune thyroiditis	48 (1.9)	65 (1.4)	0.046	40 (1.8)	40 (1.8)	<0.001
Other diagnoses						
Disorders of lipoprotein metabolism and other lipidemias	1,582 (64.2)	3,094 (65.0)	0.016	1,376 (63.6)	1,360 (62.8)	0.015
Long term (current) use of NSAIDs	73 (3.0)	113 (2.4)	0.037	66 (3.0)	65 (3.0)	0.003
Low socioeconomic status[a]	162 (6.6)	274 (5.8)	0.034	128 (5.9)	138 (6.4)	0.019
Nicotine dependence	344 (14.0)	608 (12.8)	0.035	301 (13.9)	291 (13.4)	0.013
Overweight and obesity	1,008 (40.9)	1,650 (34.7)	0.129	850 (39.3)	846 (39.1)	0.004
Rash and other nonspecific skin eruption	255 (10.3)	439 (9.2)	0.038	204 (9.4)	216 (10.0)	0.019
Tobacco use	379 (15.4)	717 (15.1)	0.009	320 (14.8)	316 (14.6)	0.005
Urinary tract infection	510 (20.7)	1,311 (27.5)	0.160	450 (20.8)	441 (20.4)	0.010

(Continued)

Table 1. (*Cont'd*)

	Before propensity score matching					After propensity score matching				
	SGLT2i (n = 2,464)		DPP4i (n = 4,761)		SMD	SGLT2i (n = 2,165)		DPP4i (n = 2,165)		SMD
Procedure, n (%)										
Inpatient consultations	71 (2.9)		176 (3.7)		0.046	62 (2.9)		65 (3.0)		0.008
Emergency department services	1,027 (41.7)		2,305 (48.4)		0.136	917 (42.4)		914 (42.2)		0.003
Medications, n (%)										
Aspirin	312 (12.7)		623 (13.1)		0.013	269 (12.4)		268 (12.4)		0.001
Ibuprofen	445 (18.1)		639 (13.4)		0.128	369 (17.0)		377 (17.4)		0.010
Naproxen	227 (9.2)		427 (9.0)		0.008	196 (9.1)		206 (9.5)		0.016
Glucocorticoids	1,518 (61.6)		2,980 (62.6)		0.020	1,332 (61.5)		1,342 (62.0)		0.010
Hydroxychloroquine	748 (30.4)		1,397 (29.3)		0.022	642 (29.7)		641 (29.6)		0.001
Azathioprine	114 (4.6)		209 (4.4)		0.011	96 (4.4)		98 (4.5)		0.004
Cyclophosphamide	≤10 (0.4)		≤10 (0.2)		0.035	≤10 (0.5)		≤10 (0.5)		<0.001
Mycophenolate mofetil	78 (3.2)		208 (4.4)		0.063	71 (3.3)		80 (3.7)		0.023
Leflunomide	70 (2.8)		119 (2.5)		0.021	58 (2.7)		62 (2.9)		0.011
Rituximab	≤10 (0.4)		28 (0.6)		0.026	≤10 (0.5)		≤10 (0.5)		<0.001
Methotrexate	228 (9.3)		393 (8.3)		0.035	193 (8.9)		200 (9.2)		0.011
Belimumab	31 (1.3)		43 (0.9)		0.034	25 (1.2)		24 (1.1)		0.004
Tacrolimus	17 (0.7)		86 (1.8)		0.101	15 (0.7)		≤10 (0.5)		0.031
Cyclosporine	83 (3.4)		185 (3.9)		0.028	76 (3.5)		79 (3.7)		0.007
Dulaglutide	176 (7.1)		66 (1.4)		0.288	65 (3.0)		60 (2.8)		0.014
Semaglutide	34 (1.4)		≤10 (0.2)		0.132	≤10 (0.5)		≤10 (0.5)		<0.001
Liraglutide	258 (10.5)		126 (2.6)		0.320	114 (5.3)		114 (5.3)		<0.001
Insulin	814 (33.0)		1,181 (24.8)		0.182	652 (30.1)		649 (30.0)		0.003
Metformin	1,436 (58.3)		2,376 (49.9)		0.169	1,221 (56.4)		1,264 (58.4)		0.040
Glimepiride	274 (11.1)		516 (10.8)		0.009	238 (11.0)		242 (11.2)		0.006
Pioglitazone	146 (5.9)		204 (4.3)		0.075	108 (5.0)		107 (4.9)		0.002
Glyburide	67 (2.7)		155 (3.3)		0.032	58 (2.7)		63 (2.9)		0.014
Glipizide	339 (13.8)		680 (14.3)		0.015	302 (13.9)		304 (14.0)		0.003
Measures and laboratories[b], mean ± SD or n (%)										
Body mass index	34.5 ± 5.5	1,092 (44.3)	32.8 ± 6.0	1,852 (38.9)	0.301	34.5 ± 5.4	911 (42.1)	33.7 ± 5.8	892 (41.2)	0.133
Hemoglobin A1c	8.0 ± 1.8	379 (15.4)	7.6 ± 1.6	514 (10.8)	0.278	7.9 ± 1.7	294 (13.6)	7.9 ± 1.6	286 (13.2)	0.038
Hemoglobin, g/dl	13.1 ± 1.6	288 (11.7)	12.8 ± 1.9	377 (7.9)	0.177	13.1 ± 1.5	220 (10.2)	13.1 ± 2.0	213 (9.8)	0.001
Creatinine in blood, mg/dL	1.2 ± 5.5	268 (10.9)	2.6 ± 11.2	367 (7.7)	0.155	1.3 ± 6.2	205 (9.5)	1.5 ± 7.7	200 (9.2)	0.021
Albumin in blood, g/dL	4.1 ± 0.4	266 (10.8)	4.0 ± 0.4	355 (7.5)	0.207	4.1 ± 0.4	206 (9.5)	4.0 ± 0.5	199 (9.2)	0.188
Glomerular filtration rate, mean (SD)	78.4 ± 22.0	277 (11.2)	66.3 ± 27.3	396 (8.3)	0.489	78.2 ± 22.3	212 (9.8)	75.6 ± 22.8	211 (9.7)	0.114

* Study population limited to patients with at least two diagnostic records for SLE before treatment with SGLT2i or DPP4i. CKD, chronic kidney disease; DPP4i, dipeptidyl peptidase 4-inhibitors; eGFR, estimated glomerular filtration rate; ICD-10, *International Classification of Diseases, Tenth Revision*; NSAID, nonsteroidal anti-inflammatory drug; PS, propensity scoring; SD, standard deviation; SGLT2i, sodium–glucose cotransporter 2 inhibitors; SLE, systemic lupus erythematosus; SMD, standardized mean difference; T2D, type 2 diabetes.

[a] ICD-10 code: Z55–Z65 (Persons with potential health hazards related to socioeconomic and psychosocial circumstances).

[b] Body mass index values at baseline available for 44.3% and 38.9% of patients treated with SGLT2i and DPP4i pre-PS matching and 42.1% and 41.2% post-PS matching. Hemoglobin A1c at baseline available for 15.4% and 10.8% of patients treated with SGLT2i and DPP4i pre-PS matching and 13.6% and 13.2% post-PS matching. Hemoglobin at baseline available for 11.7% and 7.9% of patients treated with SGLT2i and DPP4i pre-PS matching and 10.2% and 9.8% post-PS matching. Creatinine at baseline available for 10.9% and 7.7% of patients treated with SGLT2i and DPP4i pre-PS matching and 9.5% and 9.2% post-PS matching. Albumin at baseline available for 10.8% and 7.5% of patients treated with SGLT2i and DPP4i pre-PS matching and 9.5% and 9.2% post-PS matching. eGFR at baseline available for 11.2% and 8.3% of patients with SGLT2i and DPP4i pre-PS matching and 9.8% and 9.7% post-PS matching.

and percentages for categorical variables. PS was calculated using logistic regression to model the propensity of receiving either SGLT2i or DPP4i. PS matching was performed using greedy nearest-neighbor matching.[24] We matched new prescriptions of SGLT2i 1:1 to new prescriptions of DPP4i with a caliper width of 0.1 standardized mean difference (SMD) between the two groups to determine the adequacy of covariate balance; an SMD of <0.1 between cohorts was considered to be well matched.[22]

Hazard ratios (HRs) and 95% confidence intervals (CIs) were obtained by Cox proportional hazards models using an intent-to-treat approach. Patients were observed from the day after the index date to the occurrence of the first of each of the prespecified outcomes in separate analyses, death, loss to follow-up, or the end of the follow-up period (December 31, 2020). We performed an intent-to-treat analysis simulating past trials, in which patients were assigned to treatment group based on the first dispensing of either SGLT2i or DPP4i.[25] A sensitivity analysis that examined patients with three or more diagnostic records for SLE on separate days before or on the index date was conducted to assess using a more specific SLE definition. Given that outcomes of interest were associated with death, another sensitivity analysis considering death as a competing risk was also performed. Additionally, as race and ethnicity are known to affect the risks and severity of SLE, subgroup analysis stratified by race and ethnicity was also conducted. As the dataset contained only completely deidentified data, Institutional Review Board approval is not required for this study. The study was conducted in accordance with the Declaration of Helsinki. Data can be provided upon reasonable request.

RESULTS

Study population and baseline characteristics. We identified a total of 7,225 patients, with 2,464 receiving SGLT2i (mean 57.6, SD 10.6 years; 89.7% female) and 4,761 receiving DPP4i (mean 62.2, SD 11.8 years; 88.9% female) before PS matching (Table 1). Before PS matching, the SGLT2i group had a higher mean body mass index (BMI) and lower serum creatinine values and were less likely to have an existing diagnosis of AKI or CKD. After 1:1 PS matching, 2,165 patients starting SGLT2i were compared with 2,165 patients starting DPP4i, with most baseline characteristics well balanced with SMDs < 0.1 (Table 1).

Among matched patients starting SGLT2i versus DPP4i, mean ages were similar (58.2 versus 58.3 years, SMD 0.002), 89.5% versus 90.2% (SMD 0.023) were female, 11.8% versus 10.5% (SMD 0.041) had CKD, and 10.5% versus 10.0% (SMD 0.017) had heart failure at baseline. The mean eGFR was 78.2 versus 75.6 (SMD 0.114) in the two emulated trial arms. The proportion of using hydroxychloroquine was low in both groups (29.7% vs 29.6%, SMD 0.001), but a majority of both groups (61.5% vs 62.0%, SMD 0.010) had received one or more prescriptions for glucocorticoids in the past year and around 9% received methotrexate in the baseline period. Other immunosuppressants were administered as follows: around 4% of patients were treated with azathioprine, approximately 1% of patients were treated with belimumab, 0.5% of patients were treated with cyclophosphamide, less than 4% of patients were treated with cyclosporine, around 2.8% of patients were treated with leflunomide, approximately 3.5% of patients were treated with mycophenolate, less than 1% of patients were treated with tacrolimus, and 0.5% of patients were treated with rituximab. Mean BMIs were 34.5 versus 33.7 (SMD 0.133) and mean HbA1c values were 7.9 versus 7.9 (SMD 0.038).

Outcomes with prescription of SGLT2i versus DPP4i in patients with SLE and concomitant T2D. After a mean follow-up period of 753.1 (±479.2) years, SGLT2i versus DPP4i recipients had comparable all-cause mortality (HR 0.89, 95% CI 0.65–1.21) (Table 2). Renal outcomes showed reduced risks of AKI (HR 0.49, 95% CI 0.39–0.63), CKD (HR 0.61, 95% CI 0.50–0.76), and ESRD (HR 0.40, 95% CI 0.20–0.80), with no difference in the risk of incident lupus nephritis (HR 0.67, 95% CI 0.38–1.15). For CVD outcomes, no significant difference was observed for myocardial infarction (HR 0.81, 95% CI 0.54–1.23) or stroke (HR 1.03, 95% CI 0.74–1.44), but there was a reduced risk of heart failure (HR 0.72, 95% CI 0.56–0.92). SGLT2i prescription was also associated with reduced emergency department visits (HR 0.90, 95% CI 0.82–0.99) but no difference in hospitalization risk (HR 0.76, 95% CI 0.51–1.12). For infectious outcomes, the risk of severe sepsis was significantly lower among SGLT2i initiators (HR 0.61, 95% CI 0.39–0.94). Whereas there was an increased risk of genital infections (HR 1.31, 95% CI 1.07–1.61), the risks for UTIs (HR 0.90, 95% CI 0.79–1.03), and herpes zoster infection (HR 1.27, 95% CI 0.84–1.92) were similar. Importantly, no significant difference was observed in the risk of diabetic ketoacidosis (HR 1.07, 95% CI 0.53–2.14), fractures (HR 0.95, 95% CI 0.66–1.36), and new prescription of both mycophenolate (HR 1.00, 95% CI 0.62–1.63) and rituximab (HR 1.21, 95% CI 0.58–2.54) between SGLT2i and DPP4i prescription.

Sensitivity analysis. In repeated analyses using three or more diagnostic records for SLE to test the robustness of our administrative definition, comparing a total of 5,692 patients with both SLE and T2D, findings remained similar with comparable all-cause mortality (HR 0.71, 95% CI 0.50–1.00), as well as significantly reduced risks of AKI (HR 0.51, 95% CI 0.39–0.66), CKD (HR 0.67, 95% CI 0.52–0.86), and ESRD (HR 0.35, 95% CI 0.17–0.70), but there was no difference in the risk of incident lupus nephritis (HR 0.93, 95% CI 0.53–1.64). Similarly, there were again no differences in the risks for myocardial infarction (HR 0.90, 95% CI 0.57–1.40) or stroke (HR 0.98, 95% CI 0.67–1.41), and there was a reduced risk of heart failure (HR 0.68, 95% CI 0.52–0.90). Also, there was no difference in the risk of emergency

Table 2. Comparison of outcomes in patients with SLE with concomitant T2D prescribed SGLT2i versus DPP4i*

Outcomes	Before propensity score matching					After propensity score matching				
	SGLT2i (n = 2,464) Events	DPP4i (n = 4,761) Events	HR	95% CI	*P* value (log-rank)	SGLT2i (n = 2,165) Events	DPP4i (n = 2,165) Events	HR	95% CI	*P* value (log-rank)
All-cause mortality	72	364	0.464	(0.360–0.597)	<0.001	68	95	0.891	(0.652–1.217)	0.469
Renal outcomes										
Acute kidney failure	105	493	0.425	(0.345–0.525)	<0.001	94	222	0.493	(0.387–0.627)	<0.001
Chronic kidney disease	138	481	0.513	(0.424–0.619)	<0.001	126	239	0.614	(0.495–0.762)	<0.001
End-stage renal disease	13	108	0.273	(0.154–0.486)	<0.001	11	34	0.403	(0.204–0.796)	0.007
Lupus nephritis	24	82	0.657	(0.417–1.035)	0.068	20	36	0.665	(0.384–1.149)	0.141
Cardiovascular outcomes										
Myocardial infarction	42	147	0.647	(0.459–0.912)	0.012	37	55	0.812	(0.535–1.233)	0.328
Stroke	69	188	0.824	(0.625–1.087)	0.170	63	76	1.032	(0.738–1.442)	0.854
Heart failure	113	389	0.602	(0.488–0.743)	<0.001	102	170	0.717	(0.560–0.916)	0.008
Safety outcomes										
Emergency visits	833	2,126	0.803	(0.741–0.870)	<0.001	735	906	0.903	(0.819–0.995)	0.040
Hospitalization	50	139	0.813	(0.588–1.124)	0.209	41	65	0.758	(0.513–1.122)	0.165
Genital infection	240	315	1.740	(1.471–2.059)	<0.001	193	175	1.308	(1.066–1.606)	0.010
Urinary tract infection	464	1,295	0.759	(0.683–0.844)	<0.001	400	511	0.899	(0.789–1.025)	0.111
Severe sepsis	36	172	0.476	(0.332–0.682)	<0.001	31	62	0.607	(0.394–0.935)	0.022
Herpes zoster infection	55	119	1.034	(0.751–1.424)	0.837	47	44	1.270	(0.841–1.917)	0.255
Diabetic ketoacidosis	19	53	0.831	(0.492–1.404)	0.488	15	17	1.065	(0.531–2.135)	0.860
Fracture	58	187	0.694	(0.517–0.932)	0.015	53	68	0.949	(0.662–1.360)	0.777
New mycophenolate mofetil use	33	87	0.826	(0.553–1.233)	0.348	30	36	1.000	(0.615–1.625)	0.999
New rituximab use	14	22	1.489	(0.761–2.914)	0.241	14	14	1.211	(0.576–2.543)	0.613

* The study population was limited to patients with at least two diagnostic records for SLE before the index date. CI, confidence interval; DPP4i, dipeptidyl peptidase-4 inhibitors; HR, hazard ratio; SGLT2i, sodium–glucose cotransporter 2 inhibitors; SLE, systemic lupus erythematosus; T2D, type 2 diabetes.

department visits (HR 0.93, 95% CI 0.83–1.04) and hospitalizations (HR 0.92, 95% CI 0.61–1.39). Increased risk of genital infections (HR 1.61, 95% CI 1.28–2.01) persisted, but no significant difference was observed for severe sepsis (HR 0.68, 95% CI 0.42–1.10), UTIs (HR 0.92, 95% CI 0.80–1.06), and herpes zoster infection (HR 0.95, 95% CI 0.62–1.46). There was also no difference in the risk of diabetic ketoacidosis (HR 0.77, 95% CI 0.40–1.47), fractures (HR 0.78, 95% CI 0.53–1.16), and new prescription of both mycophenolate (HR 0.94, 95% CI 0.56–1.57) and rituximab (HR 1.43, 95% CI 0.62–3.31) in the sensitivity analysis (Supplementary Table 2). In the analysis considering competing risk of death, the effect of SGLT2i on reduced risks of AKI (HR 0.58, 95% CI 0.47–0.72), CKD (HR 0.72, 95% CI 0.60–0.88), and ESRD (HR 0.74, 95% CI 0.56–0.98) persisted (Supplementary Table 3).

Subgroup analysis. Among patients who were reported to be White, the reduced risks of AKI (HR 0.53, 95% CI 0.36–0.79) and CKD (HR 0.56, 95% CI 0.38–0.81) associated with SGLT2i persisted. In patients who were reported to be Black or African American, the reduced risks of CKD (HR 0.34, 95% CI 0.15–0.75) and heart failure (HR 0.36, 95% CI 0.15–0.85) with SGLT2i persisted, whereas other outcomes did not reach statistical significance (Supplementary Table 4).

DISCUSSION

In this head-to-head emulated target trial of SGLT2i versus DPP4i for patients with both SLE and T2D within in a large, US insurance-based cohort, treatment with SGLT2i was associated with strikingly and significantly reduced risks of AKI, CKD, ESRD, heart failure, and emergency department visits. The risk of genital infection increased by 30%, while risks of UTIs, herpes zoster, and sepsis were not. This suggests that the known renal and cardiovascular benefits of SGLT2i in T2D translate to patients with SLE, who are at high risk for these comorbid outcomes.

The reno-protective benefits of SGLT2i were first discovered in trials involving patients with T2D who had established or high risk for atherosclerotic CVD. The Canagliflozin Cardiovascular Assessment Study trial demonstrated that canagliflozin reduced the progression of albuminuria,[8] and post hoc analysis of the Empagliflozin, Cardiovascular Outcomes, and Mortality in T2D trial showed that empagliflozin was associated with reduced risks for nephropathy progression, doubling of serum creatinine, and transition to renal replacement therapy.[26] Heerspink et al also reported that SGLT2i was associated with slower eGFR decline in patients with T2D,[27] and Au et al demonstrated a reduced risk of ESRD and acute renal failure with SGLT2i compared to DPP4i.[28] In our cohort of patients with both SLE and T2D, SGLT2i

were also found to reduce the risks of AKI, CKD, and ESRD by over 30%.

Subsequent renal outcome trials suggested that the benefits of SGLT2i extend to CKD patients with CKD without T2D. The Dapagliflozin and Prevention of Adverse Outcomes in CKD (DAPA-CKD) trial, while enrolled patients with albuminuric CKD regardless of T2D status, found that dapagliflozin significantly reduced a composite outcome of eGFR decline, ESRD, and renal death.[14] The kidney-protective mechanisms of SGLT2i are not fully understood. Initially, there was concern that SGLT2i might increase AKI because of the observed initial dip of eGFR,[29] but subsequent studies have demonstrated this was likely because of a reduction of glomerular pressure that was reversible with cessation of SGLT2i.[26] The risk of AKI has been reported to be lower with SGLT2i prescription in trials and meta-analyses,[30–32] consistent with our findings. Several kidney protective mechanisms of SGLT2i have been suggested, including the induction of a metabolic shift, reduction of inflammation, and decrease of glomerular hyperfiltration.[33] The natriuresis that accompanies the osmotic diuresis by SGLT2i can lead to reduced glomerular pressure and glomerular hyperfiltration, resulting in slower kidney disease progression.[34] The kidney protective effect of SGLT2i may also act through a glucose-independent mechanism. This is supported by the findings from the DAPA-CKD trial, which showed that the cardiovascular and kidney protective effects persisted in patients without diabetes.[14] In a preclinical study, treatment with empagliflozin in lupus-prone MRL/lpr mice was associated with decreased levels of anti-double-stranded DNA antibodies, creatinine, and proteinuria, as well as improved preservation of glomerular and tubulointerstitial structure. Additionally, activation of complement and the phosphatidylinositol 3-kinase/protein kinase B/mechanistic target of rapamycin pathway were also markedly reduced.[35] A recently published study in a subset of the TriNetX database also investigated renal and cardiovascular outcomes among patients with both SLE and T2D who reported a more striking reduced risk of lupus nephritis, dialysis, and death among 1,775 SGLT2i prescriptions compared to 1,775 with those not prescribed SGLT2i.[36] However, their study was susceptible to confounding by indication due to lack of an active comparator, leading to significant differences between the two groups at baseline. Different index dates were used for those prescribed SGLT2i (date of first prescription) and those not receiving them (date of either SLE or T2D diagnosis, whichever was first), likely leading to an important imbalance in the chronicity and severity of underlying SLE and T2D. Even after PS matching, many factors were not well balanced between the two groups, including the presence of CKD and small vessel atherosclerotic disease, as well as that of all concurrent medication use. For example, in their study after PS matching, the use of GLP1 agonists and DPP4 inhibitors among SGLT2i prescriptions versus those not using them was 16.4% versus 4.5% (SMD 0.399) and 11.7% versus 4.8% (SMD 0.251).

CVD, hospitalization, and mortality outcomes. Early studies on the cardiovascular benefits of SGLT2i were primarily limited to T2D with established or high risk for atherosclerotic CVD.[8,9] Subsequent studies suggested that the cardiovascular benefits of SGLT2i, in particular on heart failure, may extend to patients without known CVD.[37,38] In our head-to-head comparison among patients with SLE and T2D, SGLT2i versus DPP4i initiation was associated with a reduced risk of heart failure but not with reduced all-cause mortality, myocardial infarction, or stroke. SGLT2i may reduce CVD risks, in particular that of heart failure, common in patients with SLE and those with T2D, through several proposed mechanisms, including lowering myocardial oxygen demand by inhibiting the sympathetic nervous system, increasing circulating ketones to provide additional fuel source for the heart, reducing intraventricular volumes and blood pressure through promoting osmotic diuresis, increasing myocardial oxygen supply through higher hemoglobin level by increasing erythropoietin, and potentially through the attenuation of inflammation.[39] All these mechanisms are being actively investigated and should be examined in patients with SLE.

Infectious outcomes. In our study, the use of SGLT2i was associated with increased risks of genital infections. The increased risk of genital infections in those with T2D taking SGLT2i has been reported consistently in several other trials and studies and is attributed to glucosuria.[22,32] Interestingly, no increased risk was observed for UTIs and diabetic ketoacidosis following SGTL2i. Historically, SGLT2i were reported to be associated with increased risks of both conditions, prompting the US Food and Drug Administration to add warnings in 2015. The reasons for the observed increased risks of genital infections but no difference in the risk of UTIs in our study are unclear. Recent studies have also shown inconsistent results regarding the risk of UTIs when comparing SGLT2i to other second-line hypoglycemic agents (DPP4i or GLP1 agonists). A large cohort study[40] reported no significant difference in UTI risk between SGLT2i and DPP4i (HR 0.96, 95% CI 0.89–1.04) in one cohort and even a reduced risk of UTI when comparing SGLT2i to GLP1 receptor agonists (HR 0.91, 95% CI 0.84–0.99) in another cohort. However, the authors acknowledged that these findings may be attributable to chance or residual confounding factors, including differences in clinical practice; for example, patients with a history of UTIs may be less likely to receive SGLT2i. In this study, we also controlled for risk factors for UTIs, including sex, history of UTIs, and use of immunosuppression. We also did not observe a greater risk of severe sepsis, herpes zoster infection, fractures, or new prescription of mycophenolate or rituximab in patients taking SGLT2i versus DPP4i.[22,41]

Limitations. This study has several limitations. First, baseline covariates and outcomes were identified through billing codes, and variations in coding practices among physicians

could introduce misclassification bias that could affect the validity of the results.[42] Second, we lacked data on the duration of SLE and T2D before the initiation of the oral hypoglycemics studied. Given the large sample size, it is likely that these durations were similar in the two groups, but there may have been some imbalance for which we did not account. This is also true for SLE disease severity and activity measures, which we were unable to assess at baseline or follow-up, although we did include baseline SLE immunosuppressant medications in our PS matching. For the same reason, we were unable to observe patients on therapy for a specific follow-up period or perform an as-treated analysis, censoring patients when they were no longer receiving therapy. However, we examined the distribution of the follow-up, and more than 90% of the patients in each group had a follow-up of ≥60 months.

Third, results of renal biopsies establishing the diagnosis of lupus nephritis were not available for this analysis. Although prior studies have shown that ICD-9 and ICD-10 codes for lupus nephritis have acceptably high positive predictive value and specificity for lupus nephritis,[43,44] biopsy information might provide further insights into the role of SGLT2i in reducing the risk of lupus nephritis, and it would be interesting to see if the distribution of incident lupus nephritis biopsy classes was shifted as well among those taking SGLT2i versus DPP4i. Given overlapping billing codes for CKD and ESRD, it was also not possible to assess whether the reduction in these renal outcomes was because of decreased risk or progression of lupus nephritis or diabetic nephropathy. Lastly, compared with a clinical trial, the emulated trial is more susceptible to residual confounding factors because of the absence of true randomization.

SGLT2i versus DPP4i therapy for glucose control in patients with both T2D and SLE was associated with a reduced risk of incident AKI, CKD, ESRD, and heart failure in this large, US-based emulated clinical trial with several years of data in which outcomes of several thousand comparable patients were assessed. With known cardiorenal benefits and potential effects on reducing ESRD and heart failure, SGLT2i could become a new, widely accepted add-on therapy for SLE. Prospective studies and clinical trials are warranted to validate and extend these findings to patients with SLE without T2D.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Ma, Dr. Lo, and Dr. Costenbader had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ma, Lo, Kyttaris, Tsokos, Costenbader.

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FoxO1 Deficiency in Monocytic Myeloid-Derived Suppressor Cells Exacerbates B Cell Dysfunction in Systemic Lupus Erythematosus

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Objective. Myeloid-derived suppressor cells (MDSCs) contribute to the pathogenesis of systemic lupus erythematosus (SLE), in part due to promoting the survival of plasma cells. FoxO1 expression in monocytic MDSCs (M-MDSCs) exhibits a negative correlation with the SLE Disease Activity Index score. This study aimed to investigate the hypothesis that M-MDSC–specific FoxO1 deficiency enhances aberrant B cell function in aggressive SLE.

Methods. We used GEO data sets and clinical cohorts to verify the clinical significance of FoxO1 expression and circulating M-MDSCs. Using Cre-LoxP technology, we generated myeloid FoxO1 deficiency mice (m*FoxO1*$^{-/-}$) to establish murine lupus–prone models. The transcriptional stage was assessed by integrating chromatin immunoprecipitation (ChIP)–sequencing with transcriptomic analysis, luciferase reporter assay, and ChIP–quantitative polymerase chain reaction. Methylated RNA immunoprecipitation sequencing, RNA sequencing, and CRISPR-dCas9 were used to identify N^6-adenosine methylation (m^6A) modification. In vitro B cell coculture experiments, capmatinib intragastric administration, m^6A-modulated MDSCs adoptive transfer, and sample validation of patients with SLE were performed to determine the role of FoxO1 on M-MDSCs dysregulation during B cell autoreacted with SLE.

Results. We present evidence that low FoxO1 is predominantly expressed in M-MDSCs in both patients with SLE and lupus mice, and mice with myeloid FoxO1 deficiency (m*FoxO1*$^{-/-}$) are more prone to B cell dysfunction. Mechanically, FoxO1 inhibits mesenchymal-epithelial transition factor protein (Met) transcription by binding to the promoter region. M-MDSCs FoxO1 deficiency blocks the Met/cyclooxygenase2/prostaglandin E_2 secretion pathway, promoting B cell proliferation and hyperactivation. The Met antagonist capmatinib effectively mitigates lupus exacerbation. Furthermore, alkB homolog 5 (ALKBH5) targeting catalyzes m^6A modification on *FoxO1* messenger RNA in coding sequences and 3' untranslated regions. The up-regulation of FoxO1 mediated by ALKBH5 overexpression in M-MDSCs improves lupus progression. Finally, these correlations were confirmed in untreated patients with SLE.

Conclusion. Our findings indicate that effective inhibition of B cells mediated by the ALKBH5/FoxO1/Met axis in M-MDSCs could offer a novel therapeutic approach to manage SLE.

INTRODUCTION

Myeloid-derived suppressor cells (MDSCs), comprising a diverse population including monocytic MDSCs (M-MDSCs) and granulocytic/polymorphonuclear MDSCs (G-MDSCs), are increasingly implicated in the pathogenesis of systemic lupus erythematosus (SLE). Depletion of the total MDSCs results in significantly milder lupus symptoms, characterized by lower levels of serum anti–double-stranded DNA antibodies and reduced proteinuria, underscoring the indispensable role of MDSCs in vivo.[1,2] B cells are essential for autoantibody-mediated inflammatory responses in SLE.[3] MDSCs could directly promote the

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survival of plasma cells by providing B cell activating factor, which exacerbates lupus autoimmunity.[4,5] This suggests that MDSCs may be an important cell population affecting adaptive immunity in lupus.

In addition to their well-known immunosuppressive activity, MDSCs have been identified by our laboratory and others to possess certain proinflammatory effects, exacerbating chronic inflammation and promoting immune disorder in patients with SLE and in murine lupus models.[1,2,6,7] Interestingly, it has been observed that MDSCs can transition into the M-MDSC subtype and acquire proinflammatory characteristics.[8,9] Recent studies have indicated that M-MDSCs are expanded, rather than inflammatory monocytes, in lupus mice, newly diagnosed patients with SLE, and individuals with cutaneous lupus, with their abundance positively correlating with disease severity.[10–12] Furthermore, analysis of peripheral blood samples from pristane-induced lupus mice reveals a significant increase in the proportion of M-MDSC subgroups, by a reduction in G-MDSCs within $CD11b^+$ myeloid cells.[13] These findings suggest a significant involvement of M-MDSCs in autoimmune inflammation in SLE, but the key factors governing their augmentation and function remain largely elusive.

FoxO1 has recently been indicated to have abnormally low gene expression in peripheral blood mononuclear cells (PBMCs), which is correlated with increasing lupus activity.[14,15] Furthermore, a significant reduction in FoxO1 expression in spleen cells of lupus mice leads to increased production of interferon-γ, a crucial pathogenic cytokine in lupus.[16] Based on an analysis of peripheral blood chips from patients with lupus from the NCBI–GEO database, FoxO1 has been hypothesized to serve as a novel marker or therapeutic target for diagnosing and treating SLE.[17] In our clinical analysis, FoxO1 expression in M-MDSCs exhibits a negative correlation with the SLE Disease Activity Index (SLEDAI) score.[18] Further, the up-regulation of FoxO1 promotes the differentiation of M-MDSCs into M2 macrophages in lupus-like diffuse lung injury.[19] However, the precise role of FoxO1 in modulating M-MDSCs in lupus remains incompletely understood.

N^6-adenosine methylation (m^6A) modification stands as the most prevalent internal alteration detected in the messenger RNA (mRNA) of eukaryotes and governs gene expression by influencing mRNA splicing, nuclear export, translation, and decay.[20] Clinical investigations have unveiled that the lower overall m^6A level and decreased m^6A methylation modulatory genes (methyltransferase-like 3 [*METTL3*], methyltransferase-like 14 [*METTL14*], Wilms tumor 1-associating protein [*WTAP*], alkB homolog 5 [*ALKBH5*], fat mass and obesity-associated protein [*FTO*], etc) have been noted in patients with SLE compared to controls.[21–23] Furthermore, the inhibition of *METTL3* contributes to SLE pathogenesis by participating in the activation of $CD4^+$ T cells and the imbalance of Teff cell differentiation.[24] In addition, some investigations have highlighted diverse effects of m^6A modification on *FoxO1* mRNA, not only enhancing translation but also promoting degradation.[25,26] Collectively, these studies lend strong support to the notion of a novel role of m^6A modification on FoxO1 in the onset and progression of SLE.

Here, we report that human and murine M-MDSCs subsets display unique and distinct FoxO1 signatures, strongly correlated with SLE disease severity. We further demonstrate myeloid-specific FoxO1 knockout induces a large population of M-MDSCs, weakens immunosuppressive activity, exacerbates B cell dysfunction, and worsens lupus mice autoimmune phenotypes. Of note, we uncover a new negative regulatory effect of M-MDSC–derived FoxO1, which is regulated by ALKBH5 m^6A modification, leading to its deficiency in promoting mesenchymal-epithelial transition factor protein (Met) transcription and exacerbating B cell impairment by blocking the cyclooxygenase2 (COX-2)/prostaglandin E_2 (PGE_2) secreting pathway. We also highlight the therapeutic potential of targeting Met in M-MDSCs by capmatinib (Cap) in the context of SLE.

MATERIALS AND METHODS

Mice and treatments. Female C57BL/6, MRL/MpJ, and MRL/*lpr* mice were procured from Cavens Biotechnology Co, Ltd. Gene-edited mice were obtained from GemPharmatech Co, Ltd. *FoxO1* floxed (*FoxO1*$^{f/f}$) mice were bred with myeloid-specific *Lyz2*-Cre (*Lyz2*^{Cre}) mice to produce *FoxO1*$^{f/f}$*Lyz2*^{Cre} (m*FoxO1*$^{-/-}$) mice, with *FoxO1*$^{f/f}$ littermates serving as controls. Murine genotypes were determined via polymerase chain reaction (PCR) analysis of tail-snip DNA (Supplementary Table 1). Mice were housed under specific pathogen-free conditions with a 12:12-hour light–dark cycle. All animal experiments were approved by Institutional Animal Care and Use Committee, of the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2023AE01036), and all experimental procedures were performed in accordance with Brazilian Federal Law 1.794/2008 for the scientific use of animals.

The following mouse models were induced by random grouping. For imiquimod (IMQ)–induced lupus model, mice received 1.25 mg of 5% IMQ cream on the right ear three times weekly. Starting four weeks after IMQ induction, mice were orally administered 10 mg/kg of Cap (MCE) or vehicle every other day. Adoptive transfer was conducted four weeks after IMQ induction, with 1.2×10^6 MDSCs transfected with overexpression of ALKBH5 plasmid or control, resuspended in 100 μL of phosphate buffered saline (PBS), and injected via tail vein every two weeks. Mice were euthanized eight weeks after induction. For the pristane-induced lupus model, C57BL/6 mice received a single intraperitoneal injection of 0.5 mL of pristane or PBS and were euthanized after seven months. For the spontaneous lupus model, MRL/MpJ and MRL/*lpr* mice were euthanized at 19–20 weeks of age. MRL/*lpr* mice were orally administered 10 mg/kg of Cap or vehicle every other day at 14 weeks of age and euthanized at 20 weeks. In transgenic mice expressing human B cell

activation factor (hBAFF), mice were orally administered 10 mg/kg of Cap or vehicle every other day starting at 8 weeks and euthanized after 14 weeks. Euthanasia was performed by asphyxiation via carbon dioxide.

Human samples. A total of 93 patients diagnosed with SLE were recruited from the Department of Rheumatology, Nanjing Drum Tower Hospital (Nanjing, China), and 20 healthy volunteers were prospectively enrolled as normal controls (Supplementary Tables 2, 3, and 4). All patients with SLE met the revised criteria set forth by the American College of Rheumatology[27] in 1997. Exclusion criteria included other autoimmune diseases, familial hyperlipidemia or thyroid disease history, diabetes mellitus, other rheumatic diseases, and administration of lipid-lowering agents or thyroid medications. Disease activity among patients was assessed using the SLEDAI. This study was approved by the ethics committee at the Affiliated Drum Tower Hospital of Nanjing University Medical School (No.2022-563-02) and conducted in accordance with the principles outlined in the Declaration of Helsinki. On enrollment, patients underwent standardized medical history assessments, laboratory tests, and analyses, all conducted at the clinical laboratory of Nanjing Drum Tower Hospital.

Statistical analysis. Data are presented as means ± SDs. Experiments were independently repeated at least three times. The statistical significance of two groups' comparison was assessed using Student's *t*-test or the Mann-Whitney U-test. One-way analysis of variance (ANOVA) or two-way ANOVA was used when there were more than two groups. Moreover, we established the binary logistic regression models, and next used the Pearson's test for the correlation analysis, and calculated sensitivity, specificity, and area under the curve (AUC) by receiver operating characteristic (ROC) analysis. Statistical analyses were conducted using SPSS16.0 software and GraphPad Prism version 4.3, considering a P value less than 0.05 as significant.

RESULTS

Correlation of FoxO1 expression with M-MDSCs expansion in patients with SLE and lupus mice models. To gain insight into the abundance of FoxO1 in SLE MDSCs, we initially examined a public GEO data set (Supplementary Table 5) to assess the presence of FoxO1 between patients with SLE and healthy controls (HCs). As depicted in Supplementary Figure 1A, the transcription level of FoxO1 significantly decreased in peripheral blood cells or PBMCs of patients with SLE. FoxO1 expression negatively correlated with the myeloid marker CD33 and the universal M-MDSC classification marker CD14 in patients with SLE (Supplementary Figure 1B).

Furthermore, a cohort of 20 patients with SLE and 20 healthy individuals we recruited (Supplementary Table 2) revealed a significant increase in circulating M-MDSCs (defined as $CD14^+/CD66b^-/CD11b^+/HLA\text{-}DR^{low}$) frequency in patients with SLE ($P < 0.0001$), whereas G-MDSCs (defined as $CD14^-/CD66b^+/CD11b^+/HLA\text{-}DR^{low}$) didn't change significantly (Supplementary Figure 1C, D). Importantly, FoxO1 expression was significantly down-regulated in M-MDSCs from patients with SLE ($P < 0.0001$) and negatively correlated with CD14 ($P = 0.0423$, $R = -0.4580$). However, differences in FoxO1 expression in G-MDSCs and its correlation with CD66b were not observed (Supplementary Figure 1E, F). Subsequently, the mean fluorescence intensity of FoxO1 in M-MDSCs was significantly negatively correlated with SLEDAI ($P = 0.0356$, $R = -0.4721$) and IgG levels ($P = 0.0014$, $R = -0.6635$) (Supplementary Figure 1G), indicating its clinical significance in SLE. Additionally, an ROC curve was plotted, and the AUC value reached 0.9476 (95% confidence interval 0.885–1.000), suggesting good diagnostic value for SLE (Supplementary Figure 1H).

The response of M-MDSCs FoxO1 expression to SLE progression was examined in Toll-like receptor 7 (TLR7) agonist (R848)–induced bone marrow-derived MDSCs (BM-MDSCs) and three murine lupus models (MRL/*lpr*, IMQ, and pristane). We found that only M-MDSCs (defined as $CD11b^+Gr\text{-}1^{low}Ly6C^{high}$), not G-MDSCs (defined as $CD11b^+Gr\text{-}1^{high}Ly6C^{low}$), consistently exhibited reduced proportions and down-regulation of FoxO1 (Supplementary Figures 1I–L, Supplementary Figures 2A–C and 4H). The hallmark function of MDSCs in pathologic settings is the suppression of T cell activity, and we observed that BM-MDSCs immunosuppressive function on $CD4^+$ T cell proliferation was significantly impaired in lupus disease (Supplementary Figure 1M). Transfection of FoxO1 small interfering RNA (siRNA) significantly increased the proportion of M-MDSCs and attenuated inhibitory effect on $CD4^+$ T cell proliferation (Supplementary Figure 1N–P). These findings suggest that decreased FoxO1 is involved in the accumulation of M-MDSCs during SLE pathogenesis.

Myeloid FoxO1 deficiency and lupus development of TLR7-induced mice and the generation of M-MDSCs with diminished immunosuppressive capacity. To further explore the impact of FoxO1 in M-MDSCs during the lupus pathogenic process, we generated C57BL/6 mice with myeloid-specific FoxO1 knockout using the Cre-LoxP system, thereby inhibiting FoxO1 expression in MDSCs. These mice, designated as m$FoxO1^{-/-}$, were compared with control mice ($FoxO1^{f/f}$) lacking the knockout (Supplementary Figure 3A and B). We confirmed FoxO1 down-regulation in the targeted MDSC population without affecting FoxO1 expression profile in lymphocytes (Supplementary Figure 3C and D). Subsequently, we induced lupus-like symptoms in these mice by applying IMQ to assess the role of FoxO1 on M-MDSCs generation and their contribution to SLE progression. IMQ-treated m$FoxO1^{-/-}$ mice exhibited reduced survival rates; more severe splenomegaly; elevated

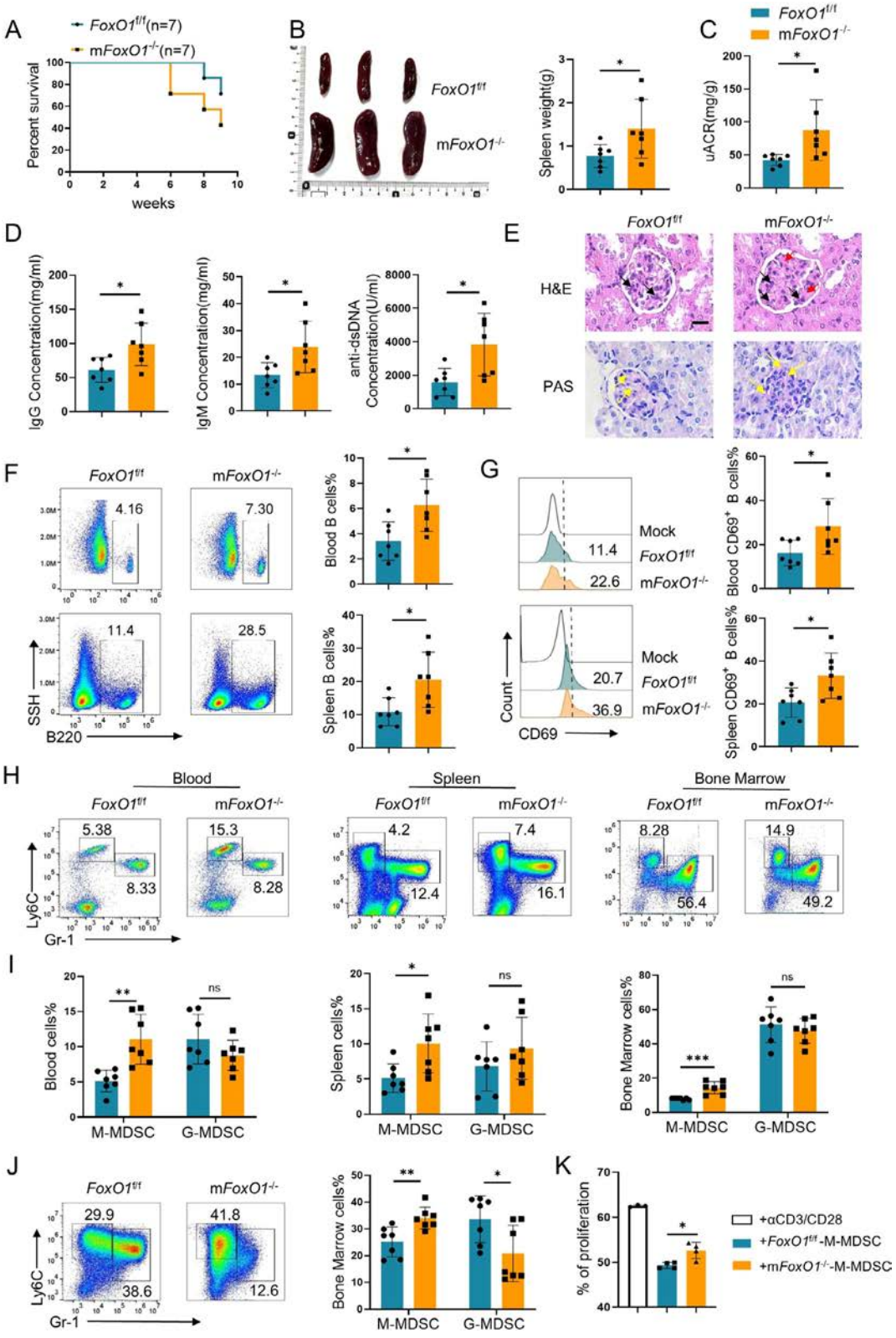

Figure 1. Myeloid FoxO1 deficiency exacerbates the development of Toll-like receptor 7–induced lupus mice and promotes the generation of M-MDSCs with diminished immunosuppressive capacity. *FoxO1*$^{f/f}$ (n = 7) and m*FoxO1*$^{-/-}$ (n = 7) were used to establish imiquimod-induced lupus mice. (A) Survival rate of mice recorded. (B) Representative photographs of spleens and spleen weights. (C and D) The uACRs and serum levels of total IgG, IgM, and anti-dsDNA measured using enzyme-linked immunosorbent assay. (E) H&E and PAS staining of kidney sections (scale bar = 20 μ*M*). (F and G) Flow cytometry analysis detected the proportion and activation level of B cells. (H and I) Percentages of

(Figure legend continues on next page.)

serum IgG, IgM, and double-stranded DNA (dsDNA) levels; increased urinary albumin/creatinine ratios (uACRs); and aggravated renal histopathology compared to *FoxO1*$^{f/f}$ mice (Figure 1A–E).

Moreover, myeloid FoxO1 knockout accelerated B cell dysfunction, as evidenced by increased proportions and absolute numbers of B220$^+$ B cells and CD69$^+$ activation marker (Figure 1F and G and Supplementary Figure 4A and B). These observations indicated that myeloid FoxO1 deficiency significantly exacerbated lupus symptoms. Crucially, FoxO1-deficient myeloid-derived cells increased M-MDSC frequency in the blood, spleen, and bone marrow (Figure 1H and I and Supplementary Figure 4C). In vitro induction experiments with BM-MDSCs further confirmed that FoxO1 absence significantly enhanced M-MDSCs differentiation, leading to a notable reduction in their immunosuppressive function on CD4$^+$ T cell proliferation (Figure 1J and K). Furthermore, we extended our findings to the pristane-induced lupus model, which faithfully recapitulates SLE symptoms in vivo (Supplementary Figure 4D–H). Thus, myeloid FoxO1 deficiency indeed increased M-MDSC differentiation and diminished immunosuppressive capacity.

Met as a direct target gene of FoxO1, the deficiency of which triggers Met transcription in lupus M-MDSC subsets. FoxO1 serves as a pivotal transcription factor specific to innate inflammation.[28] To explore the regulation mechanism of FoxO1 in M-MDSCs, we isolated splenic M-MDSCs from *FoxO1*$^{f/f}$ and m*FoxO1*$^{-/-}$ lupus mice and conducted RNA sequencing (RNA-seq) to identify FoxO1-regulated key modulatory molecules. The heat map revealed differential expression of approximately 3,896 genes, including 1,922 up-regulated and 1,974 down-regulated genes, with well-known genes such as *Met*, *Cxcl9*, *Ccl2*, and *Ccr7* (Supplementary Table 6). To further elucidate the mechanisms underlying FoxO1 regulation of target genes, we performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis in 293T cells and identified 3,260 genes in FoxO1-binding promoter regions (Supplementary Table 7), which were integrated with genes differentially expressed in M-MDSCs from lupus mice and genes significantly changed in R848-induced BM-MDSCs (Supplementary Table 8). This analysis identified 21 potential downstream regulatory genes, including *Met*, epsin 2 (*Epn2*), *Ffar1*, *Cd200*, and *Nrg2* (Supplementary Table 9). Gene Set Enrichment Analysis indicated that FoxO1 knockdown triggered an up-regulated inflammatory response (Supplementary Table 10).

Through further literature review, we identified two candidate genes, *Met* and *Epn2*, for subsequent validation (Figure 2A). After verifying their differential expression in vitro (Figure 2B), we obtained the FoxO1 potential binding sequences of mouse *Met* and *Epn2* genes through ChIP-seq data analysis and homologous alignment. ChIP-quantitative PCR (qPCR) confirmed the binding of FoxO1 at the *Met* and *Epn2* promoters (Figure 2C and D). Dual luciferase reporter assays demonstrated that R848 stimulation enhanced transcriptional activity of *Met*, not *Epn2*, and showed FoxO1 dose-dependent inhibition (Figure 2E and F). According to the significant enrichment of FoxO1 in the promoter region of human *Met* gene (Figure 2G), we predicted a potential FoxO1 binding site (TGGAAAAAGCT) within the mouse *Met* gene sequence Chr6: 17419562-17419947. Mutation and deletion of this site resulted in no difference in luciferase activity, indicating the importance of this motif for FoxO1-mediated transcriptional inhibition of Met on R848 stimulation (Figure 2H, I).

Met, a transmembrane tyrosine kinase receptor, is involved in various biologic processes such as cerebellar development, hematopoietic differentiation, and tissue inflammation by transducing signals from extracellular matrix into cytoplasm on binding to hepatocyte growth factor.[29–31] Our findings showed higher Met levels in lupus-derived M-MDSCs, which further increased with FoxO1 deficiency (Figure 2J and K). The siRNA–mediated the down-regulation of Met in MDSCs resulted in a reduction in the proportion of M-MDSCs and an alleviation of the differentiation process induced by FoxO1 loss. Notably, this effect was not observed in G-MDSCs (Figure 2L and M). Met down-regulation also reversed the promotion effect of FoxO1 knockout on T cell proliferation (Figure 2N). These results indicated that the region Chr6: 17419774-17419785 of the *Met* gene is bound by the transcription repressor FoxO1, providing a mechanism by which FoxO1 deficiency leads to *Met* promoter activation, consequently mediating an increase in Met levels in lupus-derived M-MDSCs.

Up-regulated Met in M-MDSCs association with B cell proliferation and hyperactivation via blocking COX-2/PGE$_2$ secreting signaling. To elucidate the key signaling pathways downstream of Met regulation, we conducted Venn

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CD11b$^+$Ly6C^{high}Gr-1^{low} M-MDSCs and CD11b$^+$Ly6C^{low}Gr-1^{high} G-MDSCs of total cells in *FoxO1*$^{f/f}$ and m*FoxO1*$^{-/-}$ mice shown. (J) BM-MDSCs from *FoxO1*$^{f/f}$ and m*FoxO1*$^{-/-}$ lupus mice isolated and induced in vitro, and the ratio of M-MDSCs and G-MDSCs detected by flow cytometry. (K) M-MDSCs separated from BM-MDSCs collected for coculture with spleen cells. CD4$^+$ T cell proliferation evaluated by staining with 5,6-carboxyfluorescein succinimidyl ester. Data represent the mean scores ± SDs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$, using Student's *t*-test (B, C, D, F, and G), one-way analysis of variance (ANOVA) (K), and two-way ANOVA (I and J). anti-dsDNA, anti–double-stranded DNA; BM-MDSC, bone marrow-derived MDSC; FoxO1$^{f/f}$, FoxO1 floxed; G-MDSC, granulocytic/polymorphonuclear myeloid-derived suppressor cell; H&E, hematoxylin and eosin; m*FoxO1*$^{-/-}$, myeloid FoxO1 deficiency mice; M-MDSC, monocytic myeloid-derived suppressor cell; ns, not significant; PAS, periodic acid–Schiff; uACR, urinary albumin/creatinine ratio.

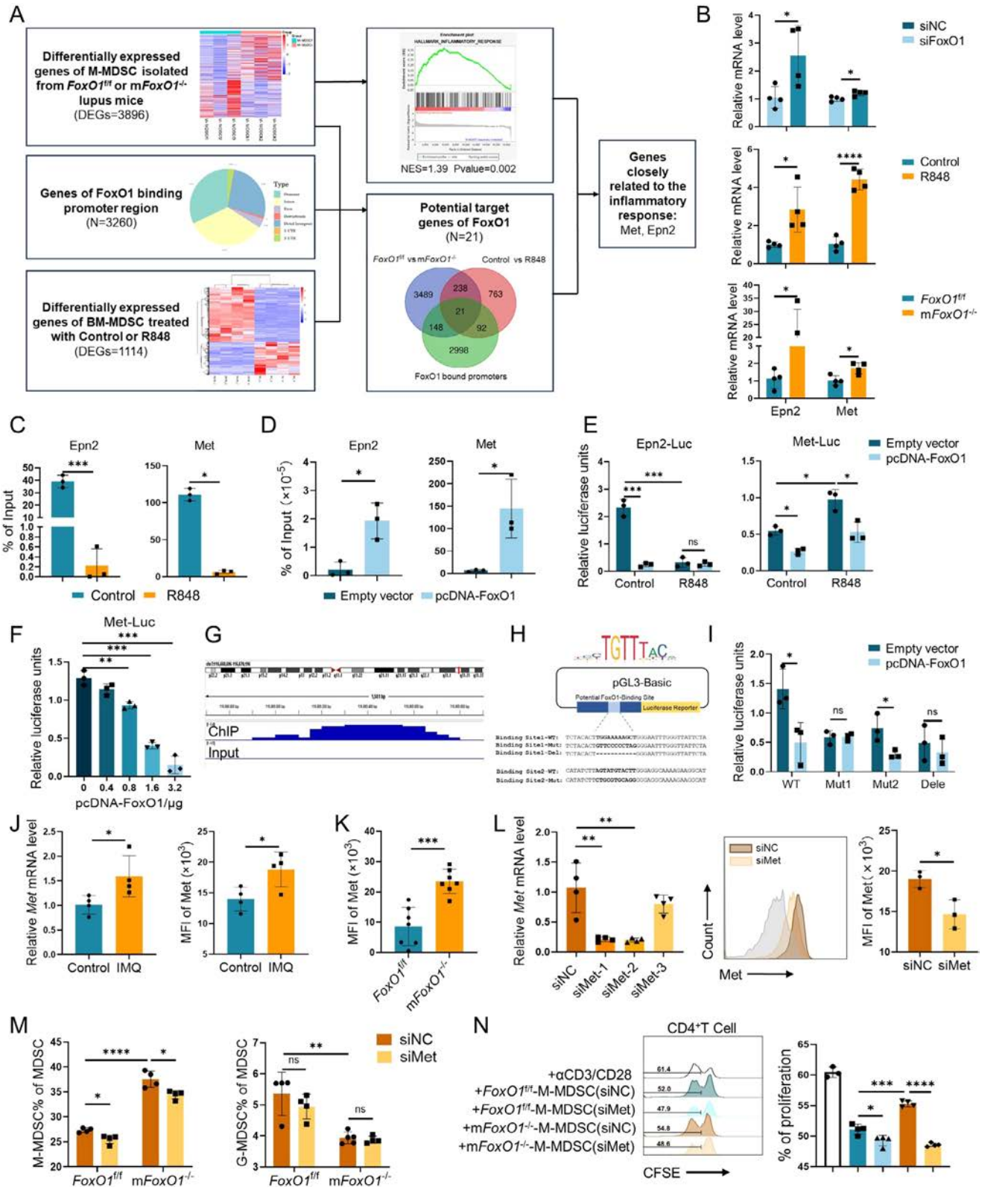

Figure 2. FoxO1 deficiency triggers Met transcription in lupus M-MDSCs subsets. (A) Bioinformatics analysis identified two potential downstream targets regulated by FoxO1 in M-MDSCs under lupus pathology. (B) Quantitative reverse transcriptase–polymerase chain reaction used to detect expression of Epn2 and Met in M-MDSCs. (C and D) ChIP–quantitative polymerase chain reaction used to analyze changes of FoxO1 binding level at different target binding sites. (E) Dual luciferase reporter assay used to detect luciferase activity of MDSCs. (F) Luciferase activity detected of MDSCs transfected with different doses of FoxO1 expression plasmid. (G) ChIP-seq results showed FoxO1 significantly enriched in

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diagram analysis, which revealed 259 overlapping genes between FoxO1 knockdown M-MDSCs and R848-stimulated BM-MDSCs (Figure 3A, Supplementary Table 11). Gene ontology (GO) analysis identified 16 genes closely associated with inflammatory response, including *Met*, *Nos2*, *Il23r*, *Ptgs2*, *Ccl2*, and *Cxcl2* (Figure 3B). Protein–protein interaction network analysis predicted interactions between *Met* with *CCL2* and *Ptgs2* (Figure 3C). Notably, *Ptgs2*, encoding COX-2, exhibited the most significant down-regulation with FoxO1 knockdown (Figure 3D). We confirmed that among the enzymes involved in PGE_2 production, only the key rate-limiting enzyme COX-2 was consistently down-regulated in lupus-derived and FoxO1 knockout M-MDSCs (Figure 3E, F). Subsequently, a significant reduction in PGE_2 secretion was observed in the serum of SLE patients and m*FoxO1*$^{-/-}$ lupus mice (Figure 3G). Silencing Met in BM-MDSCs resulted in significantly increased COX-2 expression and PGE_2 concentrations (Figure 3H, I).

Furthermore, GO analysis suggested that FoxO1 deficiency might play a crucial role in the crosstalk between M-MDSCs and B cells (Figure 3J). In a B cell coculture model, down-regulation of B cell proliferation and activation-related molecules CD69, CD80, CD86, CD40, and CD138 by Met siRNA (siMet) in M-MDSCs was observed, while PGE_2 receptor antagonists for EP2 (AH6809) and EP4 (AH23848) reversed this effect (Figure 3K, L). Thus, these data indicate that Met, by downregulating PGE_2 secretion via COX2 in M-MDSCs, promotes B cell proliferation and activation.

Met antagonist Cap treatment and lupus deterioration in myeloid FoxO1-deficient mice. To confirm FoxO1's regulatory role in M-MDSCs on Met/COX-2, we obtained BM-derived M-MDSCs from lupus mice and observed decreased COX-2 expression after FoxO1 deficiency (Figure 4A). siMet transfection reversed the PGE_2 level and the promotion of B cell proliferation and activation (CD69, CD80, CD86, CD40, and CD138) caused by FoxO1 deficiency (Figure 4B–D). Cap, an ATP-competitive inhibitor with high selectivity for Met kinase, commonly used in patients with solid tumors, was then administered intragastrically to IMQ-induced lupus mice. The results showed that Cap significantly reduced splenomegaly, serum IgG, IgM, dsDNA, and uACR levels, increased serum PGE_2 levels, and ameliorated kidney damage in *FoxO1*$^{f/f}$ lupus mice. Notably, myeloid FoxO1 deficiency–induced exacerbation of lupus symptoms was significantly alleviated by Cap (Figure 4E–I). Accordingly, Cap treatment significantly reduced the proportion and absolute numbers of B cells and their hyperactivation in m*FoxO1*$^{-/-}$ lupus mice (Figure 4J and K and Supplementary Figure 5A). Furthermore, Cap administration significantly blocked M-MDSC accumulation, but not G-MDSC accumulation, and facilitated the immunosuppressive capacity in a naive T cell coculture model (Supplementary Figure 5B and C).

To further elucidate the critical role of M-MDSCs in B cells during lupus progression, we sorted M-MDSCs from lupus mice and found that Cap administration significantly increased the expressions of COX-2 and PGE_2 in M-MDSCs and notably improved M-MDSCs' inhibition of B cell proliferation and activation in m*FoxO1*$^{-/-}$ lupus mice (Supplementary Figure 5D–F). Additionally, we further confirmed that Cap can significantly relieve lupus symptoms by gavage to spontaneous lupus mice (Supplementary Figure 6A–E). The ratio and immunosuppressive function of M-MDSCs, as well as the ratio and activation level of B cells, were significantly inhibited by the application of Cap, which yielded consistent results with IMQ-induced lupus mice (supplementary figure 6F–L). When we administered Cap to hBAFF transgenic mice, the activation of downstream B cells blocked the therapeutic effect of Cap on lupus, although it still reduced the ratio of M-MDSCs (Supplementary Figure 7A–D). Finally, we also cocultured M-MDSCs with PBMCs from patients with SLE, which further confirmed that blocking the Met signal in M-MDSCs significantly inhibited the proliferation and activation level of B cells (Supplementary Figure 7I and J). Altogether, we verified that Cap, a blocker of Met, prevents myeloid FoxO1-deficiency–induced aberrant B cell proliferation and activation and attenuates the clinical symptoms in lupus mice, suggesting a potential clinical treatment for SLE.

Regulation of *FoxO1* mRNA by ALKBH5 via m^{6}A sites in MDSCs. The reason for the decrease of FoxO1 in MDSCs from lupus remains clear. Recent studies showed that m^{6}A-modifying enzymes attach to the 3' untranslated region (3'UTR) or coding sequences (CDS) region of *FoxO1* mRNA, triggering m^{6}A

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promoter region of Met. (H and I) Strategy diagram for construction of WT, Mut, or Dele luciferase reporter plasmids at FoxO1 binding site. Dual luciferase reporter system used to detect luciferase activity of MDSCs. (J) Changes of Met expression gene and protein expression in BM-MDSCs of lupus and control mice. (K) Protein level of Met in M-MDSCs from *FoxO1*$^{f/f}$ or m*FoxO1*$^{-/-}$ lupus mice. (L–N) BM-MDSCs transfected with siNC or siMet, and proportion of M-MDSCs and G-MDSCs, CD4$^+$ T cell proliferation evaluated. Data represent the mean scores ± SDs. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; and $^{****}P < 0.0001$, using Student's *t*-test (C, D, J, K, and L), one-way analysis of variance (ANOVA) (F, L, and N), or two-way ANOVA (B, E, I, and M). BM-MDSC, bone marrow-derived MDSC; CFSE, 5,6-carboxyfluorescein succinimidyl ester; ChIP, chromatin immunoprecipitation; DEG, differentially expressed gene; Dele, deletion; Epn2, epsin 2; FoxO1$^{f/f}$, FoxO1 floxed; G-MDSC, granulocytic/polymorphonuclear myeloid-derived suppressor cell; IMQ, imiquimod; Luc, luciferase reporter plasmids; Met, mesenchymal-epithelial transition factor protein; MFI, mean fluorescence intensity; m*FoxO1*$^{-/-}$, myeloid FoxO1 deficiency mice; M-MDSC, monocytic myeloid-derived suppressor cell; mRNA, messenger RNA; Mut, mutant; pcDNA, pcDNA3.1 eukaryotic expression vector; siFoxO1, FoxO1 small interfering RNA; siMet, Met small interfering RNA; siNC, non-coding small interfering RNA; WT, wildtype. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43046/abstract.

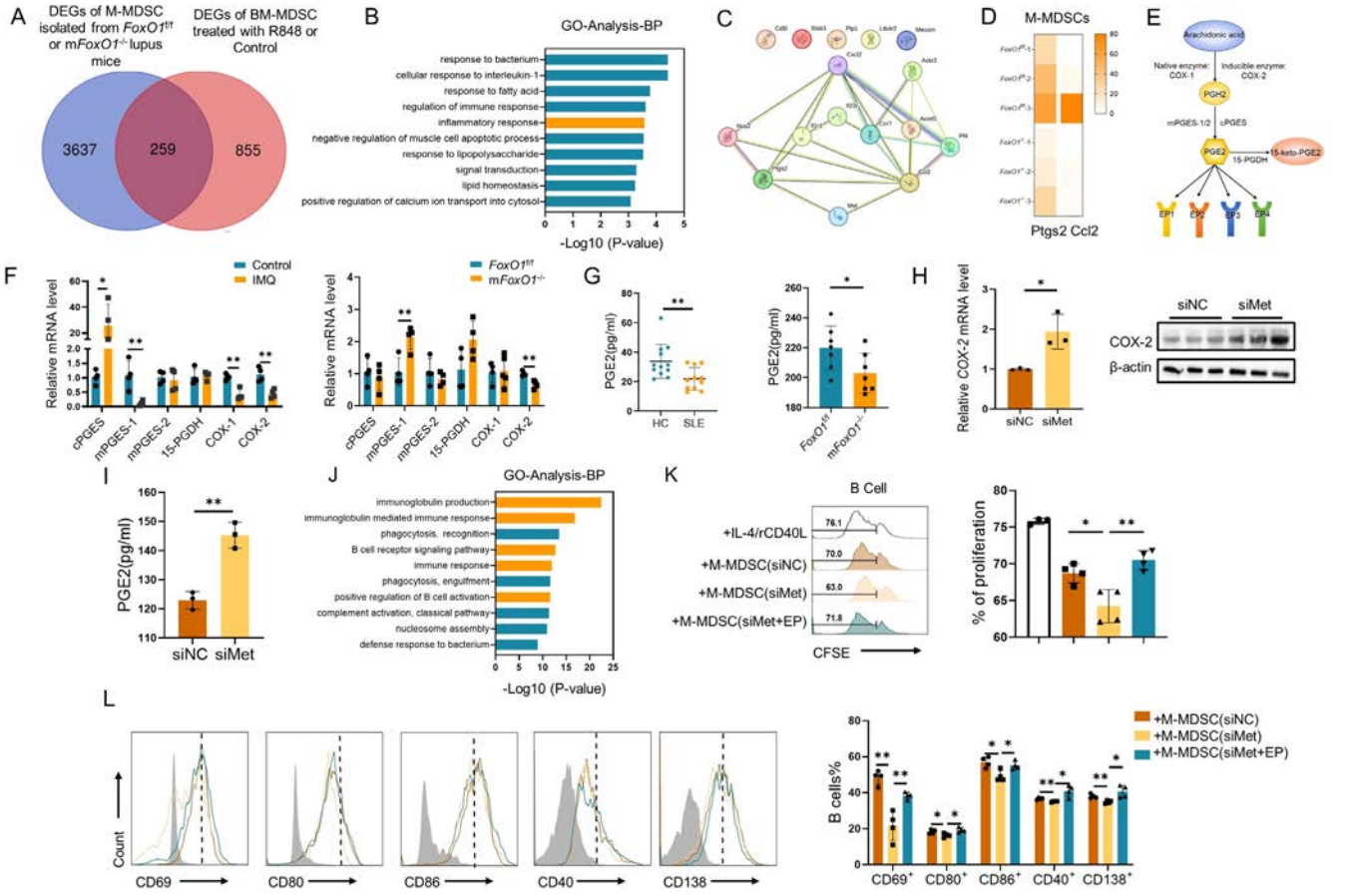

Figure 3. Up-regulated methionine in M-MDSCs promotes B cell proliferation and hyperactivation via blocking COX-2/PGE_2 secreting signaling. (A) Overlapping number of differentially expressed genes in M-MDSCs from *FoxO1*$^{f/f}$ or m*FoxO1*$^{-/-}$ lupus mice and MDSCs with or without R848 stimulation analyzed. (B) Top 10 significantly enriched terms in BPs from GO terms analysis. (C) Protein–protein interaction network between differentially expressed molecules related to inflammatory response was established using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. (D) Expression levels of genes potentially directly regulated by methionine in RNA sequencing. (E) Regulatory enzymes and their receptors involved in PGE_2 production. (F) qRT-PCR used to detect changes in gene expression of PGE_2 production–related enzymes in M-MDSCs. (G) Serum PGE_2 concentrations detected with specific enzyme-linked immunosorbent assay. (H and I) MDSCs transfected with siNC or siMet, and concentration of PGE_2 in culture supernatant measured. (J) Top 10 significantly enriched terms in BPs of differentially expressed genes in M-MDSCs from *FoxO1*$^{f/f}$ and m*FoxO1*$^{-/-}$ lupus mice from GO terms analysis. (K and L) B cell proliferation and activation evaluated by flow cytometry. Data represent the mean scores ± SDs. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; and $^{****}P < 0.0001$, using Student's *t*-test (G, H, and I), one-way analysis of variance (ANOVA) (K), or two-way ANOVA (F and L). BM-MDSC, bone marrow-derived MDSC; BP, biological process; COX, cyclooxygenase; DEG, differentially expressed gene; EP, prostaglandin E receptor; FoxO1$^{f/f}$, FoxO1 floxed; GO, gene ontology; HC, healthy control, IL-4, interleukin-4; IMQ, imiquimod; m*FoxO1*$^{-/-}$, myeloid FoxO1 deficiency mice; M-MDSC, monocytic myeloid-derived suppressor cell; mRNA, messenger RNA; PGE_2, prostaglandin E_2; PGH_2, prostaglandin H_2; qRT-PCR, quantitative real-time polymerase chain reaction; siMet, Met small interfering RNA; siNC, non-coding small interfering RNA; SLE, systemic lupus erythematosus. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43046/abstract.

methylation, and altering FoxO1 abundance by impacting mRNA stability and translation processes.[25,26,32] We investigated whether the disrupted expression of FoxO1 was governed by m^6A in MDSCs during lupus progression. In patients with SLE, overall m^6A levels in RNA from peripheral blood were significantly elevated compared to those in HCs (Figure 5A) and were inversely associated with FoxO1 expression in M-MDSCs ($P = 0.0256$, $R = -0.4976$) (Figure 5B). Moreover, m^6A levels positively correlated with erythrocyte sedimentation rate, neutrophil count (NEUT), neutrophil percentage (NEUT%), and white blood cell count and negatively correlated with SLEDAI score, hematocrit level, red blood cell count, and lymphocyte percentage (LY%) (Supplementary Figure 8A). The heightened level of m^6A modification was also confirmed in blood and BM-MDSC samples from lupus mice (Figure 5C and D). Treatment of BM-MDSCs with the methylation donor betaine decreased FoxO1 expression, promoted M-MDSC differentiation, and compromised their immunosuppressive capability (Supplementary Figure 8B–E), suggesting that m^6A modification might regulate FoxO1 expression during M-MDSC differentiation.

Subsequently, we assessed the expression of key m^6A modification enzymes in MDSCs of lupus and found that

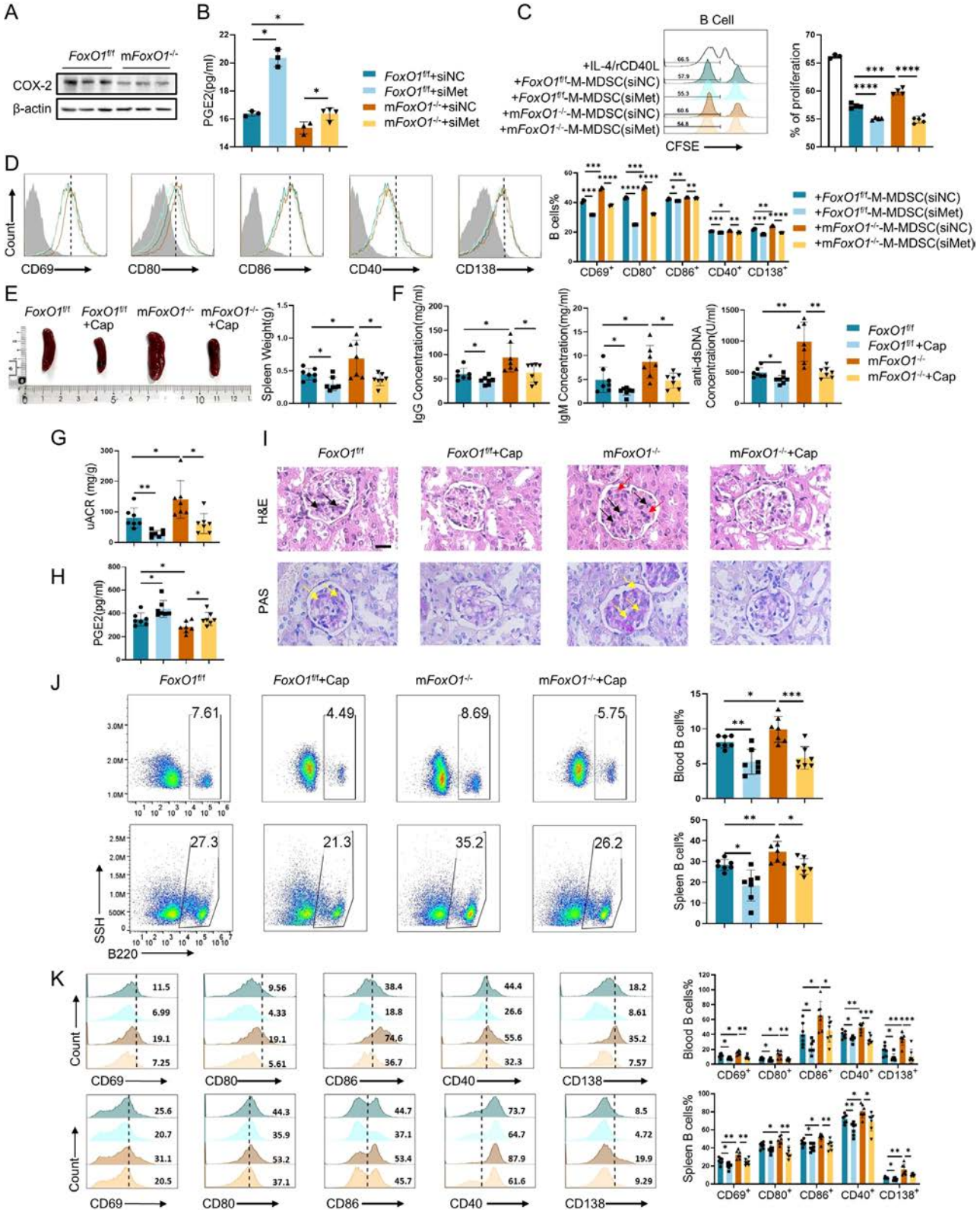

Figure 4. Methionine antagonist Cap treatment relieves lupus deterioration in myeloid FoxO1-deficient mice. (A) Changes in COX-2 protein expression in M-MDSCs from *FoxO1*$^{f/f}$ or m*FoxO1*$^{-/-}$ lupus mice. (B–D) M-MDSCs transfected with siNC or siMet, and concentration of PGE_2 in culture supernatant, B cell proliferation, and activation evaluated. *FoxO1*$^{f/f}$ lupus mice with (n = 7) or without (n = 7) Cap administration and m*FoxO1*$^{-/-}$ lupus mice with (n = 7) or without (n = 7) Cap administration were established for analysis. (E) Representative photographs of spleens and spleen weights. (F–H) The uACRs, serum levels of total IgG, IgM, anti-dsDNA and PGE2 measured using enzyme-linked immunosorbent

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demethylases ALKBH5 significantly decreased (Figure 5E and F). Knocking down ALKBH5 in MDSCs reduced FoxO1 expression and increased its mRNA degradation, suggesting a crucial role of ALKBH5 in FoxO1 expression (Figure 5G–I). Furthermore, ALKBH5 enrichment on *FoxO1* mRNA decreased after R848 stimulation or ALKBH5 knockdown (Figure 5J). Analysis of Methylated RNA immunoprecipitation sequencing (MeRIP-seq) showed highly enriched m^6A levels peak around the stop codon in both groups, with the GGAC motif highly enriched in the m^6A site (Figure 5K and L). The Integrative Genomics Viewer map revealed six putative m^6A modification sites observed in *FoxO1* mRNA (Figure 5M). Methylated RNA immunoprecipitation-qPCR (MeRIP-qPCR) confirmed a significant increase in m^6A modification level at site 2 located in the CDS region and site 5 located in the 3'UTR of *FoxO1* mRNA after R848 stimulation or ALKBH5 knockdown (Figure 5N).

To validate the necessity of m^6A modification at site 2 in the CDS region, we engineered a dcas13b-FTO fusion construct to target demethylation, resulting in decreased *FoxO1* mRNA degradation, increased protein levels, and inhibited M-MDSC differentiation (Figure 5O–R). Luciferase reporter assays were conducted to substantiate the requirement of m^6A modification for site 5 in the 3'-UTR (Figure 5S). The luciferase activity in wildtype group intensified in a dose-dependent manner with ALKBH5, whereas the mutant group showed resistance to the impact of ALKBH5. Moreover, luciferase activity significantly decreased after R848 stimulation (Figure 5T), indicating that FoxO1 regulation is influenced by ALKBH5-guided m^6A modification.

ALKBH5-m^6A–regulated FoxO1 in M-MDSCs and the development and pathogenesis of lupus. To investigate the function of ALKBH5 in FoxO1-deficiency–induced M-MDSC differentiation, we transfected BM-MDSCs with ALKBH5 overexpression plasmid and FoxO1 siRNA in vitro. High ALKBH5 expression induced FoxO1 expression, reduced M-MDSCs, and increased G-MDSCs. FoxO1 knockdown didn't affect ALKBH5 expression but diminished its regulatory effect (Supplementary Figure 9A–C). Additionally, ALKBH5-modulated MDSCs' enhanced inhibitory effect on $CD4^+$ T cell proliferation was completely abolished by FoxO1 knockdown (Supplementary Figure 9D).

To determine the impact of ALKBH5 expression in MDSCs on myeloid FoxO1 deficiency in lupus progression, we conducted an adoptive transfer experiment with ALKBH5-modulated MDSCs in myeloid-specific FoxO1 knockout mice treated with IMQ. Infusion of MDSCs with ALKBH5 overexpression restored the survival rate and alleviated lupus symptoms, including splenomegaly, elevated serum IgG, IgM, uACRs levels, and kidney injury in *FoxO1*$^{f/f}$ lupus mice. Interestingly, adoptive transfer of ALKBH5-overexpressed MDSCs into lupus m*FoxO1*$^{-/-}$ mice similarly reduced lupus symptoms, although it remained more severe than that of wildtype lupus mice (Figure 6A–E). Furthermore, the higher proportion and absolute numbers of B cells and $CD69^+$ hyperactivity in *FoxO1*$^{f/f}$ lupus mice were attenuated by ALKBH5-MDSCs treatment (Figure 6F and G and Supplementary Figure 9E and F). Overall, these data confirm that endogenous ALKBH5 induces FoxO1 expression, inhibiting M-MDSC differentiation with increased immunosuppression and ameliorating lupus severity.

Correlation of ALKBH5, FoxO1, and Met in M-MDSCs from both patients with SLE and lupus mice models. To further validate our findings, we collected peripheral blood samples from patients with SLE (n = 60) for flow detection. The results indicated a positive correlation between ALKBH5 expression in MDSCs and FoxO1 in M-MDSCs (P = 0.0255, R = 0.2882). Furthermore, in M-MDSCs, we observed a negative correlation between FoxO1 and Met (P = 0.0185, R = –0.3033), consistent with our observations in lupus mice (Figure 6H). Additionally, ALKBH5 expression in MDSCs negatively correlated with SLEDAI and monocyte percentage but positively correlated with NEUT. Notably, we further confirmed that FoxO1 expression in M-MDSCs negatively correlated with SLEDAI, IgG levels, NEUT %, and NEUT but positively correlated with LY%. Moreover, Met expression in M-MDSCs positively correlated with SLEDAI, complement component 4 and platelet levels (Figure 6I). Analysis of the human SLE blood RNA-seq data set (GSE61635) showed that, consistent with our results, FoxO1 and ALKBH5 expression were significantly down-regulated, whereas Met expression was increased. Additionally, FoxO1 expression positively correlated with ALKBH5 ($P < 0.0001$, R = 0.4288) and negatively with Met ($P \leq 0.0001$, R = –0.5141) (Figure 6J and K).

Furthermore, we confirmed our findings in different lupus mouse models. In the spontaneous lupus mouse model and the pristane-induced lupus model, ALKBH5 was significantly

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assay. (I) H&E and PAS staining of kidney sections (scale bar = 20 μ*M*). (J and K) Flow cytometry analysis detected the proportion and activation level of B cells in peripheral blood and spleen. Data represent the mean scores ± SDs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$, using one-way analysis of variance (ANOVA) (B, C, E, F, G, H, and J), or two-way ANOVA (D and K). anti-dsDNA, anti–double-stranded DNA; Cap, Capmatinib; CFSE, 5,6-carboxyfluorescein succinimidyl ester; COX, cyclooxygenase; FoxO1$^{f/f}$, FoxO1 floxed; H&E, hematoxylin and eosin; IL-4, interleukin-4; m*FoxO1*$^{-/-}$, myeloid FoxO1 deficiency mice; M-MDSC, monocytic myeloid-derived suppressor cell; PAS, periodic acid–Schiff; PGE_2, prostaglandin E_2; siMet, Met small interfering RNA; siNC, non-coding small interfering RNA; uACR, urinary albumin/creatinine ratio. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43046/abstract.

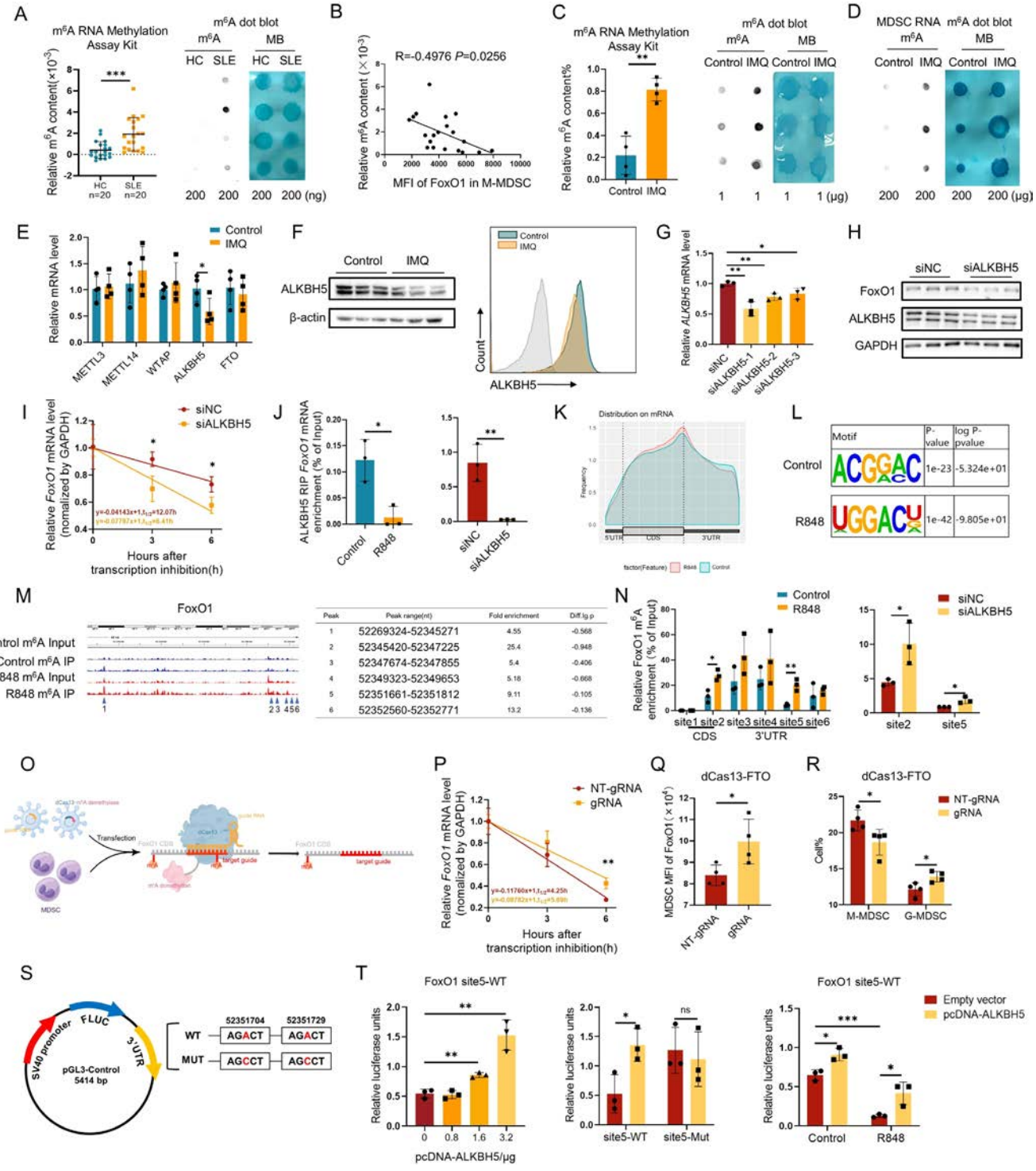

Figure 5. *FoxO1* mRNA is regulated by ALKBH5 via m^{6}A sites in MDSCs. (A) Relative m^{6}A content of RNA in peripheral blood detected with m^{6}A RNA methylation assay kit or dot blot. (B) Correlation analysis of RNA m^{6}A modification level and expression level of FoxO1 in M-MDSCs. (C and D) Relative m^{6}A content of RNA in peripheral blood or BM-MDSCs detected. (E) Expression changes of m^{6}A modification–related enzymes. (F) Protein expression changes of ALKBH5 in BM-MDSCs analyzed. (G–I) *FoxO1* mRNA degradation treated with 5 μ*M* actinomycin-D for indicated time analyzed. (J) Relative enrichment of *FoxO1* mRNA associated with ALKBH5 protein identified by RIP assays using anti-ALKBH5 antibodies. (K) Distribution of the m^{6}A peaks across the length of the mRNAs. (L) Consensus motif map with Methylated RNA immunoprecipitation sequencing (MeRIP-seq) peaks identified by Hypergeometric Optimization of Motif EnRichment (HOMER) analysis. (M) MeRIP-seq of the distribution of m^{6}A peaks along *FoxO1* mRNA. (N) MeRIP–quantitative polymerase chain reaction used to analyze changes of m^{6}A modification level at different m^{6}A modification sites. (O–R) Schematic representation of MDSCs transfected with lentivirus encoding RNA methylation editor and gRNA

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reduced in MDSCs and M-MDSCs in both peripheral blood and spleen. Additionally, the level of Met was significantly increased in M-MDSCs, and we observed a significant increase in the proportion of B cells and up-regulation of CD138 expression in lupus mice compared with control mice (Supplementary Figures 4F and 10A–H).

DISCUSSION

The imbalance of M-MDSCs is closely associated with the onset and progression of SLE. Abnormal expression of FoxO1 in M-MDSCs may contribute to SLE progression, but the mechanism remains unclear. Our study confirmed that myeloid FoxO1 knockout significantly exacerbates the lupus phenotype. Further mechanistic investigations revealed that FoxO1 inhibits Met transcription, consequently modulating M-MDSCs' inhibitory effect on B cells through the COX-2/PGE_2 axis. Additionally, low expression of ALKBH5 in MDSCs enhances *FoxO1* mRNA degradation by increasing m^6A modification. In summary, our findings unveil a novel mechanism in M-MDSCs regulates B cell proliferation and activation under SLE pathology (Figure 6L).

Although FoxO1 has been reported to regulate various immune cells, its regulatory mechanism of M-MDSC phenotype and function is not fully understood. Our previous studies have suggested that FoxO1 silencing promotes M-MDSC accumulation in vitro.[18,19] Here, we confirmed that myeloid FoxO1-specific knockout markedly exacerbates lupus symptoms, increases the proportion of M-MDSCs, which may be related to the acceleration of cell cycle transformation (Supplementary Figure 2D, E), and alters their proinflammatory function. Additionally, besides the significant increase in B cell activation, FoxO1 knockout also induces T cells and macrophages to shift toward a proinflammatory phenotype (Supplementary Figure 4I–K). This underscores the multifaceted proinflammatory phenotype induced by myeloid-specific FoxO1 knockout under lupus pathology.

In our investigation into the downstream molecular, we discovered that FoxO1 directly binds to the promoter region of Met, exerting a role of transcriptional inhibition. Met, known as a proto-oncogene, is expressed not only in cancer cells but also in immune cells. Studies have highlighted the close association between the expansion and infiltration of MDSCs with Met expression.[33,34] The up-regulation of Met is primarily attributed to aberrant transcriptional regulation, and proper control of transcription is indispensable for the normal functioning of the gene.[35] One study has elucidated that FoxO1 negatively regulates Met at the transcriptional level in gastric cancer cells,[36] a finding consistent with our observations in M-MDSCs. However, the authors of that study also indicated that FoxO1 expression was negatively regulated by Met at the posttranscriptional level, an aspect that warrants further investigation and refinement in our subsequent research endeavors.

The Met/COX-2/PGE_2 axis exhibits a positive regulatory relationship among them in tumor-related research,[37] but in a model of patients with rheumatoid arthritis and Achilles tendon injury mice, Met was found to negatively regulate[38,39] PGE_2. Previous studies have suggested that G-MDSCs, rather than M-MDSCs, predominantly use PGE_2 to mediate immunosuppression in tumor research.[40] Nonetheless, PGE_2 derived from M-MDSCs also plays a crucial role in inhibiting B cell proliferation and antibody production.[41] Our study found that Met decreases COX-2 and PGE_2 in M-MDSCs, thereby promoting B cell proliferation and activation. This confirms the significant role of PGE_2 in M-MDSCs–mediated immunosuppression and supports the notion that innate immune dysregulation fuels disease onset by abnormally activating adaptive immunity, which aligns with our previous observation that MDSC abnormalities are preferentially associated with lymphocyte abnormalities.[2] In our study, treatment with the Met inhibitor Cap in m*FoxO1*$^{-/-}$ mice significantly alleviated lupus symptoms. Cap, a prescription drug for metastatic nonsmall cell lung cancer, is novel in autoimmune diseases. Our research revealed that Cap not only reduced the proportion of pathogenic M-MDSCs and B cells but also induced an anti-inflammatory T cells phenotype (Supplementary Figures 5H and I, 6M–R, and 7E–G), although it appeared to enhance a proinflammatory phenotype in macrophages (Supplementary Figures 5J, 6S and T, and 7H). These results elucidate a novel FoxO1 regulated mechanism in M-MDSCs inhibitory function through Met/COX-2/PGE_2 pathway and propose a novel clinical treatment approach using Cap in SLE.

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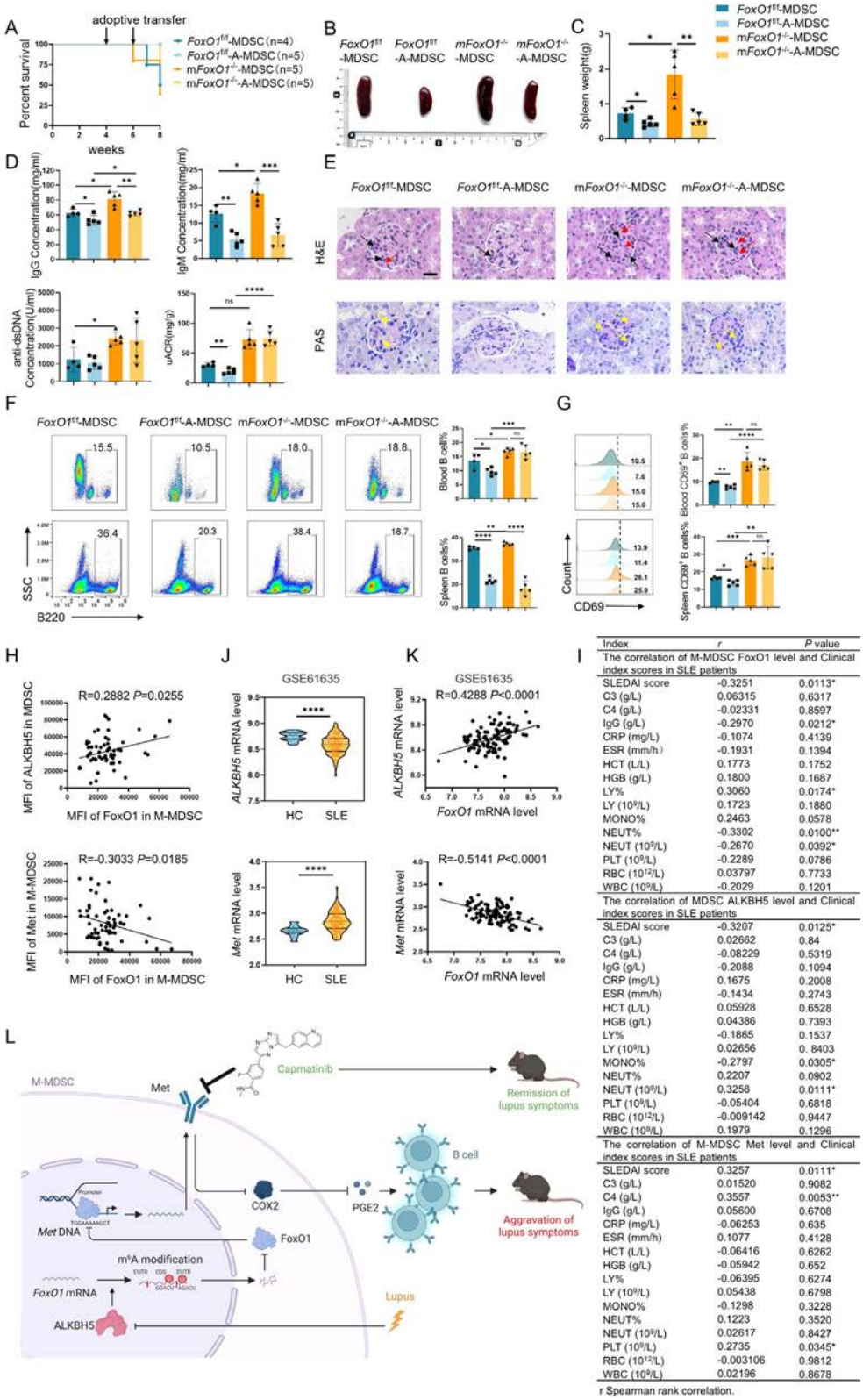
by figdraw. *FoxO1* mRNA degradation, FoxO1 expression and ratio of M-MDSCs and G-MDSCs in MDSCs analyzed. (S and T) Strategy diagram for construction of WT and MUT luciferase reporter plasmids at m^6A binding site 5. Dual luciferase reporter system used to detect luciferase activity. Data represent the mean scores ± SDs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$, using Student's *t*-test (A, C, J, and Q), one-way analysis of variance (ANOVA) (G and T), two-way ANOVA (E, I, N, P, R, and T). BM-MDSC, bone marrow-derived MDSC; ALKBH5, alkB homolog 5; CDS, coding sequences; $FoxO1^{f/f}$, FoxO1 floxed; FTO, fat mass and obesity-associated protein; gRNA, guide RNA; HC, healthy controls; IMQ, imiquimod; m^6A, N^6-adenosine methylation; MB, methylene blue; m*FoxO1*$^{-/-}$, myeloid FoxO1 deficiency mice; MDSC, myeloid-derived suppressor cell; METTL, methyltransferase; MFI, mean fluorescence intensity; M-MDSC, monocytic myeloid-derived suppressor cell; mRNA, messenger RNA; MUT, mutant; NT, non-targeting; pcDNA, pcDNA3.1 eukaryotic expression vector; siALKBH5, ALKBH5 small interfering RNA; siNC, noncoding small interfering RNA; SLE, systemic lupus erythematosus; UTR, untranslated region; WT, wildtype; WTAP, Wilms tumor 1-associating protein. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43046/abstract.



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Index	r	P value
The correlation of M-MDSC FoxO1 level and Clinical index scores in SLE patients		
SLEDAI score	-0.3251	0.0113*
C3 (g/L)	0.06315	0.6317
C4 (g/L)	-0.02331	0.8597
IgG (g/L)	-0.2970	0.0212*
CRP (mg/L)	-0.1074	0.4139
ESR (mm/h)	-0.1931	0.1394
HCT (L/L)	0.1773	0.1752
HGB (g/L)	0.1800	0.1687
LY%	0.3060	0.0174*
LY (10^9/L)	0.1723	0.1880
MONO%	0.2463	0.0578
NEUT%	-0.3302	0.0100**
NEUT (10^9/L)	-0.2670	0.0392*
PLT (10^9/L)	-0.2289	0.0786
RBC (10^{12}/L)	0.03797	0.7733
WBC (10^9/L)	-0.2029	0.1201
The correlation of MDSC ALKBH5 level and Clinical index scores in SLE patients		
SLEDAI score	-0.3207	0.0125*
C3 (g/L)	0.02662	0.84
C4 (g/L)	-0.08229	0.5319
IgG (g/L)	-0.2088	0.1094
CRP (mg/L)	0.1675	0.2008
ESR (mm/h)	-0.1434	0.2743
HCT (L/L)	0.05928	0.6528
HGB (g/L)	0.04386	0.7393
LY%	-0.1865	0.1537
LY (10^9/L)	0.02656	0. 8403
MONO%	-0.2797	0.0305*
NEUT%	0.2207	0.0902
NEUT (10^9/L)	0.3258	0.0111*
PLT (10^9/L)	-0.05404	0.6818
RBC (10^{12}/L)	-0.009142	0.9447
WBC (10^9/L)	0.1979	0.1296
The correlation of M-MDSC Met level and Clinical index scores in SLE patients		
SLEDAI score	0.3257	0.0111*
C3 (g/L)	0.01520	0.9082
C4 (g/L)	0.3557	0.0053**
IgG (g/L)	0.05600	0.6708
CRP (mg/L)	-0.06253	0.635
ESR (mm/h)	0.1077	0.4128
HCT (L/L)	-0.06416	0.6262
HGB (g/L)	-0.05942	0.652
LY%	-0.06395	0.6274
LY (10^9/L)	0.05438	0.6798
MONO%	-0.1298	0.3228
NEUT%	0.1223	0.3520
NEUT (10^9/L)	0.02617	0.8427
PLT (10^9/L)	0.2735	0.0345*
RBC (10^{12}/L)	-0.003106	0.9812
WBC (10^9/L)	0.02196	0.8678

r Spearman rank correlation.

Figure 6. ALKBH5-m^{6}A–regulated FoxO1 in M-MDSCs participates in the development and pathogenesis of lupus. *FoxO1*$^{f/f}$ lupus mice adoptive transferred with overexpression of ALKBH5 plasmid (n = 5) or control (n = 4) and m*FoxO1*$^{-/-}$ lupus mice with overexpression of ALKBH5 plasmid (n = 5) or control (n = 5) were established for analysis. (A) The survival rate of mice was recorded. (B and C) Representative photographs of spleens and spleen weights. (D) The uACRs, serum levels of total IgG, IgM, and anti-dsDNA were measured using enzyme-linked immunosorbent assay. (E) H&E and PAS staining of kidney sections (scale bar = 20 μ*M*). (F and G) Flow cytometry analysis detected the proportion and activation

(Figure legend continues on next page.)

As previously mentioned, both the CDS region and 3'UTR of *FoxO1* mRNA are known to be regulated by m^{6}A modification, influencing FoxO1 expression posttranscriptionally.[25,26] We observed an increase in m^{6}A levels in these regions following R848 stimulation, which was attributed to the demethylase ALKBH5. ALKBH5 can positively regulate other members of the Fox family,[42–44] and one report indicated a positive regulation on *FoxO1* mRNA stability.[45] Numerous studies have demonstrated that ALKBH5-regulation is closely associated with mRNA degradation.[46,47] In our study, ALKBH5 deletion in MDSCs reduced *FoxO1* mRNA stability by increasing m^{6}A level, leading to decreased FoxO1 expression. m*FoxO1*$^{-/-}$ largely nullified the beneficial effects of high ALKBH5 expression in MDSCs on lupus remission. Besides its effect on B cells, FoxO1 deficiency also attenuated the inhibitory effects of ALKBH5 overexpression in MDSCs on T cell proliferation, activation, and proinflammatory phenotype (Supplementary Figure 9G–L). The protective effects on spleen macrophage percentage and proinflammatory phenotype were completely abolished (Supplementary Figure 9M and N). These findings unveil a novel posttranscriptional modification mechanism regulating FoxO1. Nonetheless, because methylated reading proteins play a pivotal role in m^{6}A function determining, further exploration of their role in *FoxO1* mRNA regulation is warranted.

Previous studies have indicated that the role of untreated MDSCs in the treatment of lupus remains unclear.[2,48] This ambiguity is largely due to a limited understanding of the mechanisms by which MDSCs contribute to lupus pathogenesis. This research demonstrates that the adoptive transfer of MDSCs with high expression of ALKBH5 significantly alleviates lupus symptoms in mice and highlights the considerable potential of artificially modified MDSCs for clinical treatment of SLE, aligning with findings from several other studies.[13,18,49–51] Furthermore, the investigation of tumor-associated MDSCs[52] introduces a novel approach to disease treatment through in situ injection aimed at inducing MDSC modification. In summary, our study elucidates the critical molecular mechanisms by which MDSCs are involved in the progression of SLE, providing a theoretical foundation and reference for future clinical applications of MDSCs.

In summary, our findings underscore the crucial role of M-MDSCs–specific FoxO1 in lupus progression. Mechanistically, ALKBH5 guides m^{6}A modification of FoxO1 at CDS and 3'UTR regions, wherein decreased levels lead to *FoxO1* mRNA degradation. FoxO1 deficiency subsequently activates Met transcription, disrupting downstream COX2 and PGE_2 secretion. Individuals carrying the risk ALKBH5/FoxO1/Met signals exhibit heightened B cell hyperactive capacities, which, in the context of SLE, contribute to more severe disease. These findings validate the potential of Cap treatment for patients with lupus.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Dou confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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level of B cells. (H) Correlation between the expression of FoxO1 in M-MDSCs and ALKBH5 of MDSCs or Met of M-MDSCs was analyzed. (I) Correlation between the clinical characteristics and the expression of FoxO1 in M-MDSCs or ALKBH5 of MDSCs or Met of M-MDSCs was analyzed in patients with SLE. (J and K) Human SLE blood RNA sequencing data set (GSE61635) was used to analyze the expression and correlation of FoxO1 with ALKBH5 and Met. (L) Schematic representation of mechanism by which lupus-derived M-MDSCs promote B cell proliferation and activation by reducing PGE_2 production through the ALKBH5/FoxO1/Met axis created on BioRender.com. Data represent the mean scores ± SDs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$, using one-way analysis of variance (C, D, F, and G), Mann-Whitney U-test (J), and Pearson's test (H, I, and K). ALKBH5, alkB homolog 5; A-MDSC, MDSCs with ALKBH5 overexpression; anti-dsDNA, anti–double-stranded DNA; C, complement; CDS, coding sequences; COX-2, cyclooxygenase-2; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FoxO1$^{f/f}$, FoxO1 floxed; H&E, hematoxylin and eosin; HC, healthy control; HCT, hematocrit; HGB, hemoglobin level; IMQ, imiquimod; LY, lymphocyte count; LY%, lymphocyte percentage; m^{6}A, N^6-adenosine methylation; m*FoxO1*$^{-/-}$, myeloid FoxO1 deficiency mice; MDSC, myeloid-derived suppressor cell; Met, mesenchymal-epithelial transition factor protein; MFI, mean fluorescence intensity; M-MDSC, monocytic myeloid-derived suppressor cell; MONO%, monocyte percentage; mRNA, messenger RNA; NEUT, neutrophil count; NEUT%, neutrophil percentage; PAS, periodic acid–Schiff; PGE_2, prostaglandin E_2; PLT, platelets; RBC, red blood cell count; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; uACR, urinary albumin/creatinine ratio; UTR, untranslated region; WBC, white blood cell count; WT, wild-type. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43046/abstract.

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A Strong Dysregulated Myeloid Component in the Epigenetic Landscape of Systemic Sclerosis: An Integrated DNA Methylome and Transcriptome Analysis

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Objective. Nongenetic factors influence systemic sclerosis (SSc) pathogenesis, underscoring epigenetics as a relevant contributor to the disease. We aimed to unravel DNA methylation abnormalities associated with SSc through an epigenome-wide association study.

Methods. We analyzed DNA methylation data from whole-blood samples in 179 patients with SSc and 241 unaffected individuals to identify differentially methylated positions (DMPs) with a false discovery rate (FDR) <0.05. These results were further integrated with RNA sequencing data from the same patients to assess their functional consequence. Additionally, we examined the impact of DNA methylation changes on transcription factors and analyzed the relationship between alterations of the methylation and gene expression profile and serum proteins levels.

Results. This analysis yielded 525 DMPs enriched in immune-related pathways, with leukocyte cell–cell adhesion being the most significant (FDR = 4.91×10^{-9}), prioritizing integrins as they were exposed by integrating methylome and transcriptome data. Furthermore, through this integrative approach, we observed an enrichment of neutrophil-related pathways, highlighting this myeloid cell type as a relevant contributor in SSc pathogenesis. In addition, we uncovered novel profibrotic and proinflammatory mechanisms involved in the disease. Finally, the altered epigenetic and transcriptomic signature revealed an increased activity of CCAAT/enhancer-binding protein transcription factor family in SSc, which is crucial in the myeloid lineage development.

Conclusion. Our findings uncover the impaired epigenetic regulation of the disease and its impact on gene expression, identifying new molecules for potential clinical applications and improving our understanding of SSc pathogenesis.

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INTRODUCTION

Systemic sclerosis (SSc) is a rare immune-mediated inflammatory disease (IMID) characterized by immune dysregulation, vasculopathy, and cutaneous and internal fibrosis.[1] SSc is an heterogeneous disorder affecting the connective tissue from which patients can be classified based on the extension of fibrosis as having limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc or by their serological status.[2] Recent genome-wide association studies (GWASs) have identified multiple SSc-susceptibility loci, indicating the relevance of individual genetic background as a significant contributor to SSc risk.[3]

However, the significant influence of environmental or nongenetic factors in the development of SSc underscores the crucial role of epigenetics mediating gene-by-environment interactions.[4,5] In this sense, epigenetics can be considered the manifestation of the environmental influence on the regulation of gene function. In fact, epigenetic modifications not only are determined by extracellular influences but also are reversible and can change swiftly. Among these mechanisms, DNA methylation is crucial in regulating gene expression, influencing the DNA three-dimensional structure and transcription factor (TF) binding affinity.[6,7] In addition, this epigenetic mark appears as a robust and an easily measurable feature that could provide valuable insights into disease pathogenesis.[8] Over the past years, various epigenetic studies have been performed in patients with SSc,[9] and different cell type–specific DNA methylation changes have been identified.[10–13] However, these previous reports have relatively low sample size, limiting their detection power. In addition, analyzing whole blood has advantages: allowing the identification of disease biomarkers, making it valuable for clinical practice, as has been suggested in other IMIDs.[14,15] Considering this, we aim to investigate the role of DNA methylation in SSc by performing the largest epigenome-wide association study in the disease to date. In addition, the methylome data were integrated with RNA sequencing (RNA-seq) and serum proteins from the same individuals in order to investigate the functional consequences of the epigenetic variation at the gene and protein expression levels.

PATIENTS AND METHODS

Patients and samples. Samples were obtained from the multicenter, cross-sectional, clinical study PRECISESADS. A total of 428 individuals were included, 186 patients with SSc and 242 unaffected individuals as controls. In the Supplementary Material, it is the detailed list of local investigators from the participating clinical sites. Patients included in this study were aged 18 years or older and fulfilled the 1980 American College of Rheumatology classification criteria.[16] Patients who met diagnostic criteria for more than one systemic autoimmune disease were excluded from the PRECISESADS project. Controls did not have any history of autoimmune or infectious diseases and were matched by gender, age, and clinical center of origin to patients with SSc to the extent possible. At the time of blood sampling, clinical and demographic information was obtained for every patient. Demographic information is detailed in Supplementary Table 1. A consensual protocol and informed consent were approved for by local ethics committees of each participating clinical center. All patients provided written informed consent according to the Declaration of Helsinki. All the R code used for this study is available at https://github.com/Javi-Martinez-Lopez/SSc-EWAS.

Genome-wide methylation profiling. DNA was extracted from whole peripheral blood, and bisulfite conversion was performed. Afterwards, the genome for each sample was amplified, fragmented, and hybridized to the Infinium Methylation 450K BeadChip and the Infinium MethylationEPIC BeadChip (Illumina) according to sample recruitment and manufacturer's protocols. Additional information on the distribution of samples across different arrays can be found in Supplementary Table 1. The meffil R software was used to perform the quality control (QC) of the samples and probes and for data normalization steps.[17] Samples were excluded based on detection *P* criteria >99%, poor bisulfite conversion based on control dashboard check, gende mismatches according to failed chromosome X and Y clustering, and failure to match genotypic information. Probes were filtered out based on detection *P* >0.01 in >95% of samples. Additionally, all probes located at the X and Y chromosomes were filtered to avoid sex bias. Probes with genetic variants at their CpG sites with a minor allele frequency >0.05 and those that map to multiple genomic regions were also excluded following the indications of a previous study.[18]

The raw methylation beta values were background corrected and normalized using the functional normalization. DNA methylation was measured as a beta value ranging from 0 to 1. Estimated blood cells proportions were inferred from the methylation beta values using epiDish R package (https://github.com/sjczheng/EpiDISH). This analysis was conducted through the robust partial correlations method with 100,000 iterations using Reinius et al[19] as the reference panel. CpGs included in the analysis were annotated with minfi R package (https://bioconductor.org/packages/release/data/annotation/html/IlluminaHumanMethylationEPICanno.ilm10b4.hg19.html).

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Identification of SSc differentially methylated positions. Principal component analysis was performed to assess the batch effect introduced when merging the two datasets (Supplementary Figure 1). Samples deviating four SDs from the cluster centroid were removed from further analysis. The effect of sex, age, and cell composition was calculated through a type III analysis of variance test with R. CpGs associated with these covariates were estimated through regression analysis with limma.[20] The resulting significant CpGs from this analysis with an estimated false discovery rate (FDR) <0.05 were removed from further analysis to avoid any potential bias (Supplementary Table 2). In accordance, 8,978 CpGs associated with age, sex, and cell composition were removed from our final analysis (Supplementary Table 3). Afterwards, DECO, a bioinformatic tool specifically designed for analyzing heterogeneous cohorts, was employed to identify differentially methylated positions (DMPs).[21] A more detailed explanation of this process is described in the Supplementary Material.

Gene ontology enrichment analysis. For DMPs, the GREAT online tool version 4.0.4 (http://great.stanford.edu/public/html/) was used to assess gene ontology (GO) enrichment analysis for biologic processes, molecular function, and cellular components. All CpGs included in the final analysis were selected as background positions and were annotated to the nearest gene. GO terms with an adjusted P <0.05 and with at least six CpGs were considered significantly enriched. For expression quantitative trait methylations (eQTMs), GO enrichment analyses were conducted with the genes altered by DMPs using the EnrichR R package (https://maayanlab.cloud/Enrichr/). GO categories with an adjusted P <0.05 and with a minimum count of six genes were considered significant.

Epigenetic age acceleration analysis. Epigenetic age acceleration (EAA) was inferred through the online DNA Methylation Age Calculator (https://dnamage.clockfoundation.org/), using DNA methylation β values as input. EAA measures were the following: residuals from the regression between the actual age of the patient or control and the epigenetic age calculated from Horvath or Hannum reference models[22,23]; the intrinsic EAA (IEAA), which adjusts for blood cell differences, calculated for both Hannum and Horvath panels; and the extrinsic EAA, which is defined from the Hannum reference panel and considers the percentage of exhausted immune cells.[23] The quantiles from the IEAA of both clocks were used to set the thresholds to categorize individuals into accelerated and normal aging groups. This resulted in 1.83 for Hannum model and 2.55 for the Horvath model. Differences in the frequencies of individuals with accelerated epigenetic aging in each SSc and control group were evaluated through a chi-square test, accompanied by odds ratio (OR) calculation.

TF enrichment analyses. First, we conducted a TF-binding site (TFBS) enrichment analysis by using HOMER motif discovery software version 4.11.1 (http://homer.ucsd.edu/homer/). A 250-bp window upstream and downstream of the DMPs was applied, and all CpGs included in the analyses were used as background. Those TFBSs with an FDR <0.05 were considered significant. Additionally, to estimate the activity of TFs from differential expression data, we used human TF data from the collecTRI package (https://github.com/saezlab/CollecTRI). The decoupleR package was used to infer their activity in regard to differential expression results (Supplementary Table 4) following the TF activity inference in bulk RNA-seq guidelines (https://saezlab.github.io/decoupleR/). TFs with an activity score >1 in absolute value and P <0.05 were considered significant.

eQTM analysis. To integrate methylation and expression data, eQTMs were calculated through a Pearson correlation test between a DMP and a differentially expressed gene (DEG), previously obtained by a differential expression analysis as explained in the Supplementary Material. This integrative approach was applied using the MatrixEQTL R package.[24] A maximum distance of 1 Mb between DMP and DEG was defined. Those eQTMs with an FDR <0.05 were considered significant.

Serum protein correlation analysis with DMPs and DEGs. Data on serum protein levels were collected from the PRECISESADS consortium through a turbidimetric immunoassay method and were subsequently corrected and normalized as described.[25] To analyze the correlation among DMPs, DEGs, and serum proteins in patients with SSc, Spearman correlations were performed among protein levels obtained from the same patients, the methylation β values, and the trimmed mean of M-value normalized gene counts, respectively. Correlations with an FDR <0.05 were considered significant.

Data availability. All data included in our study are available upon request at ELIXIR Luxemburg with the permanent link: 10.17881/th9v-xt85, and access procedure is described on the ELIXIR data landing page. The PRECISESADS consortium committed to secure patient data access through the ELIXIR platform. This commitment was formerly given by written to all patients at the end of the project and to the involved ethical committees. The future use of the project database was framed according to the scope of the patient information and consent forms; the use of patient data is limited to scientific research in autoimmune diseases. ELIXIR reviews applicants' requests and prepares data access committee's decisions on access to data, communicates such decisions to the data providers, who have 10 days to exercise their right to veto; otherwise, access is granted to the user.

RESULTS

Alterations in methylation levels of 525 genomic positions. Our analysis involved the inspection of the DNA methylation profiles of whole-blood samples of 179 patients with SSc and 241 unaffected controls, from which we obtained information of 352,036 CpG sites. Demographic and cell composition data from the study sample are collected in Supplementary Table 1. Covariate analysis yielded a significant correlation of age and sex with DNA methylation (Supplementary Table 2). In order to avoid any bias, CpGs associated with sex, age, and cell composition were removed from further analysis (Supplementary Table 3).

The comparison between patients with SSc and controls allowed the identification of 525 DMPs. Among them, 255 were hypomethylated and 270 were hypermethylated (Supplementary Table 5). DMPs were annotated to 395 unique genes. Notably, several of the DMPs annotate at genes that have been described as risk factors from SSc GWASs,[3] including *TNFSF4*, *CD247*, *IKZF3*, and *IL12RB2* (Supplementary Table 5). DMPs were mainly located in gene bodies (41.77% in hypomethylated and 43.31% in hypermethylated), compared to all CpGs analyzed (19.16%) (Figure 1A). Accordingly, DMPs were broadly situated in open sea regions (outside the CpG islands) and their surrounding areas, and this was more pronounced in hypomethylated DMPs (Figure 1B). Interestingly, it is worth mentioning the differences between hypermethylated DMPs, which were predominantly located in the region 200 bp upstream of the transcription start site (14.79%), closer to the gene transcription start site, in comparison to hypomethylated DMPs (8,44%). This could indicate that hypomethylated DMPs could be involved in longer-range interactions than hypermethylated DMPs.

DMPs related genes involved in cell adhesion and T cell response. Following the differential methylation analysis, we aimed to explore functional mechanisms associated with these changes in the methylation pattern. To this end, we conducted GO enrichment analysis, which revealed leukocyte cell–cell adhesion as the most significant term (FDR = 4.91×10^{-9}, fold enrichment [FE] 3.65; Figure 1C), with several integrin genes in the term (*ITGAL*, *ITGB1*, and *ITGB2*). Additionally, T cell–related processes also appeared among the most significant terms, with the most significant pathway being the T cell activation (FDR = 2.56×10^{-8}, FE = 3.66), including genes such as *AIRE* and *IRF1* (Figure 1C). These results were supported by the enrichment of genes within the T cell receptor complex (FDR = 5.06×10^{-7}, FE = 44.98), which includes *CD3E* and *CD3D* genes. Other significant immune-related terms concern cytokine production (FDR = 1.86×10^{-4}, FE = 3.53), which involves genes such as *IL12RB2*. A list of the enriched pathways including genes and DMPs is detailed in Supplementary Table 6.

No evidence of accelerated epigenetic aging in patients with SSc. We calculated the differences among individuals with EAA with SSc and the control group and did not observe significant differences across all EAA measures, even regarding nominal *P* values under different thresholds (Supplementary Table 7). Furthermore, the OR directions were not consistent through the different measurements and thresholds, indicating no tendency (Supplementary Table 7). Additionally, no changes were observed in the acceleration of methylation age in patients with SSc, which could indicate that alterations in the methylation pattern of SSc are specific to the disease and not a passive change due to aging, similar to what was previously reported in psoriasis,[26] a related skin disorder.

DNA methylation on gene expression levels. Thereafter, we were interested in investigating whether methylation differences could be driving changes in the transcriptome of these patients. Accordingly, a differential expression analysis was conducted using data from the same methylation cohort (Supplementary Table 1). After QCs (Supplementary Figures 2 and 3), expression data of 15,318 genes were examined, resulting in a total of 1,352 up-regulated and 1,178 down-regulated genes between patients with SSc and controls (Supplementary Table 4). Thus, an eQTM analysis was conducted. Through the eQTM analysis, 842 significant SSc-specific cis interactions were identified (Figure 2A). These interactions involved 361 DMPs (184 hypermethylated and 177 hypomethylated) and 553 genes (348 up-regulated and 205 down-regulated; Figure 2A). Notably, these interactions exhibited both negative and positive correlations (52.49% and 47.51%, respectively; Figure 2B). In terms of their genomic location, 17 interactions (12 hypomethylated and 5 hypermethylated) were found in the gene promoter region, with 88.24% of them being negatively correlated, as expected (Figure 2A). The most significant CpG–gene correlation corresponds to cg20417024-*F2R* (r = –0.57, FDR = 1.01×10^{-24}; Supplementary Table 8). Interestingly, the hypomethylation of cg16268734 correlated with the expression of 12 different genes, including *STAT2*, located in a genomic region 1.56 Mb long on chromosome 12.

Enrichment of eQTMs in neutrophil-related pathways. We then aimed to find a functional relationship between eQTMs and SSc pathogenesis; thus, we conducted a GO term enrichment analysis. After this analysis, a total of 44 terms were significant. The most significant term was neutrophil degranulation (FDR = 9.29×10^{-11}, FE = 4.07), including 47 genes, such as *PTX3* and *CD63* (Figure 2C). These genes also belong to the following most significant terms, with neutrophil activation involved in the immune response and neutrophil-mediated immunity (Supplementary Table 9). Among these GO terms, *ITGAM* and *ITGB2* integrins were also included. The type 1 interferon (IFN) pathway (FDR = 3.32×10^{-3}, FE = 6.49), an

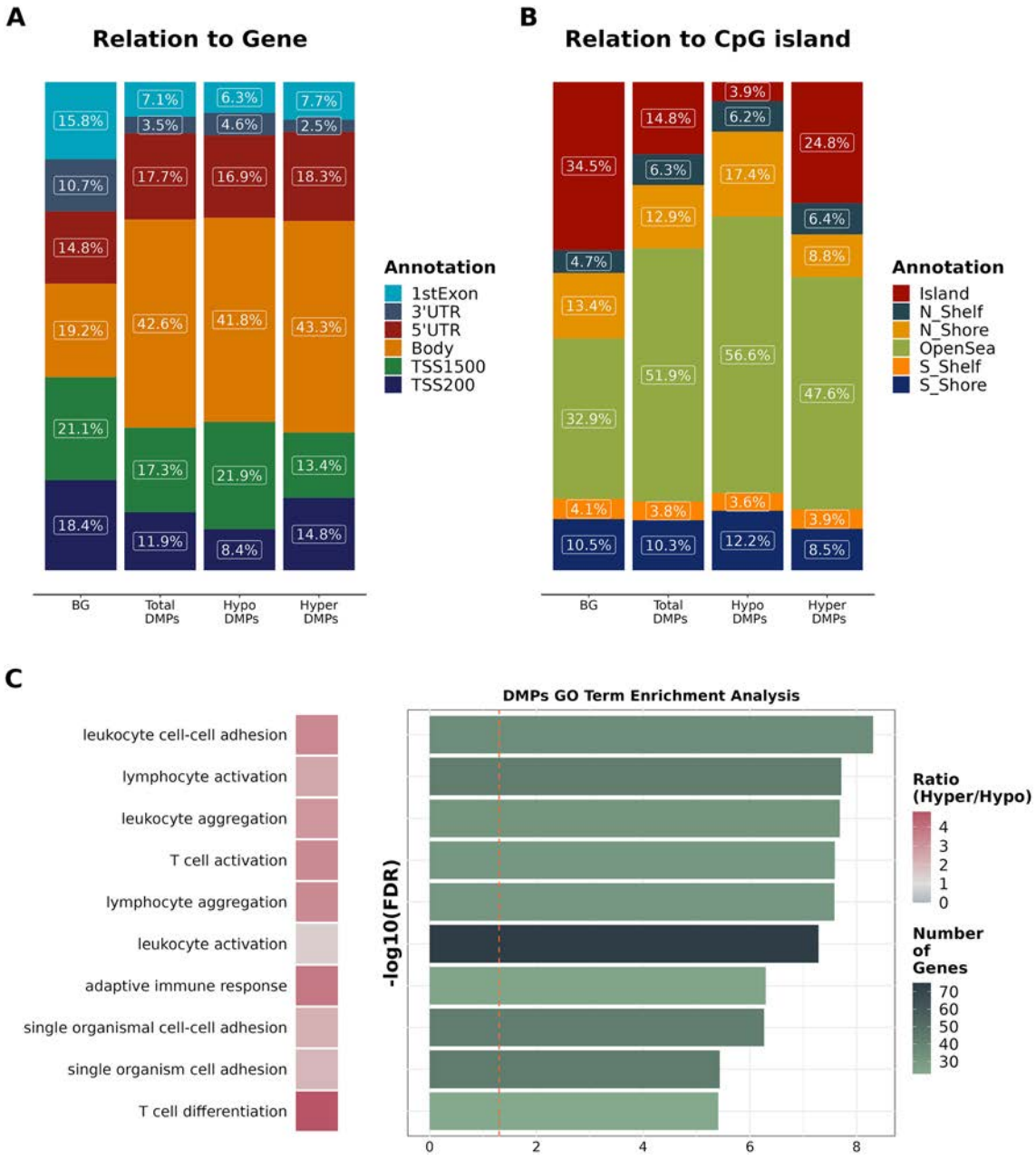

Figure 1. Differential DNA methylation analysis results. (A) Bar plot illustrating CpG positions, making a distinction between differentially methylated positions (DMPs) and the remaining analyzed CpGs in relation to genes. (B) Bar plot illustrating CpG positions, making a distinction between DMPs and the remaining analyzed CpGs regarding Islands. (C) Results of GO enrichment analysis for DMPs with GREAT software of top 10 significant terms. Boxes beside the GO term show the ratio of hypermethylated (Hyper) CpGs to hypomethylated (Hypo) CpGs within each term. The color of the bars reflects total gene count for each term. The vertical red dashed line indicates the significance threshold of false discovery rate (FDR) < related 0.05. BG, background CpGs that are not DMPs; Body, coding region of a gene; GO, gene ontology; Island, CpG island; N_Shelf, north shelf of the Island; N_Shore, north shore of the Island; OpenSea, outside the Island; S_Shelf, south shelf of the Island; S_Shore, south shore of the Island; TSS1500, 1500 bp upstream of the transcription start site; TSS200, 200 bp upstream of the transcription start site; UTR, untranslated region.

SSc-related pathway, was also enriched (Figure 2C), and several genes from this pathway, including *RSAD2* or *IRF5*, were upregulated. The results from this analysis and all genes included in the different pathways are further detailed in Supplementary Table 9.

Myeloid CEBP transcription factors in methylation and expression signatures identified in SSc. Therefore, we sought to further investigate whether these changes in the methylation pattern occur within TFBSs and thus could potentially be relevant in SSc. As a result, we observed that DMPs were

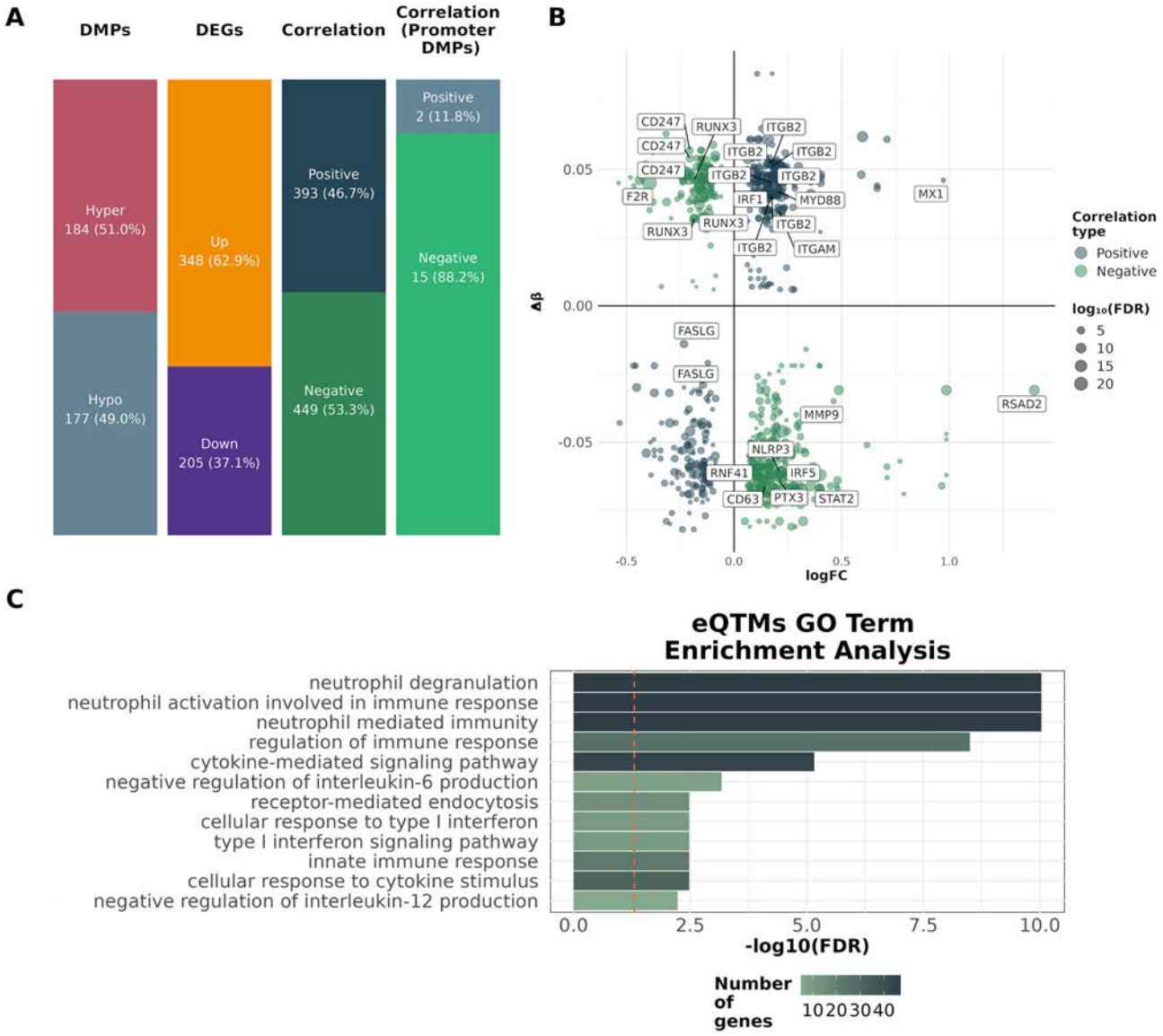

Figure 2. Integrative analysis of DNA methylation levels and gene expression. (A) Bar plot characterization of CpG–gene interactions concerning differentially methylated positions (DMPs), differentially expressed genes (DEGs), and the direction of the correlation. (B) Scatter plot illustrating significant cis interactions involving both DMPs and DEGs. The x-axis indicates the log(fold change) expression level difference between patients and controls, whereas the y-axis represents the difference in β values reflecting the shift in methylation levels. (C) Results of the GO enrichment analysis of eQTMs with EnrichR software. The top 10 most significant terms are displayed in the plot. The number of eQTMs included in each term is represented by the color. The vertical red dashed line indicates the significance threshold of false discovery rate (FDR) <0.05. Down, down-regulated; eQTM, expression quantitative trait methylation; GO, gene ontology; Hyper, hypermethylated; Hypo, hypomethylated; MMP, matrix metalloproteinase; Up, up-regulated.

preferentially located in the TFBSs of 55 different TFs (FDR < 0.05; Supplementary Table 10). The most significantly enriched TFBSs corresponded to CCAAT/enhancer-binding protein (CEBP; $P = 1 \times 10^{-12}$), a myeloid TF family related to the interleukin-6 pathway. Additionally, this overrepresentation was more prominent among the hypomethylated DMPs (Figure 3A). Furthermore, a significant enrichment was found in diverse members of the RUNX family of TFs (Figure 3A), which are involved in multiple processes including immune cell maturation, differentiation, and fibrosis.[27,28] Other relevant TFs were also enriched, such as the binding sites of Fli1 (Supplementary Table 10). This gene was previously reported to regulate the fibrotic reaction in the dermal fibroblasts of individuals with SSc.[29]

We then wanted to further investigate which TFs presented differential activity in regard to gene expression. Thus, employing differential expression results as the input, the activity of 629 different TFs was calculated. This analysis revealed 48 TFs with differential activity. Among them, 45 TFs exhibited increased inferred activity, whereas only 3 displayed a decreased activity score (Supplementary Table 11). The most significant TF was IRF9

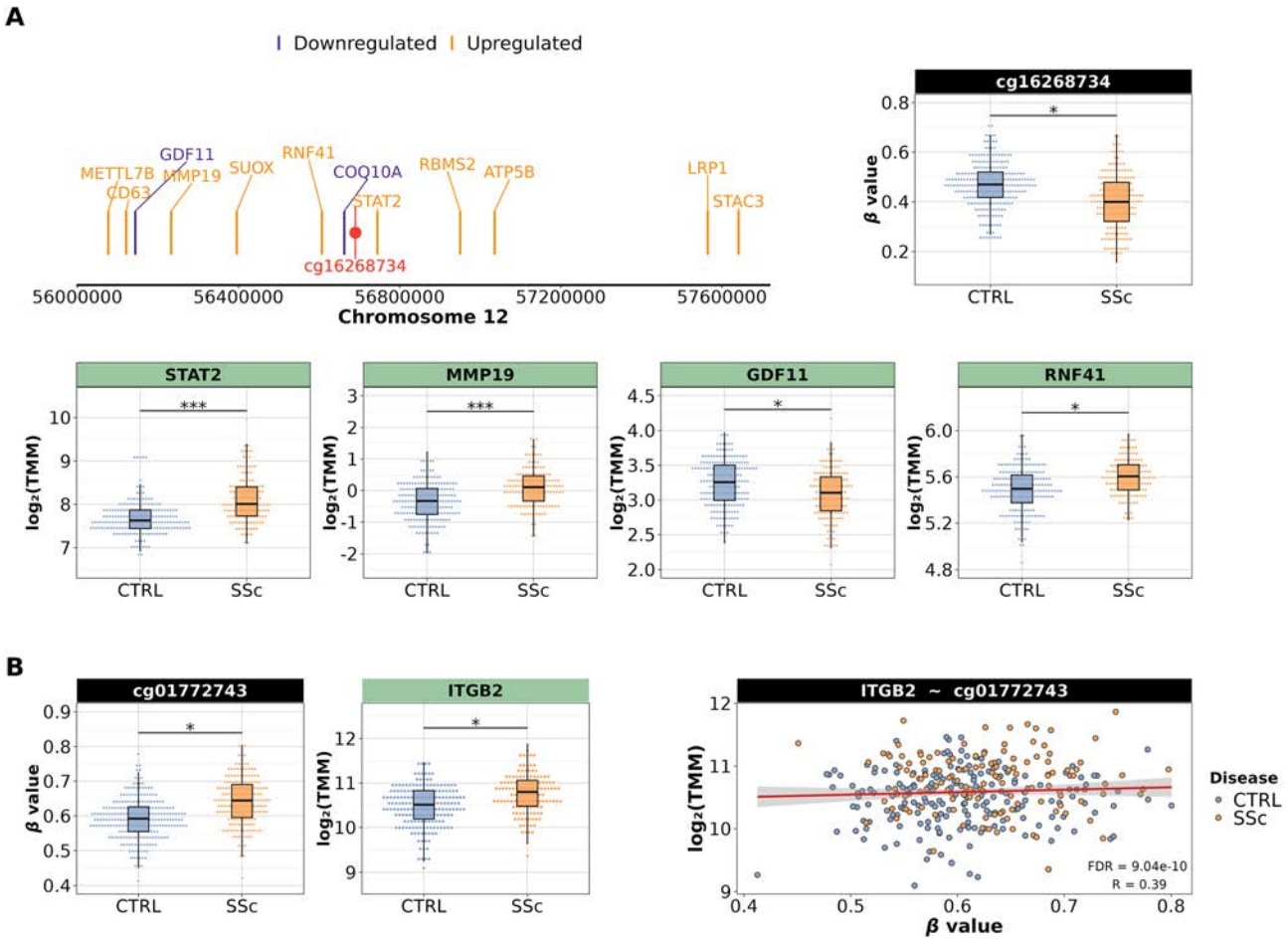

Figure 3. Expression quantitative trait methylation (eQTM) associations relevant in the analysis. (A) Graphical representation displaying the genomic position of genes located within the chr12q13.2 genomic region that includes cg16268734. Boxplots of the methylation levels of the CpGs as well as the expression of genes that are further detailed in the discussion section are also displayed. *FDR = 0.01; ***FDR < 0.0001. (B) Detailed representation of the most significant correlation *ITGB2* eQTMs among the seven significant interactions. The boxplot in the left displays β methylation values of cg01772743. A differentially methylated position that correlates with gene expression levels of *ITGB2* is depicted in the adjacent right boxplot. The scatter plot on the right-hand side illustrates the correlation between methylation (x-axis) and expression values (y-axis). *FDR = 0.01. The boxplots illustrating gene expression levels have green heading banners, whereas the boxplots with DNA methylation values have black heading banners. CTRL, unaffected individuals; FDR, false discovery rate; log_2(TMM), base 2 logarithm of the gene expression trimmed mean of M-values; MMP, matrix metalloproteinase; SSc, patients with systemic sclerosis.

($P = 3.97 \times 10^{-28}$; score 11.02), followed by other IFN-related TFs such as STAT1, IRF2, and STAT2 (Figure 3B). Additionally, the myeloid TFs CEBPA ($P = 4.71 \times 10^{-5}$; score 4.07) CEBPG ($P = 4.71 \times 10^{-5}$; score 2.91), and CEBPB ($P = 4.42 \times 10^{-2}$; score 2.01), from the CEBP TF family that resulted as the most enriched TFBS in the previous analysis, were among the significant TFs. Finally, NF-κB TF was also reported significant in this analysis (Supplementary Table 11).

Correlation of eQTMs with serum proteins, particularly matrix metalloproteinase 8, transforming growth factor β, and tumor necrosis factor α. Serum proteins levels might be seen as a good reflection of the changes occurring in this systemic disorder. Consequently, our objective was to delve into the relationship between DNA methylation and gene expression of eQTMs with serum protein levels from the same patients with SSc. We were able to retrieve data from 77 different proteins. After correlation analysis, 99 significant correlations between protein and DNA methylation levels were found, along with 101 significant correlations between protein and gene expression levels (Supplementary Tables 12 and 13). Regarding this, 22.99% of DMPs and 11.21% of DEGs from the eQTM results correlated with at least one protein. In this sense, matrix metalloproteinase (MMP) 8 correlated with 65 different DMPs (Supplementary Table 12). Additionally, tumor necrosis factor (TNF) α and transforming growth factor (TGF) β correlated with 19 and 10 DMPs, respectively. On the other hand, TGFβ correlated with the levels of 51 different DEGs, whereas TNFα correlated with 39 DEGs (Supplementary Table 13). Interestingly, *ITGB2*, a gene related with cell adhesion, correlated with both TGFβ and TNFα.

DISCUSSION

In this work, we conducted an epigenome-wide association study with the largest sample size reported to date in SSc. We identified relevant processes implicated in SSc pathology such as cell adhesion and proinflammatory and profibrotic pathways. Afterwards, we sought to explore how these DNA methylation changes might be influencing the gene expression profile and serum protein levels in the same individuals. The results of these integrative analyses highlight the importance of the epigenetic regulation of the myeloid component, with strong evidence of neutrophil involvement in SSc pathogenesis.

Interestingly, our findings highlight the potential role of integrins in SSc. Integrins are a family of proteins involved in a wide range of functions, such as leukocyte adherence and migration to affected tissues.[30] In the context of the disease, it has been described that their ablation is related to a milder fibrotic process and abnormalities in the extracellular matrix assembly.[31] Accordingly, we have identified DNA methylation alterations in several integrins such as *ITGAL* and *ITGB1*. In addition, DNA methylation also appears to influence the expression levels of *ITGAM* and *ITGB2*. Consistent with our results, three of these integrins (ITGAM, ITGAL, and ITGB2) were up-regulated in monocytes of patients with SSc, leading to increased adhesion levels in intercellular adhesion molecule (ICAM) 1–coated plates.[32] In fact, two DMPs act as eQTMs of *ICAM1*, a ligand for integrins containing ITGB2. Considering this and that indeed leukocyte cell adhesion was the most significant pathway, our findings support the rationale of a proadhesive phenotype of immune cells in the context of the disease. Efalizumab, which inhibits ITGB2–ICAM1 binding, is approved to treat plaque psoriasis, a related skin disease, underscoring the potential of targeting integrins as a therapeutic strategy for patients with SSc.[33]

Our results show an altered epigenomic pattern evidencing both a proinflammatory and a profibrotic response in patients with SSc. Concerning this, the disease is characterized by an initial inflammatory process followed by fibrosis in affected organs, a pattern illustrated in the different molecular signatures observed in patients with SSc.[34,35] Regarding the proinflammatory component, TF analysis from differential gene expression data revealed an increased inferred activity of NF-κB in patients with SSc. Other genes associated with this inflammatory cascade were also found to be up-regulated and correlated with DMPs. Furthermore, IFN-related genes extensively studied in patients with SSc were also up-regulated as a consequence of the alterations in DNA methylation (eg, *IRF1*, *IRF5*, and *STAT2*).[10,36,37] This matches well with the increased inferred activity in IFN-related TFs, which underlines the relevance of the IFN signature in patients with SSc. In the context of the profibrotic component, we identified markers of M2 monocyte polarization, which have been described to be crucial in skin fibrosis in the disease.[34] Among these markers, we reveal the hypomethylation and up-regulation of *CD163*, *LILRB4*, and *FCGR3A*.[38,39] Notably, we observe an enrichment in the TFBSs of diverse members of the RUNX family and three hypermethylated DMPs that negatively correlate with *RUNX3* expression. The down-regulation of this gene in plasmacytoid dendritic cells has been described to exacerbate skin fibrosis in patients with SSc.[28] Moreover, we identify a DMP that regulates the up-regulation of *MMP19*, which encodes a metalloproteinase that is related to idiopathic pulmonary fibrosis.[40] Considering the aforementioned results, we hypothesized that the dichotomy between inflammation and fibrosis in patients with SSc might be regulated by similar mechanisms, and these could be illustrated with the following examples: (i) there is a single DMP located in chr12q13.2, which regulates 12 different genes, including *STAT2* and *MMP19* (Figure 4A), and (ii) the integrin *ITGB2* correlated with both TGFβ and TNFα serum levels. The DNA methylation changes in these two relevant genomic loci could be considered crucial regulatory mechanisms in SSc pathogenesis.

The results from the enrichment analysis of the genes significantly modulated by eQTMs pinpoint to neutrophil-related pathways as relevant in the context of the disease. This cell type has been previously implicated in the immune, vascular, and fibrotic hallmarks of SSc.[41] It has been reported that the interaction of neutrophils with platelet microparticles leads to their differentiation into a proinflammatory and neutrophil extracellular trap (NET)-producing phenotype, exacerbating vascular damage in patients with SSc.[42] In this sense, our results show the hypermethylation of a DMP coupled with the down-regulation of *F2R*, the thrombin receptor for coagulation factor II, emphasizing the importance of the coagulation system in this vascular mechanism.[42] In accordance, our results indicate that alterations in DNA methylation levels influence an up-regulation of *PTX3*, a gene encoding pentraxin 3. This gene plays a crucial role in the proinflammatory response to toll-like receptor signaling in neutrophils.[43] Moreover, we identify a hypomethylated DMP negatively correlated with the expression of *CD63*, a regulator of NET formation.[44] Another molecule related to NETosis is the metalloproteinase MMP9,[45] the overexpression of which correlates with DNA methylation changes. Interestingly, neutrophils exhibiting high *MMP9* expression were associated with worse outcomes in idiopathic pulmonary arterial hypertension,[46] a regular complication observed in patients with lcSSc. Our results support the involvement of these molecules in the synthesis of NETs in patients with SSc. Thus, considering our results and the increasing relevance of NETs in other IMIDs,[47] neutrophils should be strongly considered in further molecular research of SSc.

In this line, TF analyses also highlighted the myeloid component. The most significant TFBS enrichment was observed in CEBP-binding sites, a myeloid TF family. In addition, we observed that CEBPA, CEBPB, and CEBPG (alpha, beta, and gamma subunits of this TF family), presented a significantly increased inferred activity in patients with SSc, reinforcing the results of the previous analysis. Interestingly, CEBPA, the most significant among them, is mainly expressed in myeloid cells and is required for neutrophil

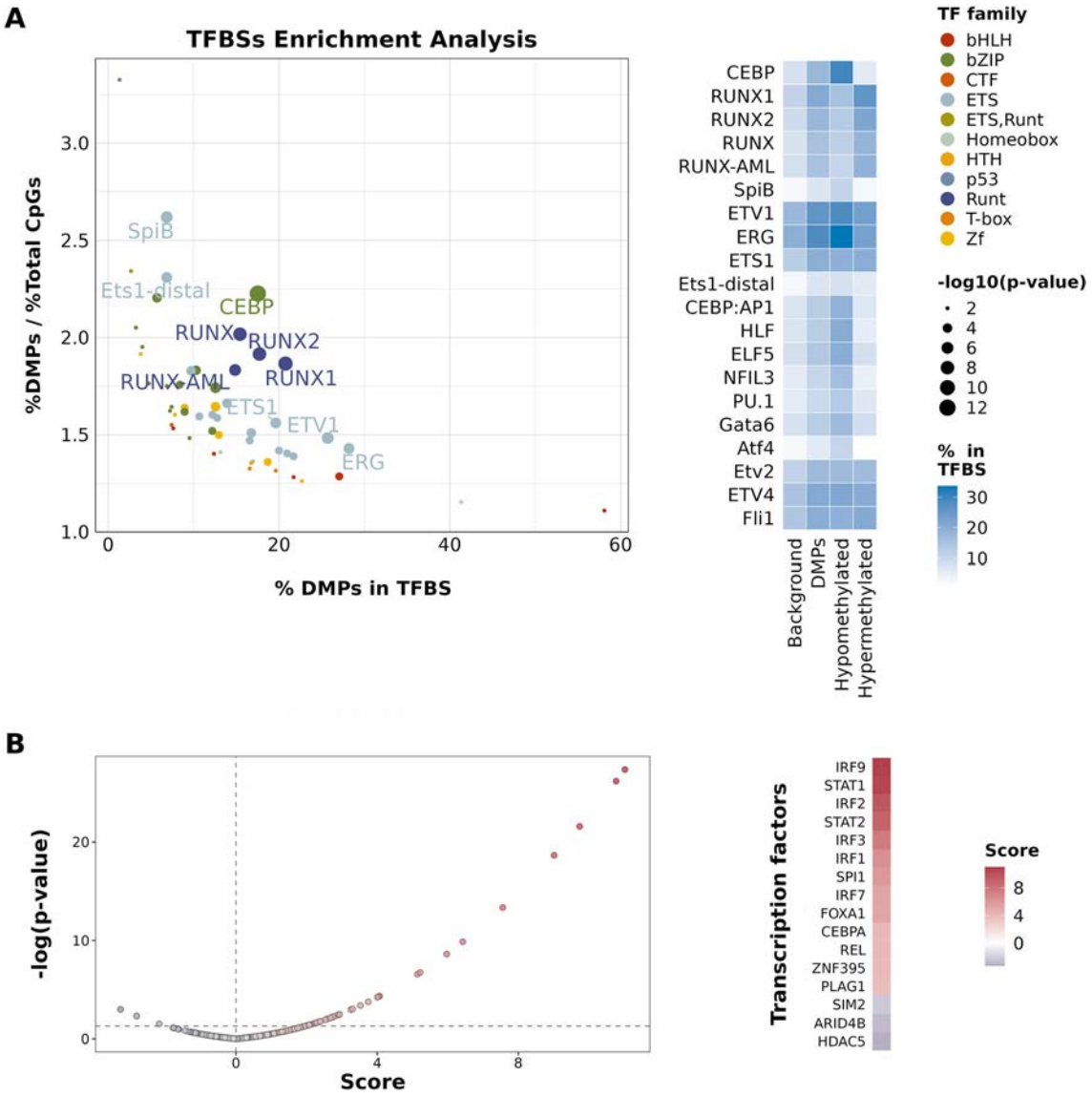

Figure 4. Results from the transcription factors (TFs) analysis. (A) Scatter plot displaying significant TFs resulting from the HOMER analysis. The x-axis exhibits the percentage of DMPs overlapping TF-binding sites (TFBSs) for each TF. The y-axis represents this value divided by the percentage of all analyzed CpGs overlapping with the same TF. The color denotes the TF's family, whereas the size reflects the significance level in terms of $\log_{10}$(*P* value). In the right part, a heatmap of the top 20 more significant TFs is displayed, stratifying between hypermethylated and hypomethylated DMPs. (B) Results of the TF activity analysis. A scatter plot illustrates the activity score on the x-axis against the significance level represented by $-\log_{10}$(*P* value) on the y-axis. Two dashed lines indicate the limits for an activity score of 0 and significance level of $P < 0.05$. On the right-hand side, a heatmap displays the activity scores for the significant TFs. Among these, only three presented a negative activity score, whereas the others constituted the top significant TFs with positive scores, completing the list of 20. CEBP, CCAAT/enhancer-binding protein; DMP, differentially methylated position; FDR, false discovery rate.

differentiation.[48] Intriguingly, CEBPB becomes active after cells are stimulated by the inflammatory response and participates in promoting the synthesis of proinflammatory molecules in macrophages.[49] Indeed, CEBPB induces the activation of the NF-κB pathway, another TF that showed increased activity in our analysis. Therefore, this goes in line with the rationale that myeloid cells, especially neutrophils, are susceptible to changes in the epigenomic landscape of SSc and might act as important contributors to the disease.

One of the limitations of our study is that we overlooked the specific variation of DNA methylation in different cell types as we analyzed whole blood. However, the accessibility of this tissue has allowed us to collect a large cohort of patients, which significantly improves the statistical power of the study. Moreover, identifying common variation in whole blood facilitates the translation of these findings into clinical practice. Additionally, although SSc is a heterogeneous disease, collecting clinical and serological data from a large cohort is challenging. Unfortunately, these data

were only available for a subset of patients (Supplementary Table 1), which significantly reduced the statistical power and limited our ability to perform stratified analyses. Finally, although we describe that both changes in the methylome and transcriptome of patients with SSc correlates with serum protein levels, further studies will be needed to unravel the exact molecular mechanism underlying this process.

Our comprehensive analyses revealed significant disparities in the epigenetic control of genes associated with key features of SSc, including immune dysregulation, vasculopathy, and fibrosis. Cell-adhesion molecules, particularly integrins, emerge as pivotal components in the regulatory landscape of the disease and may offer promising avenues for therapeutic interventions. We emphasize the importance of myeloid cell types and their proliferation and activation in the epigenetic dysregulation of SSc, as evidenced by the involvement of neutrophils and the CEBP TF family. Our findings offer a thorough insight into the intricate epigenetic regulatory mechanisms and their impact on gene expression in the disease, highlighting novel molecules as potential candidates for practical clinical applications and enhancing our understanding of SSc pathogenesis.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Acosta-Herrera confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

DATA AVAILABILITY STATEMENT

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

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Characterization of Incident Interstitial Lung Disease in Late Systemic Sclerosis

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Objective. Interstitial lung disease (ILD) is a common and potentially lethal complication of systemic sclerosis (SSc). Screening by high-resolution computed tomography (HRCT) is recommended in all patients with risk factors, including early disease. Little is known on late presentations of ILD. This study aimed to characterize the incidence, risk factors, and outcomes of late-onset SSc-ILD.

Methods. Study participants enrolled in the Canadian Scleroderma Research Group cohort from 2004 to 2020 without prevalent ILD were included. Incidence and risk factors for ILD (on HRCT) were compared according to disease duration above (late) and below (earlier) seven years from the first non-Raynaud manifestation. Risk of ILD progression was compared using Kaplan-Meier and multivariable Cox models.

Results. Overall, 199 (21%) of 969 patients developed incident ILD over a median of 2.4 (interquartile range 1.2–4.3) years. The incidence rate in late SSc (3.7/100 person-years) was lower than in earlier SSc (relative risk 0.68, 95% confidence interval [CI] 0.51–0.92). Risk factors for incident ILD included male sex, diffuse subtype, myositis, antitopoisomerase I autoantibodies, and higher C-reactive protein levels. Patients with late-onset ILD were also less frequently White and more frequently had arthritis and anti-RNA-polymerase III autoantibodies. Lung disease severity was similar between late- and earlier-onset SSc-ILD (forced vital capacity 88% and 87%, diffusion capacity of the lungs for carbon monoxide 64% and 62%, respectively). Progression rates were also similar between late- and earlier-onset SSc-ILD (log rank $P = 0.8$, hazard ratio 1.11, 95% CI 0.58–2.10).

Conclusion. ILD can present in late SSc. Risk factors and progression rates overlapped with earlier-onset SSc-ILD. Surveillance for ILD should continue in longstanding SSc. Frequency and modality of monitoring remain to be defined.

INTRODUCTION

Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease characterized by vasculopathy, immune dysregulation, and fibrosis in the skin and internal organs. Interstitial lung disease (ILD) is a frequent complication affecting more than 50% of patients with SSc[1,2] and is the leading cause of SSc-related death.[3] Early detection of SSc-ILD is essential, and screening with chest high-resolution computed tomography (HRCT) is recommended in all patients with SSc at baseline, particularly in the presence of risk factors, including early disease.[4] ILD often develops within the first three to five years of the first non-Raynaud disease manifestation[5–7] and may be more rapidly progressive in early disease.[8] Based on these observations, most

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clinical trials in SSc-ILD have restricted study inclusion to patients within seven years of SSc onset.[9–11]

However, ILD may also develop in longstanding SSc. In fact, among patients without prevalent ILD from the Canadian Scleroderma Research Group (CSRG) and the Australian Scleroderma Cohort Study registries, despite a median disease duration of 8.6 years, 153 new patients with ILD were diagnosed over a median follow-up of 4.1 years (incidence rate of 2.4 per 100 person-years),[12] with the highest incidence rates among patients with antitopoisomerase I antibodies. Recent studies have also shown similar ILD progression rates regardless of disease duration.[13,14] Moreover, immunosuppression has been reported to be effective in late SSc-ILD,[15] suggesting that the detection and treatment of ILD are relevant even in late SSc.

Although late-onset SSc-ILD may be clinically relevant, few studies have systematically assessed ILD in longstanding SSc. Therefore, in the present study, we assessed the incidence, characteristics, risk factors, evolution, and treatment outcomes of ILD developing in late SSc, in comparison with ILD developing in earlier SSc.

PATIENTS AND METHODS

Source and study populations. Our source population consisted of patients enrolled in the CSRG between 2004 and 2020 (for a list of CSRG Investigators, please see Appendix A). Briefly, study participants in the CSRG were recruited from 14 sites across Canada and one site in Mexico and had a diagnosis of SSc verified by an experienced rheumatologist, were more than 18 years of age, and were fluent in English, French, or Spanish. More than 98% of the CSRG cohort met the 2013 American College of Rheumatology/EULAR classification criteria for SSc.[16] All study participants recruited in the registry were assessed yearly by standardized clinical examinations, self-reported questionnaires, and laboratory investigations. The study population consisted of patients with SSc without a diagnosis of ILD at CSRG cohort entry (ie, no prevalent ILD). Ethics committee approval for this study was obtained at the Centre hospitalier de l'Université de Montréal and at all participating CSRG study sites. All study participants provided informed written consent to participate in the study.

Outcome measures and other covariates. The presence of ILD was determined by chest HRCT and recorded as abnormal by the recruiting physician and/or in the investigations section of the questionnaire. Chest HRCT was not performed systematically in all patients at the time of SSc diagnosis (because this was not standard clinical practice during the study period) and was most often ordered in the presence of risk factors, symptoms, or abnormal chest x-ray or pulmonary function tests. ILD was considered prevalent if present on HRCT at CSRG cohort entry, and incident if only recorded as present on HRCT over subsequent follow-up visits. Patients with a normal HRCT at any follow-up visit were assumed to have no ILD on all preceding visits, and patients without any HRCT data were considered as having missing ILD data. Chest HRCTs were performed locally, and data on lung disease characteristics and extent were extracted from radiology reports. Pulmonary function tests were performed annually at local respiratory physiology laboratories and data on forced vital capacity (FVC) and diffusion capacity of the lungs for carbon monoxide (DL_{CO}) were extracted from reports. ILD progression was defined as ≥10% relative decline in percent predicted FVC, or ≥5% to <10% relative decline in percent predicted FVC with ≥15% relative decline in percent predicted DL_{CO},[17] a definition that has been shown to predict mortality.[18]

Exposure and other variables. Disease duration was determined based on the onset of the first non-Raynaud disease manifestation as recorded by a study physician and stratified as late SSc (≥7 years) or earlier SSc (<7 years). Exposure to immunosuppression was defined as treatment with mycophenolate mofetil, cyclophosphamide, rituximab, or tocilizumab at the visit of interest or since the last registry visit. Medication history was recorded by a study physician at each visit. Demographic variables including age, sex, race and ethnicity (from a fixed set of categories), and smoking history were collected by patient self-report. Skin involvement was assessed using the modified Rodnan skin thickness score. Limited cutaneous disease was defined as skin involvement distal to the elbows and knees with or without facial involvement; diffuse cutaneous disease was defined as skin involvement proximal to the elbows and knees and/or of the trunk. Presence of inflammatory arthritis and myositis was recorded by a study physician, whereas symptoms of gastroesophageal reflux were collected by patient self-report. Autoantibody analyses were performed at baseline at the Mitogen Advanced Diagnostics Laboratory, University of Calgary, and detected by Euroline's SSc profile line immunoassay (Euroimmun GmbH) according to manufacturer's instructions. Autoantibodies were reported as absent (negative, equivocal, and low titers) and present (moderate and high titers). C-reactive protein levels were measured at local laboratories.

Statistical analysis. Incidence rates (and 95% confidence intervals [CIs]) for ILD diagnosis and progression were calculated based on the Poisson distribution and stratified by disease duration. Generalized estimating equations models with an autoregressive correlation structure were used to estimate the association between demographic, clinical, and serologic variables and the risk of incident ILD over follow-up, with a one-annual visit lag period before ILD assessment for time-varying variables. Missing variables were omitted and patterns of missingness were assessed. To estimate whether potential risk factors for incident ILD differed by timing of ILD onset, models were adjusted and stratified for disease duration, and further tested by an interaction between each risk factor and disease duration. The risk of ILD

progression was analyzed using unadjusted Kaplan-Meier and Cox proportional hazard models adjusted for FVC and DLCO, stratified by disease duration. To explore the effect of immunosuppressive drugs on lung disease progression, analyses were further stratified according to immunosuppressive drug exposure, modeled as a time-dependent current/noncurrent exposure with a one-visit lag period to minimize information bias due to reverse causality, and interaction terms were used to assess effect modification by disease duration. Study participants were observed from the time of ILD diagnosis until disease progression, or were censored due to death, permanent study drop-out, or last study visit. Baseline characteristics (at the time of ILD onset) of study participants with and without ILD progression were compared using a two-sample *t*-test, Mann-Whitney U-test, and Fisher's exact test. Sensitivity analyses were performed with disease duration defined according to time of first Raynaud or non-Raynaud disease manifestation. *P* values were considered significant if < 0.05. Multiple testing correction was not applied because analyses were considered exploratory. Statistical analyses were performed with R version 4.4.1.

RESULTS

Incidence of ILD in late SSc. Of 969 patients without prevalent ILD at baseline, 199 (21%) developed incident ILD over a median duration of 2.4 (interquartile range [IQR] 1.2–4.3) years. Of these, ILD was diagnosed at least seven years after the first non-Raynaud disease manifestation in 131 patients (66%), corresponding to an incidence rate of 3.7 (95% CI 3.1–4.3) per 100 person-years in late SSc. This incidence rate was lower than that in earlier SSc, which was at 5.4 (95% CI 4.2–6.9) per 100 person-years (relative risk 0.68, 95% CI 0.51–0.92, *P* = 0.01; Supplementary Table 1A, Supplementary Figure).

Characteristics and risk factors for late-onset ILD. Table 1 presents the characteristics of patients with and without incident ILD in late and earlier SSc. In both late and earlier SSc, patients who developed incident ILD were more frequently male and more often had diffuse cutaneous involvement, myositis, anti-topoisomerase I autoantibodies, absence of anticentromere autoantibodies, and higher C-reactive protein levels, compared to patients who did not develop ILD. In addition, patients with late-onset ILD were less frequently White and more often had arthritis and anti-RNA polymerase III autoantibodies. Lung disease severity was similar between late- and earlier-onset SSc-ILD, with comparable FVCs (88% and 87%, respectively) and DLCO (64% and 62%, respectively) values at ILD onset and similar proportions of patients having ground-glass opacities, fibrotic interstitial changes, and honeycombing on chest HRCT. Table 2 presents the associations between risk factors and incident ILD using two alternative approaches, namely analyses adjusted for disease duration and including an interaction term, and analyses stratified by late and earlier disease. These show consistent associations between risk factors in both late and earlier ILD.

Lung disease progression in late-onset SSc-ILD. Of 106 patients with incident ILD and available follow-up data, ILD progression was observed in 48 patients (45%) over a median duration of 3.1 (IQR 2.1–4.0) years, for an average incidence rate of 14.1 per 100 person-visits. The incidence of lung disease progression was not different between late- and earlier-onset ILD (Figure 1, log rank *P* = 0.8; and Table 3, adjusted hazard ratio [HR] 1.11, 95% CI 0.58–2.10). Progressors tended to be more frequently male (28% vs 8%, *P* = 0.076) and less frequently White (76% vs 95%, *P* = 0.066) in late-onset SSc-ILD, but otherwise similar regarding other demographic and disease characteristics (Table 4).

Effect of immunosuppressive drugs on lung disease progression in late-onset SSc-ILD. Over the course of follow-up, 29 person-visits (8.7%) were exposed to immunosuppressive drugs. Study participants were exposed for a median cumulative duration of 2 (IQR 1–3) annual visits. Among the exposed person-visits, 21, 11, 3, and 1 patients were exposed to mycophenolate mofetil, cyclophosphamide, tocilizumab, and rituximab, respectively. Exposed person-visits less frequently had late incident ILD (35% vs 61%, *P* = 0.01) and had lower mean FVCs (84.9% vs 93.6% predicted, *P* = 0.004) and numerically lower mean DLCO values (63.6% vs 71.3%, *P* = 0.06). Using time-dependent multivariable Cox analyses, the adjusted HR was 0.90 (95% CI 0.19–4.29) for ILD progression in study participants exposed to immunosuppression compared to nonexposed study participants in late-onset SSc-ILD, whereas in earlier-onset SSc-ILD, the adjusted HR was 0.40 (95% CI 0.05–3.27; *P* = 0.572 for interaction) (Table 3).

Sensitivity analyses using disease duration defined from time of Raynaud or non-Raynaud onset. Sensitivity analyses using disease duration defined according to time of Raynaud or non-Raynaud were done and are presented in Supplementary Tables 1–4. Results were mostly consistent with primary analyses.

DISCUSSION

In this retrospective cohort study, we aimed to study the incidence, characteristics, risk factors, evolution, and treatment outcomes of late-onset SSc-ILD. We found that ILD can present in late SSc, although its incidence rate was lower than in earlier SSc. This is consistent with previous studies showing that clinically significant ILD develops mostly within the first three to five years from disease onset.[5–7] Risk factors for developing ILD in late SSc were largely similar to those observed in earlier SSc and highly consistent with known risk factors for incident,

Table 1. Risk factors and lung disease characteristics of late- and earlier-onset SSc-ILD*

	Late SSc		Earlier SSc	
	ILD (n = 131 person-visits)	No ILD (n = 3,428 person-visits)	ILD (n = 66 person-visits)	No ILD (n = 1,154 person-visits)
Demographic characteristics				
Age at ILD onset, mean ± SD	59.2 ± 11.8	59.2 ± 11.8	54.8 ± 11.4	54.2 ± 11.9
Female, n (%)	114 (87)	3,163 (92)	50 (76)	994 (86)
White, n (%)	105 (82)	2,964 (90)	55 (86)	960 (88)
Smoking (ever), n (%)	78 (61)	1,969 (59)	41 (63)	629 (57)
SSc characteristics				
Diffuse, n (%)	51 (39)	907 (27)	32 (49)	410 (36)
mRSS, median (IQR)	7 (3–12)	4 (2–9)	8 (4–14)	6 (2–14)
Arthritis, n (%)	31 (28)	462 (17)	16 (25)	190 (18)
Myositis, n (%)	12 (11)	123 (5)	8 (13)	59 (6)
Gastroesophageal reflux, n (%)	56 (52)	1,183 (53)	21 (39)	433 (46)
Anti-centromere, n (%)	38 (30)	1,699 (54)	13 (22)	450 (45)
Anti-topoisomerase I, n (%)	26 (21)	202 (7)	15 (26)	131 (13)
Anti-RNA polymerase III, n (%)	24 (19)	354 (11)	13 (22)	215 (21)
Anti-Th/To, n (%)	2 (2)	18 (0.6)	4 (7)	11 (1)
Anti-fibrillarin, n (%)	0 (0)	22 (0.7)	2 (3)	4 (0.4)
Anti-Ro52/TRIM21, n (%)	34 (27)	726 (23)	13 (22)	226 (23)
Anti-PM/Scl-75/100, n (%)	4 (4)	158 (5)	3 (5)	36 (4)
C-reactive protein, median (IQR), mg/dL	4.0 (1.5–10.0)	2.6 (1.0–5.6)	5.4 (3.0–10.0)	2.5 (1.0–5.9)
ILD characteristics				
FVC (% predicted), mean ± SD	88.3 ± 17.2	–	87.2 ± 17.9	–
DLco (% predicted), mean ± SD	63.5 ± 18.9	–	61.8 ± 20.0	–
Ground-glass opacities, n/N (%)	19/38 (50)	–	17/32 (53)	–
Moderate to severe	4/31 (13)	–	5/29 (17)	–
Fibrotic interstitial changes, n/N (%)	31/40 (78)	–	24/34 (71)	–
Moderate to severe	5/32 (16)	–	4/28 (14)	–
Honeycombing, n/N (%)	6/34 (18)	–	6/31 (19)	–
Moderate to severe	0/30 (0)	–	2/30 (7)	–

* Anti-PM/Scl, anti–polymyositis/scleroderma; DLco, diffusion capacity of the lungs for carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; IQR, interquartile range; mRSS, modified Rodnan skin score; SSc, systemic sclerosis; TRIM21, tripartite motif–containing protein 21.

prevalent and/or severe ILD. These included male sex,[19–21] non-White race,[19, 22–25] diffuse cutaneous involvement,[6,7,23] myositis,[19,23] presence of antitopoisomerase I antibodies,[6–8,22] absence of anticentromere antibodies,[7,23] and high markers of inflammation.[19,26] In addition, in late SSc, incident ILD was also more frequent in patients with anti-RNA polymerase III antibodies[7] and arthritis.

Late- and earlier-onset SSc-ILD presented with similar lung disease severity and characteristics, with comparable FVC and DLco values as well as similar proportions of patients having ground-glass opacities, fibrotic interstitial changes, and honeycombing. However, only a small proportion of patients had data on specific HRCT characteristics, and information on ILD distribution and pattern were unavailable in the CSRG database. In a cross-sectional study done at one CSRG center, we found that lung disease pattern in 35 patients with late-onset SSc-ILD mostly consisted of nonspecific interstitial pneumonia and usual interstitial pneumonia patterns,[27] consistent with patterns classically described in SSc-ILD.[28]

Lung disease progression occurred in nearly half of patients and mostly occurred within the first four years of ILD diagnosis. The incidence of disease progression did not vary according to SSc duration at the time of ILD diagnosis. Although shorter SSc duration was previously reported to be associated with more rapid ILD progression,[19] the apparent plateau in FVC progression in patients with longer disease duration may have been attributable to survival bias,[13] and recent studies do not support early disease as a risk factor for ILD progression.[13,14]

On the other hand, other predictors of SSc-ILD progression have been identified, including HRCT extent[14,29–31] of ILD > 20%, lower or declining FVC and DLco,[29,32,33] presence of antitopoisomerase I autoantibodies,[22,31,34] diffuse cutaneous involvement,[34,35] male sex,[36–38] and high C-reactive protein levels.[26,39] In our study, progressors tended to be more frequently male and less frequently White in late-onset SSc-ILD, but otherwise similar regarding other demographic and disease characteristics.

A minority (9%) of person-visits were exposed to immunosuppressive drugs, mostly to mycophenolate mofetil and mostly in earlier-onset ILD. Patients requiring treatment had lower FVC and DLco values, which are known risk factors for ILD progression and possible indications for treatment initiation. Interestingly, on exploratory analyses, risk estimates for the effect of immunosuppressive drugs on lung disease progression suggested a

Table 2. Associations between risk factors and incident ILD expressed as ORs and 95% CIs

Risk factor	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P* value for interaction	Stratified ORs	
				Late ILD	Earlier ILD
Age, yr	1.002 (0.989–1.012)	1.003 (0.992–1.015)	0.892	1.003 (0.988–1.017)	1.004 (0.984–1.026)
Female	0.51 (0.34–0.76)	0.54 (0.36–0.80)	0.794	0.56 (0.33–0.97)	0.50 (0.27–0.93)
White	0.61 (0.41–0.91)	0.61 (0.41–0.92)	0.285	0.53 (0.33–0.85)	0.85 (0.41–1.79)
Smoking (ever)	1.13 (0.83–1.53)	1.13 (0.84–1.54)	0.584	1.07 (0.74–1.55)	1.28 (0.76–2.17)
Diffuse	1.80 (1.33–2.44)	1.74 (1.29–2.36)	0.906	1.77 (1.22–2.57)	1.70 (1.02–2.83)
mRSS (log)	1.42 (1.23–1.64)	1.40 (1.21–1.61)	0.065	1.56 (1.29–1.88)	1.19 (0.96–1.47)
Arthritis	1.78 (1.26–2.52)	1.78 (1.26–2.52)	0.526	1.93 (1.26–2.97)	1.53 (0.86–2.73)
Myositis	2.60 (1.60–4.21)	2.55 (1.58–4.12)	0.902	2.62 (1.39–4.92)	2.45 (1.12–5.40)
Gastroesophageal reflux	0.95 (0.69–1.30)	0.96 (0.70–1.32)	0.948	0.97 (0.66–1.43)	0.95 (0.56–1.62)
Anti-centromere	0.35 (0.25–0.50)	0.36 (0.26–0.51)	0.974	0.36 (0.24–0.54)	0.36 (0.19–0.68)
Anti-topoisomerase I	3.27 (2.20–4.86)	3.15 (2.11–4.69)	0.232	3.77 (2.32–6.11)	2.34 (1.23–4.45)
Anti-RNA polymerase III	1.58 (1.07–2.34)	1.50 (1.01–2.24)	0.171	1.84 (1.14–2.98)	1.07 (0.56–2.03)
Anti-Ro52/TRIM21	1.14 (0.80–1.62)	1.15 (0.81–1.62)	0.599	1.22 (0.81–1.83)	0.99 (0.52–1.89)
C-reactive protein (log)	1.430 (1.264–1.617)	1.427 (1.262–1.613)	0.456	1.377 (1.165–1.628)	1.511 (1.266–1.804)

* Analyses were first adjusted for late- or earlier-onset SSc-ILD and included an interaction term with disease duration, and separately stratified by disease duration at time of ILD onset. CI, confidence interval; ILD, interstitial lung disease; mRSS, modified Rodnan skin score; OR, odds ratio; SSc, systemic sclerosis; TRIM21, tripartite motif–containing protein 21.

numerically larger benefit in earlier-onset SSc-ILD compared to late-onset SSc-ILD. Although this difference was not statistically significant, this study was likely underpowered to detect such an interaction, with small numbers of treated patients in each subgroup. Few studies are available to inform us on the effect of immunosuppressive drugs in late-onset SSc-ILD,[15] as most clinical trials excluded patients with SSc duration above seven years.[9–11] It is possible that late-onset SSc-ILD is a condition that is pathophysiologically distinct from earlier-onset SSc-ILD and thus more refractory to immunosuppressive drugs, although our preliminary observations on similar ILD risk factors, severity, distribution, and pattern do not support this hypothesis.

There are several limitations to this study. First, patients were not systematically screened by HRCT at baseline or on follow-up yearly visits, as this was an observational cohort and, as such, procedures were performed as per standard of care in clinical practice settings. Thus, this study may have included study participants with prevalent subclinical ILD at baseline, and results should be interpreted as characterizing the incidence of clinically apparent ILD. Sensitivity analyses excluding patients without

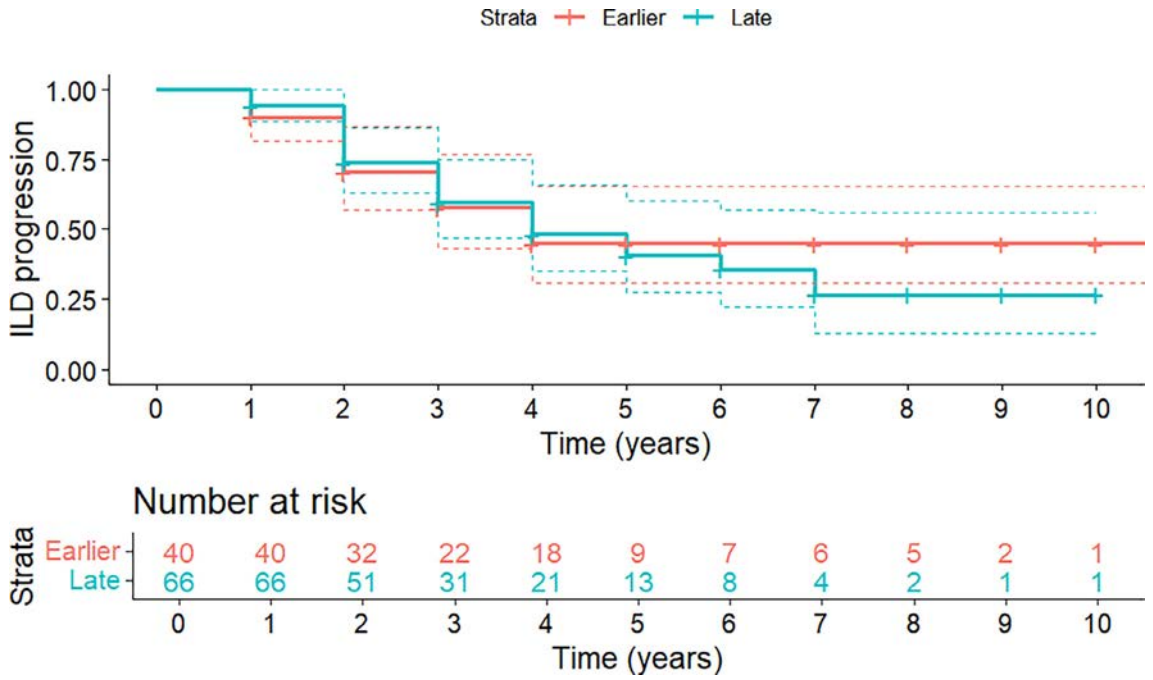

Figure 1. Kaplan-Meier curves for lung disease progression, stratified by late- and earlier-onset of systemic sclerosis–ILD (log rank $P = 0.8$). ILD, interstitial lung disease.

Table 3. Associations between the risk of interstitial lung disease progression and disease duration, expressed as HRs and 95% CIs*

	Events	Person-visits	Incidence per 100 person-visits (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)[a]
Total	48	340	14.1 (10.5–18.5)		
Late-onset	29	198	14.6 (9.9–20.6)	1.06 (0.59–1.89)	1.11 (0.58–2.10)
Treated	2	10	20.0 (3.3–61.7)	1.05 (0.24–4.57)	0.90 (0.19–4.29)
Not treated	27	184	14.7 (9.8–20.9)	1.00	1.00
Earlier-onset	19	142	13.4 (8.2–20.3)	1.00	1.00
Treated	3	19	15.8 (3.9–40.9)	1.39 (0.39–4.94)	0.40 (0.05–3.27)
Not treated	15	119	12.6 (7.3–20.1)	1.00	1.00

* Models were also stratified by the administration of immunosuppressive drugs for exploratory analyses. CI, confidence interval; HR, hazard ratio.
[a] Adjusted for forced vital capacity and diffusion capacity of the lungs for carbon monoxide. $P = 0.572$ for interaction.

proven absence of ILD on HRCT before ILD assessment showed a similar, albeit nonstatistically significant trend for lower incidence of ILD in late SSc (Supplementary Table 1C).

Second, nearly half of the study population was excluded from progression analyses due to missing pulmonary function test results on follow-up. Excluded patients had a lower DLco and a numerically higher frequency of late-onset ILD compared to included patients (Supplementary Table 5). This may have introduced some bias leading to underestimation of the risk of ILD progression in late-onset SSc-ILD.

Third, there was missingness in time-varying variables used in the study of associations between risk factors and incident ILD, most notably for C-reactive protein levels and especially among person-visits without incident ILD (Supplementary Table 6). If one hypothesizes that C-reactive protein levels are not tested systematically in routine care in the absence of active disease and that missing values are likely within normal range, then the high frequency of missing levels in person-visits without ILD would introduce bias leading to the underestimation of the association between C-reactive protein and risk of incident ILD.

Table 4. Characteristics of progressors and nonprogressors in late- and earlier-onset SSc-ILD*

	Late-onset SSc-ILD			Earlier-onset SSc-ILD		
	Progressors (n = 29)	Nonprogressors (n = 37)	*P* value	Progressors (n = 19)	Nonprogressors (n = 21)	*P* value
Demographic characteristics						
Age, mean ± SD	57.0 ± 12.2	61.4 ± 8.6	0.090	56.7 ± 8.8	52.1 ± 13.5	0.219
Female, n (%)	21 (72)	34 (92)	0.076	15 (79)	15 (71)	0.855
White, n (%)	22 (76)	35 (95)	0.066	16 (84)	18 (90)	0.951
Smoking (ever), n (%)	21 (72)	23 (62)	0.539	12 (63)	13 (62)	>0.99
SSc characteristics						
Diffuse, n (%)	13 (45)	15 (41)	0.921	10 (53)	12 (57)	>0.99
mRSS, median (IQR)	11 (4–18)	8 (4–11)	0.182	6 (4–15)	9 (4–15)	0.516
Arthritis, n (%)	6 (21)	9 (26)	0.860	2 (11)	38 (32)	0.680
Myositis, n (%)	4 (14)	2 (6)	0.546	2 (11)	20 (17)	0.447
Gastroesophageal reflux, n (%)	10 (35)	14 (40)	0.846	8 (47)	41 (38)	0.480
Anti-centromere, n (%)	5 (18)	12 (33)	0.269	2 (12)	4 (20)	0.818
Anti-topoisomerase I, n (%)	9 (32)	7 (19)	0.383	3 (18)	5 (25)	0.888
Anti-RNA polymerase III, n (%)	4 (14)	9 (25)	0.457	7 (41)	4 (20)	0.297
Anti-Th/To, n (%)	0 (0)	0 (0)	–	1 (6)	1 (5)	>0.99
Anti-fibrillarin, n (%)	0 (0)	0 (0)	–	1 (6)	0 (0)	0.934
Anti-Ro52/TRIM21, n (%)	7 (25)	11 (31)	0.834	5 (29)	3 (15)	0.509
Anti-PM/Scl-75/100, n (%)	2 (7)	0 (0)	0.365	2 (12)	1 (5)	0.883
C-reactive protein, median (IQR), mg/dL	3.5 (1.1–4.8)	4.9 (1.7–8.7)	0.131	6.0 (2.3–8.1)	3.7 (3.1–5.5)	0.334
ILD characteristics						
FVC (% predicted), mean ± SD	89.3 ± 15.0	86.7 ± 15.5	0.485	88.7 ± 16.1	91.2 ± 16.5	0.637
DLco (% predicted), mean ± SD	66.8 ± 17.3	62.8 ± 16.8	0.365	62.7 ± 20.1	70.3 ± 17.7	0.250
Ground-glass opacities, n/N (%)	1/3 (33)	1/6 (17)	>0.99	3/4 (75)	1/7 (14)	0.173
Moderate to severe	0/2 (0)	0/6 (0)	–	0/3 (0)	0/7 (0)	–
Fibrotic interstitial changes, n/N (%)	2/3 (67)	4/7 (57)	>0.99	4/4 (100)	4/7 (57)	0.406
Moderate to severe	0/2 (0)	0/7 (0)	–	0/3 (0)	0/6 (0)	–
Honeycombing, n/N (%)	0/3 (0)	0/5 (0)	–	0/3 (0)	0/8 (0)	–
Moderate to severe	0/3 (0)	0/5 (0)	–	0/3 (0)	0/8 (0)	–

* Anti-PM/Scl, anti–polymyositis/scleroderma; DLco, diffusion capacity of the lungs for carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; IQR, interquartile range; mRSS, modified Rodnan skin thickness score; SSc, systemic sclerosis; TRIM21, tripartite motif–containing protein 21.

Fourth, we were unable to repeat the analyses using the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Asociación Latinoamericana de Tórax definition of progressive pulmonary fibrosis,[40] which also considers symptoms and radiologic progression, due to significant missing data on follow-up HRCTs. Fifth, medication data were nominal for "current" or "past" exposure, with no details regarding specific start and stop dates, dose, intermittent exposure, or total duration of treatment. This could have led to exposure misclassification. Finally, for the analysis of the effect of immunosuppressive drugs on lung disease progression, a marginal structural Cox model incorporating inverse probability of treatment weights would have been preferable to account for confounding by indication and time-varying confounders; however, the small number of exposed person-visits within each disease duration stratum precluded such analyses.

In conclusion, in this multicentric retrospective cohort study, we confirmed that ILD can present in late SSc. Risk factors for developing ILD in late SSc were largely similar to those observed in earlier SSc and included male sex, non-White race, diffuse cutaneous involvement, arthritis, myositis, antitopoisomerase I antibodies, and high C-reactive protein levels. Late-onset SSc-ILD appears to present with similar lung disease severity and characteristics as in earlier SSc-ILD and may have comparable disease progression rates. Surveillance for incident ILD should continue even in patients with longstanding SSc, especially in the presence of risk factors, as patients may be equally at risk of having progressive disease regardless of disease duration at time of ILD onset. Frequency and modality of monitoring remain to be defined and should be the topic of future research.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Hoa confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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APPENDIX A: CANADIAN SCLERODERMA RESEARCH GROUP INVESTIGATORS

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Incidence and Genetic Risk of Juvenile Idiopathic Arthritis in Norway by Latitude

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Objective. We aimed to investigate the incidence of juvenile idiopathic arthritis (JIA) in the three geographic regions of Norway and whether potential regional incidence differences are explained by environmental or genetic factors across regions.

Methods. We conducted a register-based cohort study including all Norwegian children born from 2004 to 2019, with follow-up throughout 2020. The JIA diagnosis, defined by at least two *International Classification of Diseases, Tenth Revision* codes for JIA, was validated against medical records. The incidence rate (IR) and hazard ratio (HR) for JIA were estimated for all Norway and for the North, Mid, and South regions. In a subsample from the Norwegian Mother, Father, and Child Cohort Study (MoBa), the genetic risk for JIA was assessed in the three regions.

Results. After median 9.1 (range 0.3–16.0) years of follow-up, we identified 1,184 patients with JIA and 910,058 controls. The IR for JIA/100,000 person-years was 14.4 in all of Norway, 25.9 in the North region, 17.9 in the Mid region, and 12.5 in the South region. The HR (95% confidence interval [CI]) of JIA in the North region was 2.07 (1.77–2.43) and in the Mid region HR 1.43 (95% CI 1.23–1.67) compared with the South region. Adjustments for perinatal factors, socioeconomic status, and early antibiotic exposure did not change our estimates substantially. In MoBa (238 patients with JIA, 57,392 controls), the association between JIA and region of birth was no longer significant when adjusting for genetic factors.

Conclusion. We found a higher incidence of JIA with increasing latitude without evidence for available environmental factors explaining the observed gradient. In contrast, genetic factors modified the association, but further studies are warranted.

INTRODUCTION

The term juvenile idiopathic arthritis (JIA) describes a group of clinically heterogeneous diseases with onset before age 16 years, characterized by chronic joint inflammation.[1] Although its etiology is largely unknown, JIA is considered a complex condition in which one or more environmental risk factors may trigger disease in a genetically susceptible individual.[1]

JIA is the most common inflammatory rheumatic condition in childhood,[2] but the estimated incidence varies.[3,4] A systematic review from 2014 reported an incidence rate (IR) for JIA ranging from 1.6 to 23.0 cases per 100,000 person-years (PYs).[3] Some of this variation has previously been explained by differences in research methodology among studies.[4] However, epidemiologic studies still indicate a variation across geographic regions, populations, and ethnicities.[5–8] If there is a true variation across

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geographic regions, characterizing it may aid to identify disease-modifying environmental and genetic factors.

In the United States and Europe, both in the northern hemisphere, several autoimmune diseases show a notable north-south gradient with the highest incidence in the north.[9–11] This pattern is also seen in some childhood-onset autoimmune diseases like type 1 diabetes and pediatric inflammatory bowel disease (PIBD).[9,10] Similarly, in the southern hemisphere, a New Zealand study reported a higher risk of PIBD at higher southern latitudes.[12]

A possible north-south gradient in JIA has also been pointed out with the highest incidence in the northernmost countries in Europe compared with the south.[7,13] Within Norway, previous studies have reported a higher incidence of JIA in the northernmost regions than in the southeast region.[14,15] However, no previous study has investigated JIA incidence across different European countries or all regions in Norway; thus, the results are not directly comparable because of the variation in methodology. Importantly, no previous study has accounted for the effect of genetic or environmental factors on the occurrence of JIA across geographic regions.

Norway is the country situated farthest north in Europe, and it stretches across a wider range of latitudes than most other European countries, from 57°N to 71°N latitude.[16] The geography of Norway and the public health care system,[17] combined with unique health and administrative registries, position Norway as an ideal location for conducting epidemiologic research across geographic regions.

We aimed to investigate the incidence of JIA across different geographic regions in Norway and assess if there is a latitudinal gradient. To explain possible regional differences in the incidence, we also aimed to investigate the impact of environmental or genetic risk factors for JIA across regions.

PATIENTS AND METHODS

Study design, populations, and data sources. To investigate the incidence of JIA in different geographic regions, we conducted a nationwide register-based study based on data from the Medical Birth Registry of Norway (MBRN). To assess the impact of potential differences in genetic background for JIA across regions, we also included data from the Norwegian Mother, Father, and Child Cohort Study (MoBa).

The MBRN sample. From the MBRN (MBRN sample), we included all children born in Norway between January 1, 2004, and December 31, 2019, and observed them until the onset of JIA, age 16 years, or December 31, 2020, whichever occurred first. The MBRN is a national health registry based on mandatory reporting and contains information about all births in Norway.[18] Data from the MBRN were linked on an individual level using the unique national identification (ID) number with data from the Norwegian Patient Registry (NPR), Statistics Norway (SSB), and Norwegian Prescription Database (NorPD). Children who had emigrated were excluded.

The MoBa sample. From MoBa (MoBa sample), we included children who previously had been genotyped in MoBaPsychGen.[19] MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (NIPH). Participants were recruited from all over Norway from 1999 to 2008. The women consented to participation in 41% of the pregnancies. The cohort included approximately 114,500 children, 95,200 mothers, and 75,200 fathers.[20,21] We used version 12 of the quality-assured MoBa data files, which was released for research purposes in January 2019. Data on the children in MoBa were also linked on an individual level with data from the NPR. The MoBa sample partially overlaps with the MBRN sample; 42,552 children in the MoBa sample were born between 2004 and 2009 and were also included in the MBRN sample.

Study setting. In 2020, the number of inhabitants in Norway was approximately 5.4 million, with almost one million children age <16 years.[22] The health care system provides universal access to health care services, and it is free of charge for children age <16 years.[17] Children with JIA are followed either by pediatricians or rheumatologists, almost exclusively at public hospitals. The university hospitals have the main responsibility for patients with JIA, but many patients also receive intermediate follow-up care at their local hospitals.

Case definition. Cases in the MBRN sample were identified using data from the NPR, which has received data with personal ID numbers from all Norwegian public hospitals and specialists with public funding since 2008.[23] Consequently, the NPR captures data for virtually all Norwegian children with JIA. Cases were defined by at least two *International Classification of Diseases, Tenth Revision* (ICD-10) codes of M08 (juvenile arthritis) and/or M09 (juvenile arthritis in diseases classified elsewhere) reported to the NPR between January 1, 2008, and December 31, 2020, with the first registration before age 16 years. If the year of diagnosis was 2020, we only required one code of M08 or M09 because this was the last year of data from the NPR and some children most likely only had one visit before the end of the year. The age of diagnosis was calculated from the child's birth year and month to the year of their first registration of a relevant ICD-10 code (M08, M09, or M13 [other arthritis]) for those who later fulfilled the case definition. Because we only had information about which year the ICD-10 codes were registered, we averaged the month of onset to July 1. In sensitivity analyses, we also assessed a stricter case definition with at least three codes of M08 and/or M09 (at least two codes if the year of onset was 2020). The cases in the MoBa sample were identified according to the same case definition of at least two diagnostic codes for

JIA using data from the NPR registered between January 1, 2008, and December 2021. If the year of diagnosis was 2021, only one code of M08 or M09 was required.

Validation of the case definition. To assess the validity of our case definition against medical records and to evaluate potential regional variations in coding practices in Norway, we conducted a validation study that included data from four hospitals. Oslo University Hospital (OUS), St. Olav's Hospital, and the University Hospital of North Norway (UNN) are university hospitals with primary responsibility for patients with JIA living in Southeastern, Mid- and Northern Norway, respectively. Vestfold Hospital Trust (SIV) is a local hospital situated southwest of the capital Oslo.

At each hospital, patients age <16 years registered with their first M08 or M09 ICD-10 code between January 1, 2008, and December 31, 2020, were identified. Children who resided outside the primary health region of each hospital and those who were observed in other hospitals with insufficient information available in their medical records were excluded. At OUS, a random sample from the pediatric and rheumatologic departments was drawn. At SIV and UNN, all eligible patients from the pediatric department were included. At St. Olav's, all eligible patients from all departments were included.

Assessments of medical records were performed by medical doctors highly experienced with the diagnosis and follow-up of patients with JIA (SVH, MR, EN, and SA). The children were categorized either as patients with JIA (true positives) or patients without JIA (false positives). The number of ICD-10 codes of M08/and or M09 in categories (1, ≥2, or ≥3) registered between January 1, 2008, and December 31, 2022, and JIA (yes or no) were registered for each patient.

Main exposure. We divided our study samples into three geographic areas from north to south based on the mother's region of living at the time of birth as given in the MBRN: the North, Mid, and South regions. Region South was used as the reference region.

Covariates in the MBRN sample. *Potential mediators.* To assess the effect of potential mediators on the association between region of birth and JIA, we included variables from different sources. From the MBRN we included maternal age, parity, mode of delivery, maternal smoking during pregnancy, child's sex, prematurity, season of birth, year of birth, and birth weight. Both education and income are measures used for an individual's socioeconomic status.[24] From SSB we categorized maternal educational level by October 1, 2020, into three groups (low, medium, and high). If the educational level was missing (n = 286,560), the household income from SSB was categorized into three groups (<25th percentile, 25th–75th percentile, or >75th percentile) and used instead. Because the frequency of antibiotic use is known to vary across regions in Norway and early antibiotic exposure is a potential risk factor for JIA,[25–27] we included information on systemic antibiotics given in the neonatal period from the MBRN and antibiotics dispensed from a pharmacy within age 2 years from NorPD.

JIA medication. To characterize the cases, we included data from NorPD on disease-modifying antirheumatic drugs (DMARDs) dispensed from a pharmacy to the cases between 2004 and 2021. These drugs were divided into two groups: methotrexate (MTX) and other DMARDs, including abatacept, tofacitinib, baricitinib, etanercept, adalimumab, certolizumab, golimumab, anakinra, tocilizumab, canakinumab, and secukinumab.

Genetic factors in the MoBa sample. In MoBa, blood samples were obtained from children (umbilical cord) at birth. DNA was extracted and stored at the NIPH.[28] The MoBa cohort genotyping was conducted through multiple research projects for several years.[29] A novel family-based pipeline (MoBaPsychGen genotype quality control [QC] pipeline) that includes preimputation QC, phasing, imputation, and postimputation QC was implemented to handle the complex relatedness structure of the cohort while taking into account the genotyping array and genotyping batch effects.[19]

We restricted the analysis to individuals of European ancestry selected based on a visual comparison of the first seven genetic principal components (PCs) with PCs from 1,000 genome phase 1 unrelated samples (n = 1,083), as described previously.[19] For each related pair in the study with individuals having a kinship coefficient >0.05, one member was excluded. The exclusion process gave priority to the retention of cases; all other exclusions were made randomly.

To assess each individual's genetic risk for JIA, we calculated polygenic risk scores (PRSs) from a genome-wide association study of JIA.[30] For this calculation, we used PRSice (version 2.3.5)[31] with various *P* value thresholds (5e–8, 1e–6, 1e–5, 1e–4, 1e–3, 1e–2, 5e–2, 1e–1, 5e–1, and 1). For further analyses, we extracted the first PCs of PRSs across all *P* value thresholds, following a widely applied method.[32] The genetic PCs were calculated as described in Corfield et al.[19]

Statistical analysis. In the validation of our case definition, we calculated the positive predictive values (PPVs) as the proportion of children with JIA, based on an assessment of medical records, out of all the children who were registered with at least one, two, or three M08 and/or M09 ICD-10 codes at each hospital. PPVs were calculated separately at each hospital and combined to obtain pooled results.

In the MBRN sample, we calculated the IR and cumulative incidence in all of Norway and separately for each of the three regions. To estimate the hazard ratio (HR) for JIA by geographic region, we used Cox regression. Additionally, we conducted Schoenfeld proportional hazards tests and log-log survival plots to assess whether the assumption of proportional hazards was

upheld. To assess the effect of potential mediators, we included perinatal factors, socioeconomic status, and systemic antibiotics during age 0 to 24 months in the adjusted Cox regression model.

In sensitivity analyses, we included maternal smoking during pregnancy. In further sensitivity analyses, we calculated the IR of JIA in different regions and the HR by region of birth with a stricter case definition. Additionally, we performed sensitivity analyses in which children born before 2007 were excluded because (1) the NPR contains individual-level data from 2008 and onward (allowing for the age at onset calculations)[23] and (2) JIA rarely manifests before age 1 year.[1]

To compare distributions of sex, medication use, and differences in the age of diagnosis for patients with JIA by region of birth in the MBRN sample (Supplementary Table 1), we used chi-square tests and Kruskal-Wallis test. To assess whether observed differences in the IR across geographic regions of Norway may be attributed to genetic differences, we ran logistic regression analyses in the MoBa sample. First, we tested the association between the region of birth and JIA (unadjusted model). In model 1, we included the covariates sex and year of birth. In model 2, we adjusted for sex, year of birth, and PRS for JIA. In model 3, we adjusted for sex, year of birth, PRS for JIA, and the first 10 genetic PCs. All statistical analyses were performed using STATA/SE V17 statistical software[33] and R (version 4.2.3).[34]

Ethics. The use of MBRN data with relevant linkages was approved by the Regional Committee for Medical and Health Research Ethics (REK #18622) and the Norwegian Data Protection Authority. The study was exempted from individual consent because it was a registry-based study with a low risk of personal identification. Involvement of patients and the public was considered not relevant in this study.

The validation study was approved by the Data Protection Officer (DPO) at each hospital for quality improvement purposes. Additionally, secondary approval was granted for the use of results in research projects. The current study with use of data from MoBaGenetics was approved by REK Southeast 28469 as part of the MoBaRheuma project. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The pregnant women provided written informed consent. The MoBa cohort is currently regulated by the Norwegian Health Registry Act.

RESULTS

Validation of case definition. In the validation, we included 1,086 children with at least one M08 or M09 code registered between 2008 and 2020. Out of these, 959 had at least two codes registered. In the validation, 913 patients with true JIA were identified. The PPV for those with at least two relevant ICD-10 codes was 93.4%. A less strict definition of only one code resulted in a PPV of 84.1% whereas a stricter definition of at least three relevant ICD-10 codes resulted in a PPV of 95.1% (Supplementary Table 2).

The MBRN sample. *Baseline characteristics by region of birth.* In the MBRN sample, we included 911,242 children and identified 1,184 patients with JIA after a median of 9.1 years (range 0.3–16.0 years) of follow-up (for flowchart, see Supplementary Figure 1). The distributions of baseline characteristics by region of birth are presented in Table 1. The maternal age was lower in regions North and Mid compared with region South, and the socioeconomic status and use of antibiotics during age 0 to 24 months was lower in region North compared with regions Mid and South.

Characteristics of patients with JIA. Among the patients, 796 (67.2%) were born in region South, 199 (16.8%) in region Mid, and 189 (16.0%) in region North (Figure 1). Of all patients, 746 (63.0%) were girls, and the median age of diagnosis was 5.0 years (range 0.3–16.0 years). The distribution of sex and age of diagnosis did not differ across the regions. The use of MTX and other DMARDs was lowest in region North (Supplementary Table 1).

Incidence of JIA by region of birth. The national cumulative incidence was 0.13% with 0.11% in region South, 0.16% in region Mid, and 0.24% in region North. In all of Norway, we found an IR per 100,000 PYs of 14.4 (95% confidence interval [CI] 13.6–15.2) (Figure 1). The IR increased gradually by latitude from 12.5 (95% CI 11.6–13.4) in region South to 25.9 (95% CI 22.5–29.9) in region North (Figure 1). Using region South as the reference category, the JIA in region North was HR = 2.07 (95% CI 1.77–2.43) and in region Mid it was HR = 1.43 (95% CI 1.23–1.67) (Table 2).

To study whether differences in the IR across regions were mediated by other recorded variables that differ across regions, we adjusted for all variables included in Table 1 in addition to birth year. With adjustments for perinatal factors, socioeconomic status, and systemic antibiotics during age 0 to 24 months when these factors were known (n = 902,379), the HR for JIA remained almost unchanged (Table 2). In further analyses when additionally adjusting for smoking during pregnancy when this was known (n = 733,980), the HR was essentially unchanged (Supplementary Table 3). In sensitivity analyses with a stricter case definition, the IR of JIA was slightly lower than with a case definition of at least two codes (Supplementary Table 4), but the HR comparing regions Mid and North with region South remained in magnitude (Supplementary Table 5). In further sensitivity analyses including children born from 2007 (n = 742,724), we found a numerically lower IR in all regions, and the HR for region North

Table 1. Baseline characteristics by region of birth in the Medical Birth Registry of Norway sample

	Region of birth		
	South (n = 708,943)	Mid (n = 122,910)	North (n = 79,389)
Maternal factors, n (%)			
Maternal age			
<25 years	95,270 (13.4)	21,449 (17.5)	17,440 (22.0)
25 to 34 years	469,214 (66.2)	80,489 (65.5)	47,943 (60.4)
≥35 years	144,458 (20.4)	20,972 (17.1)	14,006 (17.6)
Parity			
0	303,088 (42.8)	49,149 (40.0)	31,907 (40.2)
1	258,893 (36.5)	44,823 (36.5)	28,120 (35.4)
2	105,250 (14.9)	21,108 (17.2)	13,273 (16.7)
≥3	41,712 (5.9)	7,830 (6.4)	6,089 (7.7)
Mode of delivery			
Any Cesarean section[a]	117,514 (16.6)	21,265 (17.3)	13,098 (16.5)
Type of Cesarean section[a]			
Planned	44,720 (6.3)	7,635 (6.2)	4,615 (5.8)
Emergency	72,672 (10.3)	13,496 (11.0)	8,468 (10.7)
Not specified	122 (0.02)	134 (0.1)	15 (0.02)
Socioeconomic status[b]			
Low	131,499 (18.6)	20,678 (16.8)	16,142 (20.3)
Medium	234,171 (33.0)	45,153 (36.7)	28,845 (36.3)
High	339,275 (47.9)	56,695 (46.1)	34,020 (42.6)
Missing	3,998 (0.6)	384 (0.3)	382 (0.5)
Smoking through pregnancy			
No	525,430 (74.1)	97,875 (79.6)	57,326 (72.2)
Occasionally or changed	9,195 (1.3)	3,136 (2.6)	1,256 (1.6)
Yes	29,901 (4.2)	4,560 (3.7)	5,301 (6.7)
Missing	144,417 (20.4)	17,339 (14.1)	15,506 (19.5)
Child factors, n (%)			
Sex			
Female	344,663 (48.6)	59,920 (48.8)	38,725 (48.8)
Prematurity[c]			
Yes	45,062 (6.4)	7,276 (5.9)	4,758 (6.0)
Missing	3,774 (0.5)	297 (0.2)	193 (0.2)
Season of birth[d]			
Winter	164,210 (23.2)	28,246 (23.0)	18,690 (23.5)
Spring	183,673 (25.9)	31,731 (25.8)	20,324 (25.6)
Summer	191,148 (27.0)	33,430 (27.2)	21,116 (26.6)
Autumn	169,912 (24.0)	29,503 (24.0)	19,259 (24.3)
Birth weight, g			
<2,500	33,417 (4.7)	5,051 (4.1)	3,448 (4.3)
2,500 to 3,499	304,771 (43.0)	49,780 (40.5)	33,077 (41.7)
3,500 to 4,499	348,633 (49.2)	63,549 (51.7)	40,068 (50.5)
≥4,500	22,122 (3.1)	4,530 (3.7)	2,796 (3.5)
Antibiotic exposure during age 0 to 24 months			
Yes	294,268 (41.5)	49,978 (40.7)	26,303 (33.1)

[a] Vaginal birth was the reference category.
[b] Socioeconomic status was defined by the maternal educational level by October 1, 2020, categorized into three groups (low, medium, and high). If the educational level was missing (n = 286,560), the household income from Statistics Norway was used instead and categorized into three groups (<25th percentile, 25th–75th percentile, or >75th percentile).
[c] Gestational age <37 weeks.
[d] Winter was December to February, spring was March to May, summer was June to August, and autumn was September to November. There was one missing value for maternal age. There were no missing values for parity, mode of delivery, sex, birth weight, or antibiotic exposure during age 0 to 24 months.

compared with region South was slightly attenuated (Supplementary Tables 6 and 7).

Genetic factors in the MoBa sample by region of birth. From MoBa, we included 57,630 children and identified 238 patients with JIA (for flowchart, see Supplementary Figure 2). In unadjusted analyses, and when adjusting for sex and birth year, the risk of JIA was significantly higher in region North (odds ratio [OR] = 1.76, 95% CI 1.14–2.71; $P = 0.01$) compared with region South (Figure 2 and Table 3, model 1). When additionally adjusting for PRS in model 2, we observed an attenuation of association for region North (OR = 1.63, 95% CI 1.05–2.52; $P = 0.03$) and an

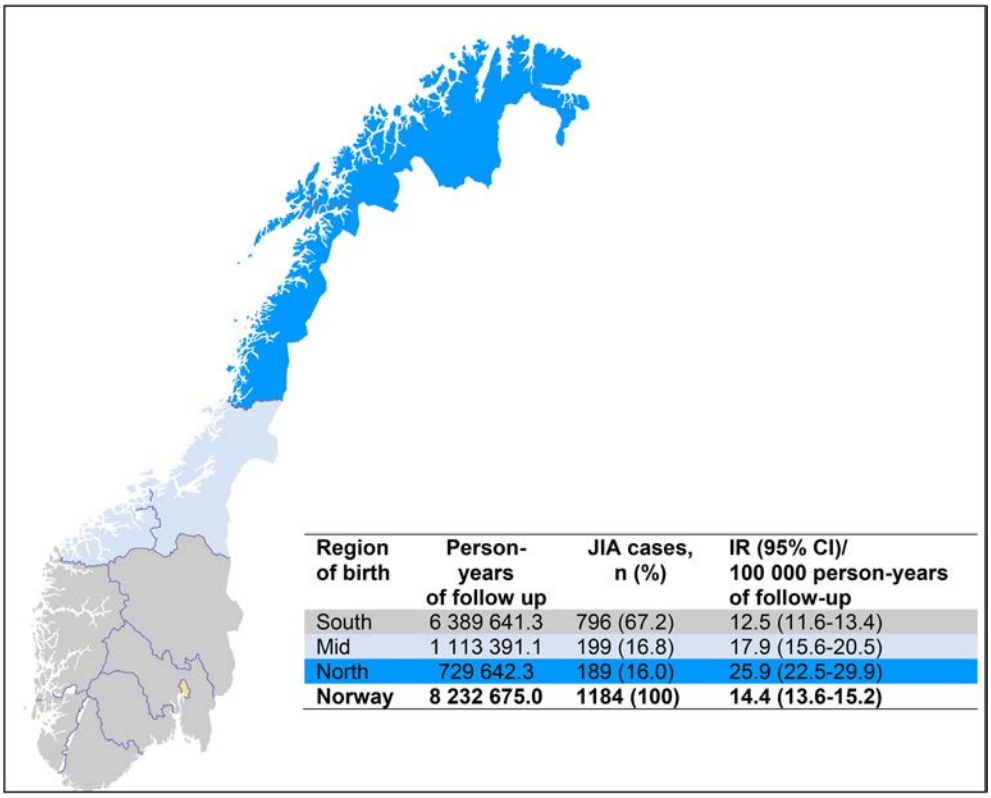

Figure 1. IR of JIA by region of birth in the Medical Birth Registry of Norway sample. CI, confidence interval; IR, incidence rate; JIA, juvenile idiopathic arthritis.

association between JIA diagnosis and PRS for JIA (OR = 1.79, 95% CI 1.57–2.03; *P* = 4.70e–19). When we further adjusted for the first 10 PCs (model 3), the association for region North became nonsignificant (OR = 1.29, 95% CI 0.73–2.27; *P* = 0.38), whereas we observed associations between JIA diagnosis and both PRSs for JIA (OR = 1.77, 95% CI 1.56–2.02; *P* = 2.77e–18) and the third genetic PC (OR = 8.53e–06, 95% CI 1.56e–09 to 0.05; *P* = 0.01).

DISCUSSION

In this nationwide study from Norway, we found an IR for JIA of 14.4/100,000 PYs with an increasing IR by increasing latitude. Adjustments for environmental factors such as perinatal factors, socioeconomic status, antibiotic use, and smoking did not substantially impact the results. In MoBa, the observed association between JIA and region of birth was no longer significant when adjusting for genetic factors.

The estimated national incidence is in line with previous studies from the Nordic countries and southeast of Norway reporting IRs of 15.0 and 14.0/100,000 PYs, respectively.[13,15] The national estimate in our study was quite close to these previous findings and is likely explained by a high proportion (78%) of the children in Norway residing in region South.

We were able to apply the same method across all regions, which makes a direct comparison of the regions feasible. Our findings support the existence of a north-south gradient, which may also exist outside of Norway. This is supported by previous

Table 2. HR for JIA by region of birth in the Medical Birth Registry of Norway sample*

Region	JIA, n (%)		HR unadjusted (95% CI)	HR adjusted (95% CI)[a]
	Yes (n = 1,184)	No (n = 940,571)		
South	796 (67.2)	708,147 (77.8)	Ref	Ref
Mid	199 (16.8)	122,711 (13.5)	1.43 (1.23–1.67)	1.42 (1.21–1.65)
North	189 (16.0)	79,200 (8.7)	2.07 (1.77–2.43)	2.11 (1.80–2.48)

* CI, confidence interval; HR, hazard ratio; JIA, juvenile idiopathic arthritis; Ref, reference.
[a] Adjusted for maternal age, parity, socioeconomic status, mode of delivery, prematurity, birth weight, systemic antibiotics during age 0 to 24 months, sex, year of birth, and season of birth. A total of 8,863 children were excluded because of missing covariate exposures.

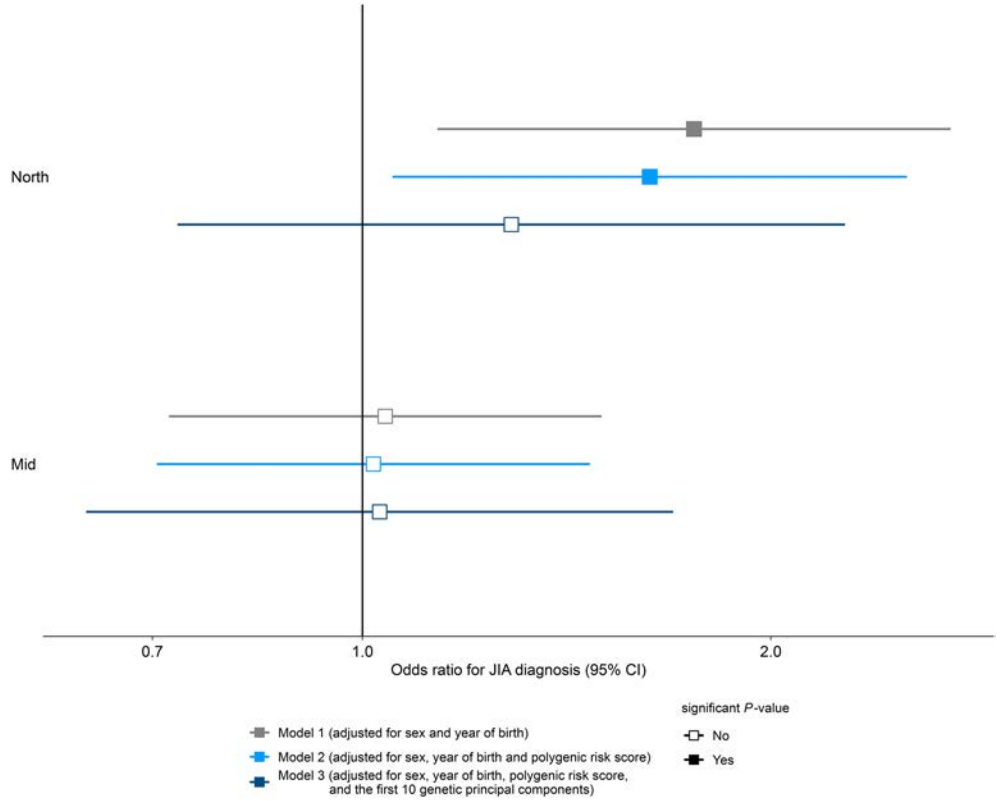

Figure 2. Associations between region of birth and JIA in the Norwegian Mother, Father, and Child Cohort Study sample. CI, confidence interval; JIA, juvenile idiopathic arthritis. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43040/abstract.

studies conducted in Nordic countries with a high IR, such as in Finland and Northern Norway,[13] compared with countries in Southern Europe, such as France and Spain, which have reported IRs in the range of 1.6 (95% CI 1.0–2.5) to 6.9 (95% CI 5.8–8.1). These rates are substantially lower compared with those observed in Nordic countries.[3,13–15] A previous study from northern parts of Norway also reported a high IR for JIA of 23.0/100,000 PYs.[14,15]

Interestingly, a recent study from the United Kingdom reported higher rates of JIA in the northern region of the United Kingdom compared with the southern regions.[35] Similarly, regional differences were also reported in Germany with a higher incidence in the north/northeast compared with the south/southwest.[36]

The existence of a north-south gradient in the incidence may indicate differences in environmental risk factors across regions.[7] We adjusted for perinatal factors, socioeconomic status, and antibiotic exposure during age 0 to 24 months as potential mediators, but we were not able to explain the observed north-south gradient by these factors.

A north-south gradient may also be caused by different genetic risk for JIA in different regions. In the MoBa sample, we found a higher occurrence of JIA in region North versus South (as observed in the MBRN sample) but no significant difference between regions Mid and South as opposed to the MBRN sample. This difference might be explained by selection bias[37,38] or insufficient power in the MoBa sample. When we adjusted for

Table 3. OR for JIA by region of birth in the Norwegian Mother, Father and Child Cohort Study sample with adjustments for genetic risk*

Region of birth	JIA, n		OR unadjusted (95% CI)	Model 1, OR adjusted (95% CI)[a]	Model 2, OR adjusted (95% CI)[b]	Model 3, OR adjusted (95% CI)[c]
	Yes (n = 238)	No (n = 57,392)				
South	181	45,854	Ref	Ref	Ref	Ref
Mid	34	8,284	1.04 (0.72–1.50)	1.04 (0.72–1.50)	1.02 (0.70–1.47)	1.03 (0.63–1.69)
North	23	3,254	1.79 (1.16–2.77)[d]	1.76 (1.14–2.71)[d]	1.63 (1.05–2.52)[d]	1.29 (0.73–2.27)

* CI, confidence interval; JIA, juvenile idiopathic arthritis; OR, odds ratio; Ref, reference.
[a] Adjusted for sex and year of birth.
[b] Adjusted for sex, year of birth, and polygenic risk score.
[c] Adjusted for sex, year of birth, polygenic risk score, and the first 10 genetic principal components.
[d] *P* value <0.05.

PRS, the observed association between region North and JIA was weakened. When further including the 10 genetic PCs, the association was no longer significant.

Taken together, our findings may indicate that genetic factors explain some of the regional differences, but other factors not examined in our study cannot be excluded. The first 10 genetic PCs represent a coarse-grained genetic background reflecting broad genetic ancestry and population admixtures. The observed association of JIA with genetic PCs might indicate higher genetic susceptibility to JIA in populations with certain genetic backgrounds or may be attributed to environmental risk factors for JIA, which are more common in certain populations but were not included in our study. These unknown underlying factors might be combinations of other environmental factors like vitamin D levels, air pollution, environmental toxicants, and infectious agents. Also, there may be interplay between genetic and environmental factors, which we have not investigated. Nonbiologic factors like health care–seeking behavior, health care services availability, or diagnostic accuracy across regions should be completely ruled out before confirming a true geographic difference. However, our validation data do not support these explanations.

A strength in our study is the public health care system and nationwide registers in Norway. We had access to prospectively collected data from population-based registers of high quality encompassing all regions with a virtually complete nationwide sample. This provided us with reliable data and the ability to make a direct comparison of rates in different regions.

Another strength was the ability to validate our outcome definition and to investigate possible regional differences in coding practices. Further, we included both relevant environmental factors and data on genetic risk as possible explanatory variables for the regional differences.

One limitation was that the age of diagnosis in the MBRN dataset was estimated by the year of first registration in the NPR and not accurately recorded. For children diagnosed before 2008, the year of their first registration was not available. Sensitivity analyses excluding children born before 2007 showed slightly lower IR in all regions and a slightly attenuated but still highly significant north-south gradient.

Because we only included children born in Norway, we had no data on children who immigrated during our study period. With a PPV of 93.4 in our case definition, approximately 7% of children might potentially be misclassified as having JIA. However, some milder cases may also not be captured if they do not seek medical care or receive a correct diagnosis. In addition, the highest PPV was found at UNN representing the northernmost region. A high PPV indicates a low risk of false positives, which underscores a true high IR in region North.

A stricter case definition of at least three codes, which showed a PPV of 95.1% would decrease the risk of false positives but also increase the risk of losing some true cases.[39] In sensitivity analyses using the strict case definition, the number of patients with JIA went from 1,148 to 1,087, but the north-south gradient remained consistent in magnitude. In the validation, all departments at St. Olav's Hospital were included, whereas in the other hospitals only the rheumatologic (OUS) and the pediatric department (OUS, SIV, and UNN) were included. These differences occurred because of different approvals by the local DPO and may have influenced the validation.

Environmental and genetic risk factors may vary across JIA categories,[2] but we lacked information regarding the specific categories within our samples. Previous studies have reported a high risk of JIA in indigenous populations of North America, Australia, and New Zealand.[1] Most of Norway's indigenous Sami live in the northern regions,[40] but we had no information about Sami ancestry in our study.

We were not able to include all environmental and genetic factors in both of our partly overlapping study samples. Because only a relatively small number of children were genotyped, our sample from MoBa may not be representative of all Norwegian children, and the results may be limited by lack of power. Thus, these results should be interpreted with caution.

In this nationwide study from Norway, we found an increasing HR for JIA with increasing latitude. The limited environmental factors available in our study did not seem to explain the gradient, whereas differences in genetic background between regions may explain some of the geographic variation. Further risk factors for JIA, including gene-environmental interplay that might explain the observed north-south gradient, should be investigated.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Hestetun confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/ Declaration of Helsinki requirements.

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Trends in New Use of Disease-Modifying Antirheumatic Drugs for Juvenile Idiopathic Arthritis Among Commercially Insured Children in the United States from 2001 to 2022

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Objective. The objective of this study is to describe recent trends in disease-modifying antirheumatic drug (DMARD) use for children with juvenile idiopathic arthritis (JIA) in the United States.

Methods. We used commercial claims data (2000–2022) to perform a serial cross-sectional utilization study of children aged 1 to 18 that were diagnosed with JIA. Initiations of conventional synthetic DMARDs (csDMARDs), biologic DMARDs (bDMARDs), or targeted synthetic DMARDs (tsDMARDs) were identified after a ≥12-month baseline and expressed as a percentage of all new DMARD initiations per year, by category, class, and individual agent. Trends were evaluated using linear regression. We also examined the first bDMARDs and tsDMARDs initiated after csDMARD monotherapy.

Results. We identified 20,258 new DMARD use episodes among 13,696 individuals (median age 14 years, 67.5% female). csDMARDs, although most used overall, declined from 89.5% of new use episodes to 43.2% (2001–2022, $P < 0.001$ for trend). In contrast, bDMARD use increased (10.5–50.0%, $P < 0.001$). For tumor necrosis factor inhibitors (TNFi), etanercept peaked at 28.3% in 2006 and declined to 4.2% in 2022 ($P = 0.002$). Meanwhile, adalimumab use doubled (7.0–14.0%, 2007–2008) after JIA approval, increasing further following a less painful formulation release (20.5% in 2022, $P < 0.001$). However, overall TNFi use has declined with increasing use of other bDMARDs and tsDMARDs, particularly ustekinumab, secukinumab, and tofacitinib. By 2022, adalimumab was the most common b/tsDMARD initiated first after csDMARDs (77.8%).

Conclusion. Among commercially insured children with JIA in the United States, new b/tsDMARD use is rising and new csDMARD use is declining. For b/tsDMARDs, adalimumab is most used and is the predominant b/tsDMARD initiated first after csDMARDs. Patterns in DMARD use for JIA have evolved relative to multiple factors, including regulatory approvals and tolerability.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common pediatric rheumatic disorder, affecting approximately 16 to 150 per 100,000 children in North America.[1] The chronic inflammation, resultant damage, and burdens of treatment associated with JIA can impact patients' daily activities and productivity.[2] Important goals of treatment in patients with JIA are to eliminate active disease, normalize physical function, preserve normal vision and growth, prevent long-term damage, maximize quality of life, and minimize short- and long-term toxicity.[2] Disease-modifying antirheumatic drugs (DMARDs) collectively represent the most common and effective treatments used for JIA and JIA-associated eye inflammation (uveitis).[3] DMARDs include conventional synthetic DMARDs (csDMARDs) such as methotrexate; biologic DMARDs (bDMARDs) such as etanercept, adalimumab,

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and tocilizumab; and targeted synthetic DMARDs (tsDMARDs) such as tofacitinib and baricitinib.

Research on trends in DMARD utilization for children enable better understanding on how selection of therapies for JIA has evolved with increasing availability of effective agents. Nonetheless, most studies on DMARD utilization have focused on adults with inflammatory arthritis.[4] One retrospective study using US data from 2019 to 2020 found that tumor necrosis factor inhibitors (TNFi) were the most prescribed b/tsDMARDs for first- and second-line treatment of rheumatoid arthritis (RA) in adults.[5] Among studies in populations with JIA, a retrospective cohort study from a single Canadian clinic (n = 325) found that the most common DMARD used from 2011 to 2019 was methotrexate, followed by etanercept.[6] In a cross-sectional analysis of the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry (2010–2011), approximately 75% of all enrolled children with JIA (median age of 12 years) in the registry (N = 2,748) received csDMARDs and 25% received bDMARDs.[7] In a retrospective cohort study using commercial insurance claims data from US children with JIA from 2008 to 2016, etanercept was the most common first bDMARD used, followed by adalimumab.[8] However, this study focused on economic outcomes and did not consider trends across years. In another US retrospective cohort study of commercially insured children and young adults with JIA or RA from 2009 to 2013, etanercept was the most commonly used TNFi.[9] In a separate retrospective cohort study using national US commercial claims data from 2005 to 2012, use of TNFi for the treatment of JIA increased two- to three-fold.[10] However, trends in individual DMARD use were not compared across calendar years, and these older studies do not reflect potential changes from the availability of multiple new drugs, new formulations, and other more recent changes in the management of JIA. A more recent registry-based study showed increasing use of bDMARDs in Canada between 2005 and 2010 and between 2017 and 2021, but analyses did not extend to specific DMARD types or classes.[11] Furthermore, most studies have not examined utilization specifically for JIA-associated uveitis, a common complication of JIA that influences choice of DMARD.

There is little research evaluating trends in DMARD use in populations with JIA over the past decade, including research on specific DMARDs and first-line b/tsDMARDs. We evaluated national trends in new use of DMARDs from the last two decades among commercially insured children with JIA in the United States. Inflection points in DMARD use were hypothesized to occur with JIA-specific approvals. We also hypothesized that bDMARD use has increased over time and that adalimumab has become the most commonly used bDMARD.

PATIENTS AND METHODS

Study design and data source. We performed a serial cross-sectional study in a cohort of commercially (privately) insured children in the United States with JIA using Merative MarketScan Commercial Claims and Encounters data from 2000 to 2022 (the most recently available data at the time of analysis). This database contains administrative claims data with information about enrollment, inpatient and outpatient encounters, and prescription drug claims.[12] Medical encounters are coded using International Classification of Disease Ninth and Tenth Revision, Clinical Modification (ICD-9-CM and ICD-10-CM) codes, Current Procedural Terminology Fourth Edition codes, Healthcare Common Procedure Coding System codes, and National Drug Codes.[13] This study of deidentified data was approved by the Rutgers University Institutional Review Board (Pro2023001171) with a waiver for informed consent and in accordance with existing data use agreements. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.[14]

Study population. During each year of analysis from 2001 to 2022, we identified a cohort of children aged 1 to 18 years old with a JIA diagnosis before age 18 who initiated a DMARD without previous use of the same DMARD in the past 365 days. The index date was defined as the date of DMARD initiation for each DMARD of interest. Eligible individuals were required to have ≥365 days of continuous health care and pharmacy eligibility before the index date. JIA was defined by ≥1 diagnosis in an outpatient or inpatient encounter in any position (ICD-9-CM 696.0x, 714.xx, or 720.xx; ICD-10-CM L40.5x, M05.x, M06.x, M08.x, or M45.x).[15,16] Patients with cancer, inflammatory bowel disease, lupus, and other systemic rheumatic diseases during the 365-day baseline period were excluded from the study because treatment for these patients is often dictated by these conditions rather than JIA. Patients were eligible to have multiple initiations of distinct DMARDs each year if each initiation event met the eligibility criteria.

DMARDs. The DMARDs evaluated in this study were csDMARDs (methotrexate, sulfasalazine, hydroxychloroquine, leflunomide), bDMARDs (etanercept, adalimumab, golimumab, infliximab, certolizumab, abatacept, tocilizumab, sarilumab, canakinumab, anakinra, rilonacept, secukinumab, ustekinumab, rituximab), and tsDMARDs (tofacitinib, ruxolitinib, baricitinib, upadacitinib) (Supplementary Table 1). DMARDs were characterized by category (csDMARD, bDMARD, or tsDMARD), class (TNFi, interleukin-6 inhibitors, interleukin-1 inhibitors, or JAK inhibitors), and specific drug. DMARDs were identified from inpatient records, outpatient records, and dispensing records.

Covariates. The covariates evaluated in this study were age, sex, region, and inpatient or outpatient diagnoses of select comorbidities at baseline. These comorbidities were chronic pain disorders, psoriasis, celiac disease, and uveitis.

Statistical analysis. To describe the study population, baseline characteristics of all eligible patients were assessed before or on the index date for each new use episode. We calculated the percentage of DMARD initiations per calendar year, classified by specific DMARD, DMARD class, or DMARD category; the denominator was represented by the number of all eligible DMARD initiations in that calendar year. To assess for statistically significant changes in DMARD use over the entire study period, we used linear regression with calendar year as the independent variable. Trends in DMARD use were also described visually based on comparison with key dates (eg, regulatory approvals, changes in formulation) (Supplementary Tables 1 and 2),[17–24] hypothesizing inflection points with Food and Drug Administration (FDA) approvals for JIA. Additional exploratory inflection points of interest corresponded to published American College of Rheumatology guidelines for JIA management in 2011, 2013, and 2019,[25–27] changes in formulations, and the start of the US COVID-19 pandemic in 2020.

Given that b/tsDMARDs are more expensive and frequently follow initial treatment with csDMARDs based on treatment recommendations,[25–27] in a secondary analysis, we assessed the first bDMARD or tsDMARD used at least 30 days after the use of csDMARD monotherapy. bDMARDs and tsDMARDs were not included in this secondary analysis if their use was within 30 days of csDMARD initiation because this could constitute combination therapy. Secondary subgroup analyses were stratified by age group (<12 or ≥12 years) and sex. We also conducted secondary subgroup analyses for patients with uveitis diagnosis and separately for patients without psoriasis diagnosis.

To assess whether utilization patterns differed based on the timing of DMARD use or definition of the study population, two sensitivity analyses were performed: evaluation of any DMARD use (incident or prevalent) within each calendar year and eligibility based on at least two JIA diagnosis codes 30 to 365 days apart. All data analyses were conducted using SAS version 9.4 (SAS Institute). *P* values less than 0.05 were considered statistically significant and were not adjusted for multiple testing because analyses were intended to be descriptive.

RESULTS

Baseline characteristics. We identified 20,258 new episodes among 13,696 children diagnosed with JIA who newly initiated at least one DMARD between 2001 and 2022. The median age was 14 (interquartile range, 10–16 years) years; most patients were between the ages of 12 and 18 years (65.5%) and female (67.5%) (Table 1). Of the four comorbidities assessed, patients most commonly had diagnoses of psoriasis (21.2%), followed by uveitis (7.5%), chronic pain disorders (5.1%), and celiac disease (0.7%).

Table 1. Baseline characteristics of children with JIA who newly initiated at least one DMARD between 2001 and 2022*

Baseline characteristics	Value	New DMARD episodes (%)
Total number of new episodes	20,258	–
Total number of distinct patients with new episodes	13,696	–
Age, median (IQR), y	14 (10–16)	–
Age, y		
<6	2,168	10.7
6–11	4,816	23.8
12–18	13,274	65.5
Sex		
Male	6,580	32.5
Female	13,678	67.5
Comorbidities[a]		
Psoriasis	4,287	21.2
Uveitis	1,524	7.5
Chronic pain disorders	1,028	5.1
Celiac disease	143	0.7
Region[b]		
Northeast	3,439	17.0
North Central/Midwest	5,181	25.6
South	7,505	37.0
West	3,885	19.2
Unknown	248	1.2

* DMARD, disease-modifying antirheumatic drug; IQR, interquartile range; JIA, juvenile idiopathic arthritis.
[a] Code lists are provided in Supplementary Table 11.
[b] Based on United States Census data from 2020, the geographic breakdown of the overall United States population is as follows: Northeast (17.3%), Midwest (20.8%), South (38.1%), and West (23.7%).[44]

Trends in DMARD use in children with JIA. Of the DMARD categories, csDMARDs were most common early in the study period until 2018, when their use was surpassed by bDMARDs (Figure 1, Supplementary Table 3). Between 2001 and 2022, use of csDMARDs declined from 89.5% to 43.2% of all DMARD initiations ($P < 0.001$ for trend). During this same period, bDMARD use increased from 10.5% to 50.0% ($P < 0.001$). For TNFi specifically, use of etanercept (first-in-class) peaked at 28.3% of all DMARD initiations in 2006 and subsequently declined to 4.2% by 2022 ($P = 0.002$) (Figure 2, Supplementary Table 4). Another TNFi, adalimumab, doubled in use from 7.0% in 2007 to 14.0% in 2008 (year of FDA approval for JIA). Adalimumab initiations increased even further following the 2018 approval of a citrate-free formulation to reduce burning, reaching 20.5% by 2022 ($P < 0.001$).

Despite the increases in individual TNFi over time, overall TNFi use has declined in recent years as the use of other b/tsDMARDs has increased (Figure 3, Supplementary Figure 1, Supplementary Table 4). Two bDMARDs initially approved for psoriasis have increased in recent years: ustekinumab, increasing from 0.1% of all DMARD initiations in 2009 (year of FDA approval for adults with psoriasis) to 2.4% in 2017 (year of FDA approval for pediatric psoriasis) to 10.5% in 2022 (year of FDA approval for pediatric psoriatic arthritis) ($P < 0.001$); and secukinumab,

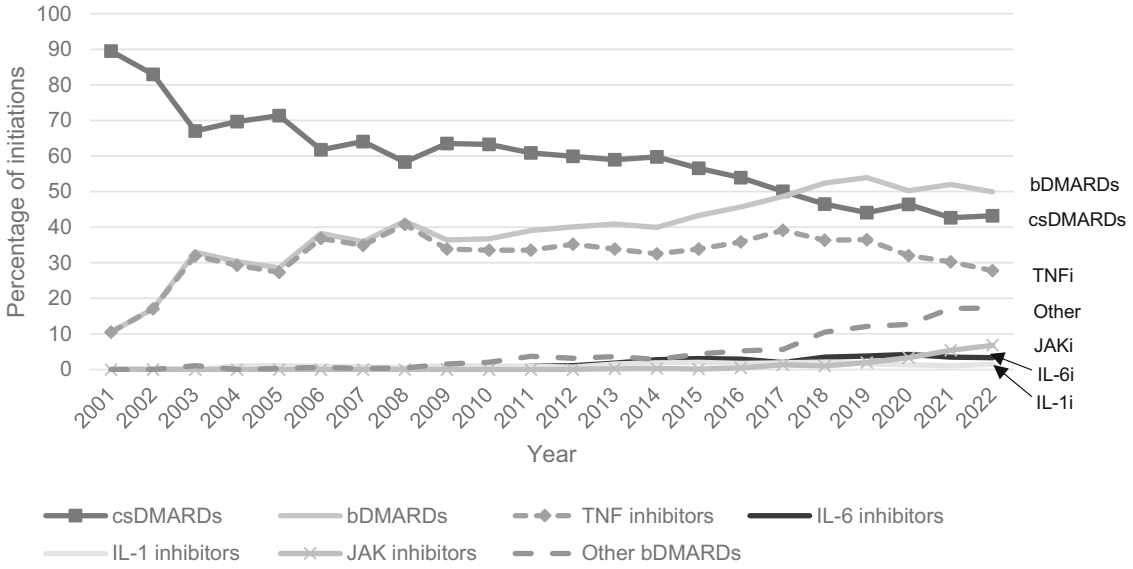

Figure 1. Initiation of DMARDs by drug class or category in children with JIA from 2001 to 2022. This figure displays the percentage of total new episodes per year of each DMARD class or category in children with JIA in MarketScan between 2001 and 2022. A DMARD claim was considered a new episode if a patient had no claim for the same DMARD within 365 days before the current DMARD claim. Each patient could contribute more than one eligible new DMARD episode. csDMARDs included methotrexate, sulfasalazine, leflunomide, and hydroxychloroquine; bDMARDs included etanercept, adalimumab, infliximab, certolizumab, golimumab, tocilizumab, anakinra, canakinumab, rilonacept, abatacept, ustekinumab, secukinumab, and rituximab; TNFi included etanercept, adalimumab, infliximab, certolizumab, and golimumab; IL-6 inhibitors included tocilizumab and sarilumab; IL-1 inhibitors included anakinra, canakinumab, and rilonacept; JAK inhibitors included tofacitinib, baricitinib, ruxolitinib, and upadacitinib; and other bMDARDs included abatacept, ustekinumab, secukinumab, and rituximab. bDMARD, biologic disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DMARD, disease-modifying antirheumatic drug; IL-1, interleukin-1; IL-6, interleukin-6; JIA, juvenile idiopathic arthritis; TNF, tumor necrosis factor.

increasing from 0.2% of all DMARD initiations in 2015 (year of FDA approval for adults with psoriasis) to 3.0% in 2021 (year of FDA approval for pediatric psoriatic arthritis) and 4.8% in 2022 ($P <$ 0.001) (Figure 3, Supplementary Table 4). Initiations of tofacitinib increased following approval from 2.9% of all DMARD initiations in 2020 to 5.2% in 2021 before dipping to 3.7% in 2022 ($P <$

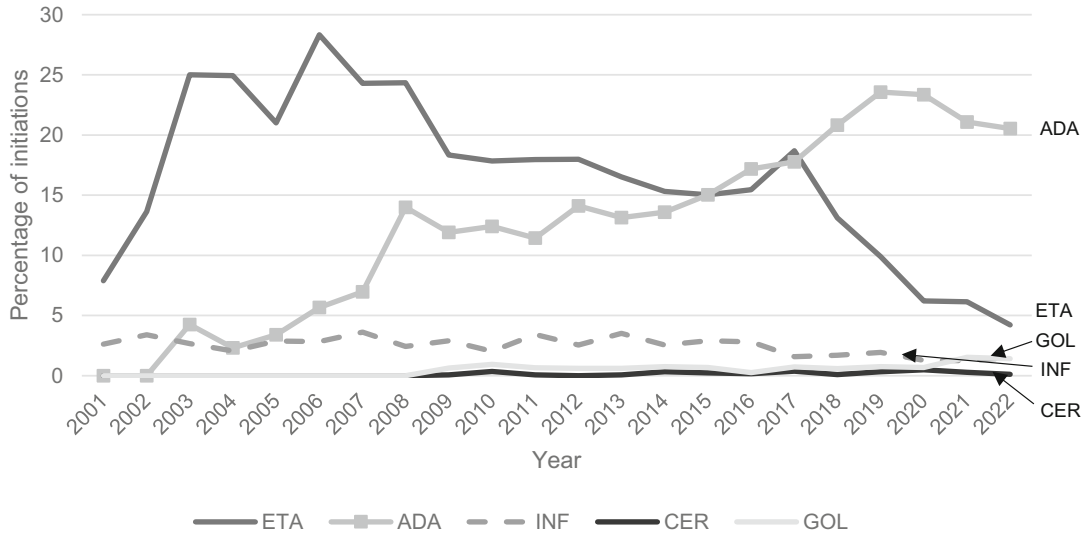

Figure 2. Initiation of TNFi in children with JIA from 2001 to 2022. This figure displays the percentage of total new episodes per year of each TNFi in children with JIA in MarketScan between 2001 and 2022. A DMARD claim was considered a new episode if a patient had no claim for the same DMARD within 365 days before the current DMARD claim. Each patient could contribute more than one eligible new DMARD episode. Select relevant events in the timeline are marked by vertical lines and corresponding labels. ADA, adalimumab; CER, certolizumab; DMARD, disease-modifying antirheumatic drug; ETA, etanercept; GOL, golimumab; INF, infliximab; JIA, juvenile idiopathic arthritis; TNFi, tumor necrosis factor inhibitors.

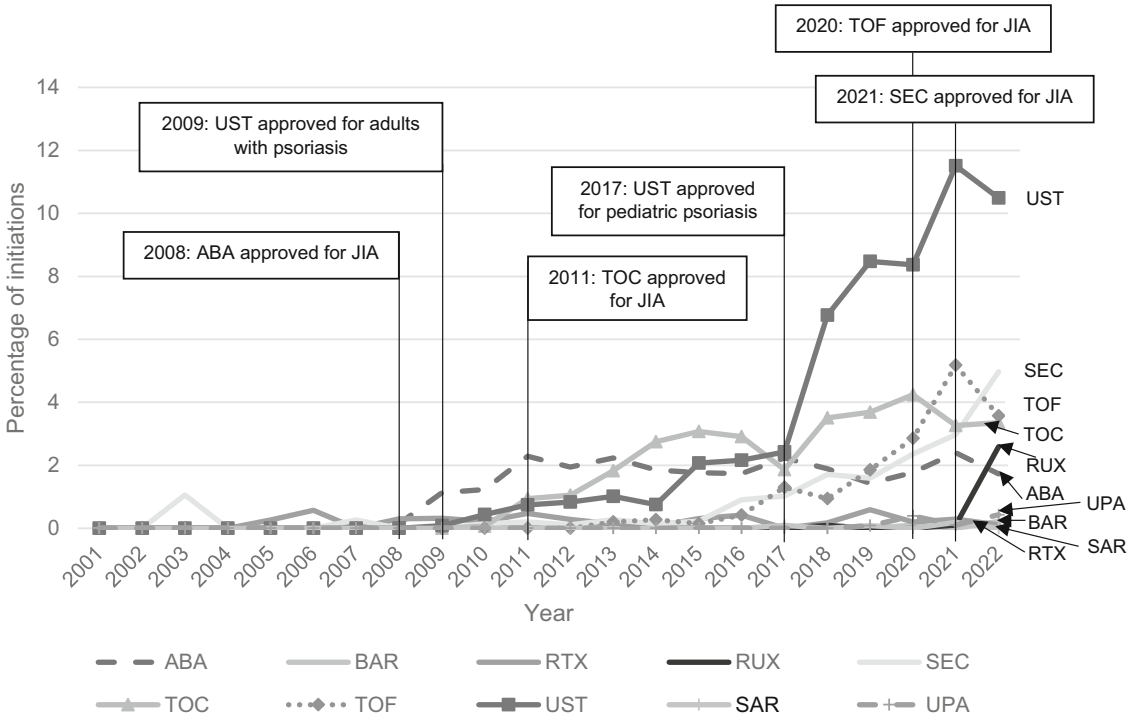

Figure 3. Initiation of select bDMARDs and tsDMARDs in children with JIA from 2001 to 2022. This figure displays the percentage of total new episodes per year of b/tsDMARDs besides TNFi and IL-1 inhibitors in children with JIA in MarketScan between 2001 and 2022. A DMARD claim was considered a new episode if a patient had no claim for the same DMARD within 365 days before the current DMARD claim. Each patient could contribute more than one eligible new DMARD episode. Select relevant events in the timeline are marked by vertical lines and corresponding labels. ABA, abatacept; BAR, baricitinib; bDMARDs, biologic disease-modifying antirheumatic drugs; IL-1, interleukin-1; JIA, juvenile idiopathic arthritis; RTX, rituximab; RUX, ruxolitinib; SAR, sarilumab; SEC, secukinumab; TNFi, tumor necrosis factor inhibitors; TOC, tocilizumab; TOF, tofacitinib; tsDMARDs, targeted synthetic disease-modifying antirheumatic drugs; UPA, upadacitinib; UST, ustekinumab.

0.001). Of all DMARDs assessed, methotrexate (csDMARD) was the most used DMARD throughout the entire study period, but it declined in use relative to all DMARDs (42.1% in 2001, 21.5% in 2022, $P < 0.001$) (Figure 4, Supplementary Table 4).

Secondary and sensitivity analyses. In the secondary analysis of the first b/tsDMARD used after csDMARDs, etanercept use went from 100% in 2001 to 2002 to 6.5% by 2022 ($P < 0.001$) (Figure 5, Supplementary Table 5). In contrast, adalimumab use went from 0% in 2001 to 2002 to 77.8% by 2022 ($P < 0.001$).

In the subgroup of those diagnosed with uveitis, the top six most used DMARDs were adalimumab, etanercept, hydroxychloroquine, infliximab, methotrexate, and sulfasalazine (Supplementary Figure 2, Supplementary Table 6). Within this subgroup, the percentage of methotrexate initiations declined from 54.8% in 2005 to 20.0% in 2006 ($P < 0.001$) as the relative use of bDMARDs increased; infliximab and etanercept were more common in earlier years, and adalimumab was more common in later years. In the subgroup of those without psoriasis, we found much lower ustekinumab use compared with the general JIA population (Supplementary Figure 3, Supplementary Table 7). Users of ustekinumab or secukinumab had a similar or larger number of baseline JIA diagnoses compared with psoriasis diagnoses across most years (Supplementary Table 8).

DMARD trends in subgroup analyses stratified by age group (Supplementary Figure 4) and sex (Supplementary Figure 5) were mostly consistent with the main analyses. However, methotrexate use was relatively more common in children aged younger than 12 than in older children. Adalimumab, etanercept, hydroxychloroquine, methotrexate, and sulfasalazine were most used by children of both sexes. Use of ustekinumab was higher among older children (≥12) and male patients, whereas use of infliximab was higher among younger children (<12) and female patients.

The results of the sensitivity analyses were consistent when assessing any DMARD use (Supplementary Figure 6, Supplementary Table 9) and when including those with at least two JIA diagnosis codes in the previous year (Supplementary Figure 7, Supplementary Table 10).

DISCUSSION

Among commercially insured children with JIA in the United States, methotrexate remains the most initiated DMARD for JIA. However, as with other csDMARDs, new methotrexate use

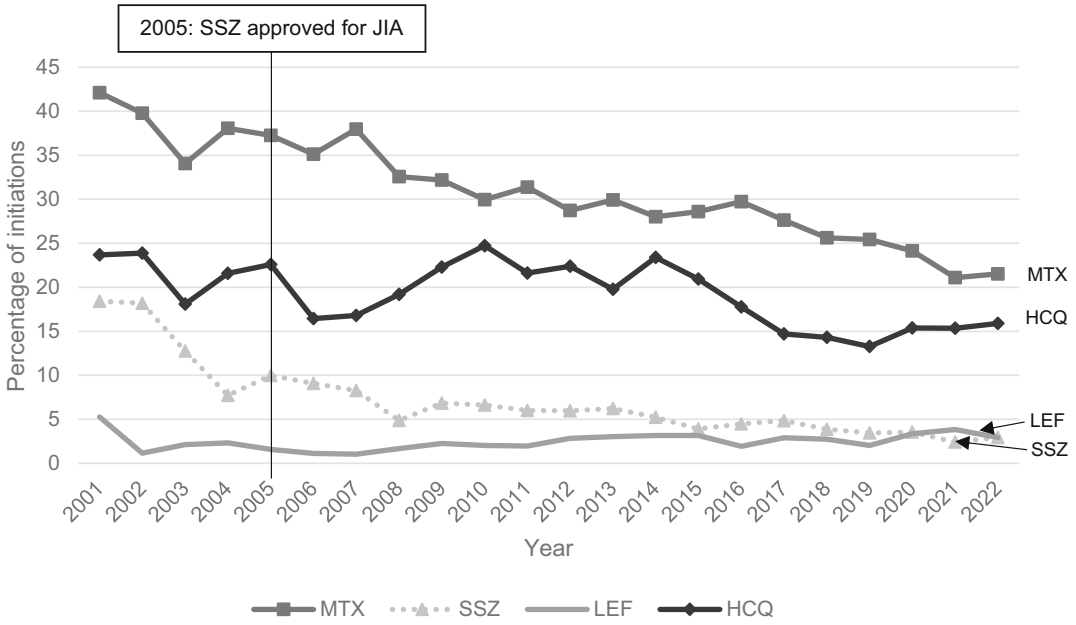

Figure 4. Initiation of csDMARDs in commercially insured children with JIA, 2001 to 2022. This figure displays the percentage of total new DMARD episodes per year of each csDMARD in children with JIA in MarketScan between 2001 and 2022. A DMARD claim was considered a new episode if a patient had no claim for the same DMARD within 365 days before the current DMARD claim. Each patient could contribute more than one eligible new DMARD episode. csDMARD, conventional synthetic disease-modifying antirheumatic drug; DMARD, disease-modifying antirheumatic drug; HCQ, hydroxychloroquine; JIA, juvenile idiopathic arthritis; LEF, leflunomide; MTX, methotrexate; SSZ, sulfasalazine.

has been declining as b/tsDMARD use has been steadily increasing over time. In recent years, adalimumab has been the most used b/tsDMARD and the predominant b/tsDMARD initiated first after csDMARDs. Use of other b/tsDMARDs, particularly ustekinumab and secukinumab, which are indicated for psoriatic arthritis, have sharply risen in recent years.

The findings from our study expand on the available literature on DMARD use for patients with JIA. In a previous retrospective

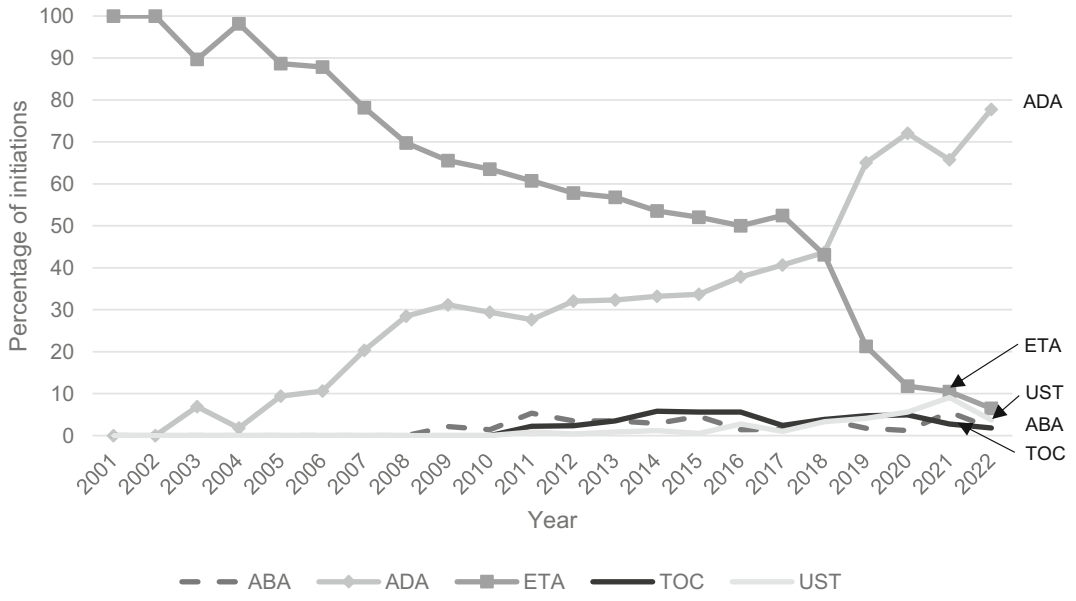

Figure 5. Initiation of first b/tsDMARD used after csDMARDs in children with JIA, 2001 to 2022. This figure displays the percentage of total new episodes per year of each first b/tsDMARD used after csDMARDs in children with JIA in MarketScan between 2001 and 2022, only including DMARDs that reached 5% in a given year. Each patient could contribute more than one eligible new DMARD episode. ABA, abatacept; ADA, adalimumab; b/tsDMARD, biologic or targeted synthetic disease-modifying antirheumatic drug; csDMARDs, conventional synthetic disease-modifying antirheumatic drug; ETA, etanercept; JIA, juvenile idiopathic arthritis; TOC, tocilizumab; UST, ustekinumab.

study of patients with systemic JIA using the German National Pediatric Rheumatologic Database, use of csDMARDs predominated from 2003 to 2005, but bDMARD use in this population increased, exceeding csDMARDs from 2011 to 2013.[28] Our larger study including all types of JIA in the United States showed a similar if delayed trend, with bDMARD initiations surpassing csDMARD initiations by 2018. In a prospective cohort of young adults with JIA in Germany from 2007 to 2019, the most frequently used DMARD was etanercept, followed by methotrexate and adalimumab.[29] However, the study did not focus on children and had a small sample size. In another retrospective cohort study using MarketScan data from 2008 to 2016, etanercept was the most common first bDMARD used in children with JIA, although trends over time were not evaluated.[8] In contrast to these smaller studies using older data, we found that by 2022, adalimumab was by far the most commonly started b/tsDMARD (20.5%), whereas etanercept only represented 4.2% of initiations. Our findings also extend the findings of a previous single-center study, in which adalimumab was the most frequently prescribed bDMARD by 2018.[30]

In the early 2000s, methotrexate, hydroxychloroquine, and sulfasalazine represented the vast majority of DMARD initiations for JIA. However, as hypothesized, relative csDMARD use steadily fell over time as b/tsDMARDs became available. Although b/tsDMARDs are more expensive for patients, they are more targeted than csDMARDs and frequently better tolerated than methotrexate. Although methotrexate and adalimumab both had changes in formulation during the study period, only adalimumab showed an increase in initiations, following a new, less painful formulative release in 2018. That same year also represented an inflection point, after which adalimumab became the preferred b/tsDMARD following csDMARDs. The rising use of adalimumab initiations compared with etanercept could also relate to other conveniences of administration, including fixed-dose formulations for children and the every-other-week dosing regimen.[31] Throughout the study period, we observed off-label use of novel b/tsDMARDs for JIA after their initial FDA approval for adults with inflammatory arthritis or psoriasis and before approval for JIA. Use of many b/tsDMARDs noticeably increased following their approval in children, including adalimumab, golimumab, tocilizumab, canakinumab, abatacept, ustekinumab, secukinumab, and tofacitinib, suggesting that labeling and corresponding marketing does influence prescribing and use of DMARDs for JIA.

Of note, nearly a quarter of the patients in this cohort had a documented diagnosis of psoriasis, which is higher than the expected prevalence of psoriatic arthritis in JIA.[32] This could in part relate to dual use of b/tsDMARDs for JIA and psoriasis, including agents approved specifically for psoriatic arthritis in adults and children, such as ustekinumab. Notably, ustekinumab increased considerably in use over time, becoming the second most common b/tsDMARD (after adalimumab) used in the study population by the end of the study period. Ustekinumab was predominantly prescribed for patients diagnosed with both JIA and psoriasis rather than patients with psoriatic arthritis without a diagnosis of psoriasis. Although the reasons for the rise in ustekinumab use are unclear, some potential explanations are that patients with psoriatic arthritis frequently have chronically uncontrolled disease, which may require additional therapy with agents such as ustekinumab[33]; preferential prescribing of ustekinumab by dermatologists who see patients with psoriasis and arthritis; or misclassified coding of JIA in patients who may have had psoriasis and joint pain without frank arthritis—though few patients had more baseline psoriasis diagnoses than JIA diagnoses. Additionally, TNFi have been associated with the development of psoriasis in some children,[34] and given the broad use of TNFi in the population with JIA, diagnosis of this potential treatment complication may also have contributed to the unexpectedly high prevalence of psoriasis.

We did not observe a major impact of the US COVID-19 pandemic on DMARD prescribing except perhaps a reversal of a downward trend of hydroxychloroquine use starting in 2020, following its brief emergency use authorization for COVID-19 before its effectiveness was disproven. In one recent study, commercially insured children with acute COVID-19 were observed to have higher rates of nonrecommended prescriptions, including hydroxychloroquine.[35]

In the subpopulation of JIA diagnosed with uveitis, we found greater use of adalimumab and infliximab and lower use of etanercept than in the general JIA population, consistent with the relative effectiveness of these respective agents in treating uveitis as well as the 2019 guidelines for JIA-associated uveitis, which recommend monoclonal TNFi over etanercept.[36,37] Notably, use of etanercept declined steeply after the publication of a negative clinical trial for JIA-associated uveitis by Smith et al.[38] It is possible that some DMARDs that are not recommended for uveitis were used to treat other aspects of JIA, such as etanercept for arthritis or sulfasalazine for enthesitis.

Evaluation of individual agents in other subgroups also revealed important insights. Methotrexate use was relatively more common in children aged younger than 12 than in older children, possibly because younger children may be less likely to experience methotrexate-associated gastrointestinal side effects or more likely to respond to methotrexate monotherapy.[39,40] Another possibility is that relatively more patients in the younger age group were newly diagnosed and started on methotrexate as first-line therapy. To our surprise, hydroxychloroquine was commonly used by children of both sexes and in both age groups, despite a negative landmark trial and lack of approval for JIA.[41] Additionally, use of ustekinumab was higher among older children (≥12) and male patients, whereas use of infliximab was higher among younger children (<12) and female patients. This could be due to the higher incidence of uveitis in younger children and higher incidence of psoriatic disease in older children.[42] Older children with psoriatic arthritis are also more likely to be male than younger children.[43]

Our study had several strengths, including the analysis of a large national population of commercially insured children in the United States, which provided more generalizable findings than in previous single-center and registry-based populations. Our inclusion of data spanning the last two decades enabled us to describe trends over a period of dramatic expansion in the market availability of treatments for JIA, particularly b/tsDMARDs. We also present novel findings on the recent dominance of adalimumab as a first-line b/tsDMARD for JIA as well the recent increases in the use of ustekinumab, tofacitinib (first targeted oral agent FDA-approved for JIA, 2020), and secukinumab in this population, making this study timely and informative for understanding real-world treatment patterns for JIA.

This study also had certain limitations. Our findings from a privately insured population may not be generalizable to other populations because DMARD prescribing and use may differ in patients with public insurance, patients without insurance, or patients outside of the United States. Additionally, we did not have access to data on race, ethnicity, or socioeconomic status to investigate the presence of disparities in DMARD use. We also lacked clinical details about the population with JIA, including JIA type (eg, systemic JIA, polyarticular JIA), disease severity, prescribers' specialty, and other factors that could impact patterns of DMARD prescribing and use. There was also the potential of diagnostic misclassification of JIA and comorbidities, such as uveitis or psoriasis. These utilization data also do not reveal the reasons behind the observed patterns of DMARD use, including the high recent uptake of ustekinumab.

In summary, in a large population of commercially insured children with JIA in the United States, we found a steady decrease in initiations of csDMARDs and a corresponding increase in initiations of b/tsDMARDs from 2001 to 2022. Adalimumab has become the most widely used b/tsDMARD, particularly as a first-line agent after csDMARDs. Use of ustekinumab, secukinumab, and tofacitinib has also increased in recent years. These real-world treatment patterns give us insight into how selection of therapies for JIA has evolved with increasing availability of effective agents and help prepare for future studies on comparative DMARD safety and effectiveness.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Horton confirms that all authors have provided the final approval of the version to be published, and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Helsinki Declaration requirements.

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Clinical Characteristics of Anti-Synthetase Syndrome: Analysis From the Classification Criteria for Anti-Synthetase Syndrome Project

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Objective. Anti-synthetase syndrome (ASSD) is a rare systemic autoimmune rheumatic disease (SARD) with significant heterogeneity and no shared classification criteria. We aimed to identify clinical and serological features associated with ASSD that may be suitable for inclusion in the data-driven classification criteria for ASSD.

Methods. We used a large, international, multicenter "Classification Criteria for Anti-synthetase Syndrome" (CLASS) project database, which includes both patients with ASSD and controls with mimicking conditions, namely, SARDs and/or interstitial lung disease (ILD). The local diagnoses of ASSD and controls were confirmed by project team members. We employed univariable logistic regression and multivariable Ridge regression to evaluate clinical and serological features associated with an ASSD diagnosis in a randomly selected subset of the cohort.

Results. Our analysis included 948 patients with ASSD and 1,077 controls. Joint, muscle, lung, skin, and cardiac involvement were more prevalent in patients with ASSD than in controls. Specific variables associated with ASSD included arthritis, diffuse myalgia, muscle weakness, muscle enzyme elevation, ILD, mechanic's hands, secondary pulmonary hypertension due to ILD, Raynaud phenomenon, and unexplained fever. In terms of serological variables, Jo-1 and non–Jo-1 anti-synthetase autoantibodies, antinuclear antibodies with cytoplasmic pattern, and anti-Ro52 autoantibodies were associated with ASSD. In contrast, isolated arthralgia, dysphagia, electromyography/magnetic resonance imaging/muscle biopsy findings suggestive of myopathy, inflammatory rashes, myocarditis, and pulmonary arterial hypertension did not differentiate between patients with ASSD and controls or were inversely associated with ASSD.

Conclusion. We identified key clinical and serological variables associated with ASSD, which will help clinicians and offer insights into the development of data-driven classification criteria for ASSD.

INTRODUCTION

Anti-synthetase syndrome (ASSD) is a rare systemic autoimmune rheumatic disease (SARD) usually characterized by the presence of autoantibodies against aminoacyl-transfer RNA synthetases (ARSs).[1,2] Until now, eight anti-ARS autoantibodies have been identified, namely, anti–Jo-1, PL-7, PL-12, EJ, OJ, KS, Zo, and Ha autoantibodies,[3] and other possible anti-ARS

autoantibodies have been recently recognized.[4–6] The clinical manifestations of ASSD include the classic "triad" of arthritis, myositis, and interstitial lung disease (ILD), along with other typical clinical features including fever, Raynaud phenomenon, and mechanic's hands/hiker's feet.[7,8]

Although ASSD is commonly categorized as a subtype of idiopathic inflammatory myopathies (IIMs), not all patients with ASSD exhibit myositis. In fact, most studies have shown a higher prevalence of ILD than myositis, particularly among patients with non–Jo-1 anti-ARS autoantibodies.[9,10] Furthermore, a study from the American and European Network of Anti-synthetase Syndrome (AENEAS) cohort reported that 24% of patients with anti–Jo-1–positive ASSD presented with isolated arthritis, and these patients were often classified as having rheumatoid arthritis (RA).[11] In this study, only 20% of patients had the complete "triad" at presentation.[12] For these reasons, patients with ASSD, in particular those presenting with isolated arthritis or ILD and non–Jo-1 anti-ARS autoantibodies, may not meet the 2017 EULAR/American College of Rheumatology (ACR) classification criteria for adult and juvenile IIMs and their major subgroups,[13] in which muscle involvement is weighted heavily, whereas arthritis, ILD, and non–Jo-1 anti-ARS autoantibodies are not included.[14] Moreover, ~20% of patients with ASSD present with inflammatory rashes and can be diagnosed with dermatomyositis (DM).[9] Whether these patients are better characterized as having DM or ASSD needs to be explored further, especially due to differences in the pathophysiologic findings between DM and ASSD.[15,16]

Serological testing of anti-ARS autoantibodies is considered crucial for ASSD diagnosis; however, the availability, methodology, and accuracy of anti-ARS autoantibody detection vary significantly among different centers and countries.[17] Because of the lack of standardized and reliable anti-ARS autoantibody testing,[18] defining ASSD based solely on the positivity of the antibodies may lead to both under- and overclassification. Given these disparities, there is an increasing consensus on the need for specific clinical or clinic-serologic classification criteria for ASSD that are distinct from other forms of IIMs or ILD.[19,20] Although several classification criteria for ASSD have been proposed by different groups,[21–23] they lack a data-driven foundation and have not been validated, nor are they widely accepted. The

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lack of data- and consensus-driven classification criteria for ASSD has hindered the development of international, multicenter studies and clinical trials for this rare and potentially life-threatening condition.

The Classification Criteria for Anti-synthetase Syndrome (CLASS) project is an international collaborative study funded by EULAR/ACR to develop and validate data and consensus-driven classification criteria for ASSD. For the data-driven process, the CLASS database, comprising 2,035 ASSD cases and 2,140 control diseases from 92 centers across 30 countries worldwide, has been developed. In this manuscript, we report the results of univariable and multivariable analysis in the CLASS database to identify clinical and serological variables associated with ASSD. The identified variables will be incorporated into the process leading to data-driven classification criteria for ASSD.

PATIENTS AND METHODS

The CLASS project. The CLASS project is an international, multicenter, retrospective observational study funded by EULAR/ACR to develop and validate classification criteria for ASSD (co–principal investigators [PIs]: RA and LC). The complete list of the CLASS project investigators is provided in Appendix A as well as in Supplementary Table S1. We recruited centers with databases or registries of patients with ASSD, IIMs, SARDs, or ILD. A total of 350 investigators from 92 centers worldwide participated in the CLASS project (Supplementary Table S1). We also invited international experts on ASSD, IIMs, and ILD to join the project as members of the steering committee, which included 12 rheumatologists, 4 pulmonologists, 2 dermatologists, and 2 neurologists from North America, South America, Europe, Asia, and Australia (Supplementary Table S2). The project was approved by the Ethical Committee of the IRCCS Policlinico S. Matteo Foundation of Pavia, Pavia, Italy (P-201190088730; Prot. 20190094533) and the local institutional review boards in each participating center. The complete study process was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients at each center.

Data collection. Participating centers were requested to report retrospectively or prospectively collected data from patients with ASSD (cases) or other conditions mimicking ASSD (controls), according to their clinical diagnosis. For controls, we considered all conditions for which clinicians may consider ASSD in the differential diagnosis or those that have overlapping clinical features with ASSD. A comprehensive list of variables potentially associated with ASSD covering clinical manifestations, laboratory data, imaging studies, and autoantibody testing was provided to each center on REDCap, a secure, web-based data capture platform hosted at the University Hospital of Ferrara (https://redcap.ospfe.it/). The list of variables was developed based on the systematic literature review that we performed previously,[24] as well as expert opinions from the steering committee members, which included multiple clinical (joint, muscle, lung, skin, cardiac, and others) and serological domains (Figure 1 and Supplementary Table S3). The data collection process began in August 2020 and ended in April 2021.

Data reviewing process. All imputed patient data underwent quality control, and the diagnoses of both ASSD and other SARD were verified by the CLASS project working group. Each record was assessed by a minimum of two reviewers: one working group member and one of the two PIs (RA and LC). We sent queries to the participating centers regarding missing data or reports with discrepancies. The participating centers were allowed to revise or enter new data if needed to confirm the variables or diagnoses. Equivocal cases were reviewed by the two PIs, and the final decision to include the patients as an ASSD case or control SARD was based on the consensus of both PIs. Because the disease concept of ASSD has not been established yet, patients diagnosed with both ASSD and other SARDs were classified as ASSD (cases), especially given the positivity of anti-ARS autoantibodies and treating physicians' diagnoses. Inconsistent patient records were excluded from the analysis (Supplementary Figure S1).

Statistical analyses. 50% of the verified cases and controls were randomly selected using the *sample* function provided in the base R package; the remaining 50% were used for the validation analyses. First, we performed univariable logistic regression analyses to investigate the association between each clinical or serological variable and the diagnosis of ASSD. For each variable (e.g., arthritis), the comparator was those lacking the specific item analyzed (e.g., no arthritis). We also performed sensitivity analyses using another comparator definition: those completely lacking the corresponding organ involvement (e.g., no joint involvement) (see Supplementary Table S4 for detailed definitions of comparators). We generated several macro variables in some clinical domains based on input from the steering committee members. For instance, a macro variable "inflammatory rashes" was composed of Gottron signs/papules, heliotrope rash, V-sign, shawl sign, and malar rash, and "Other myositis-specific autoantibodies (MSAs)/myositis-associated autoantibodies (MAAs)" included anti–Mi-2, anti–transcription intermediary factor 1-γ, anti–melanoma differentiation–associated gene 5, anti–small ubiquitin-like modifier-1 activating enzyme, anti–nuclear matrix protein 2/MJ, anti–signal recognition peptide, anti–3-hydroxy-3-methylglutaryl-CoA reductase, anti–PM-Scl, anti-U1-RNP, and anti-Ku autoantibodies. Detailed definitions of the macro variables are presented in Supplementary Table S5.

Additionally, we performed subgroup analyses in four distinct subcohorts that included all cases and controls with specific

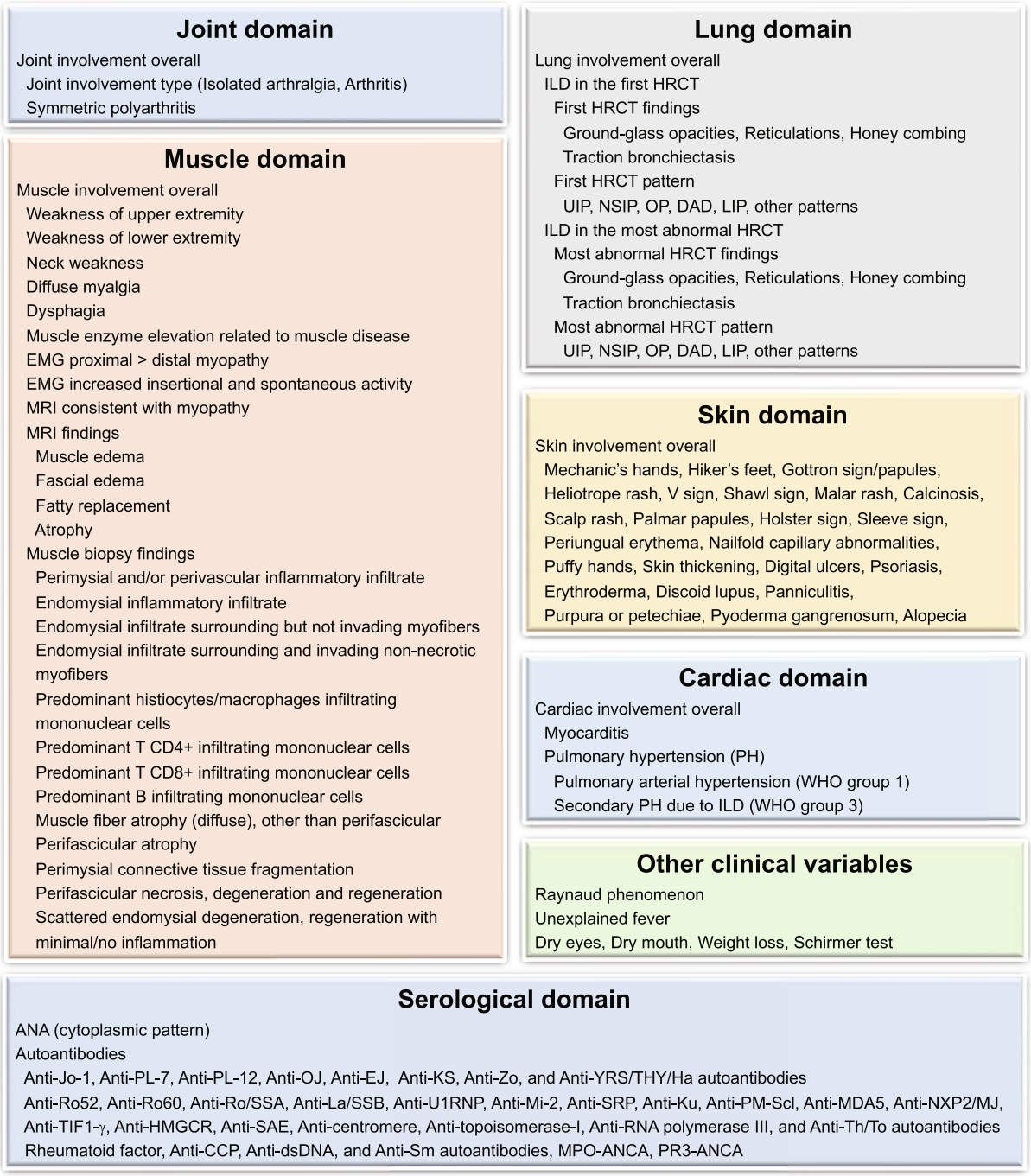

Figure 1. Clinical and serological variables included in each domain. ANA, antinuclear autoantibody; ANCA, anti–neutrophil cytoplasmic antibody; CCP, cyclic citrullinated peptide; DAD, diffuse alveolar damage; dsDNA, double-stranded DNA; EMG, electromyography; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; LIP, lymphoid interstitial pneumonia; MDA5, melanoma differentiation–associated gene 5; MPO, myeloperoxidase; MRI, magnetic resonance imaging; NSIP, nonspecific interstitial pneumonia; NXP2, nuclear matrix protein 2; OP, organizing pneumonia; PH, pulmonary hypertension; PR3, proteinase 3; SAE, small ubiquitin-like modifier-1 activating enzyme; SRP, signal recognition peptide; TIF1-γ, transcription intermediary factor 1-γ; UIP, usual interstitial pneumonia; WHO, World Health Organization.

organ involvement, namely, joint, muscle, lung, or skin involvement, which we refer to as the joint, muscle, lung, or skin cohort, respectively, to better understand the association between certain variables and the diagnosis of ASSD in patients with a specific organ involvement (e.g., the joint cohort included all patients with ASSD and controls who had joint involvement). Specifically, we repeated univariable logistic regression analyses in these four cohorts. For example, to evaluate the performance of

inflammatory rashes in discriminating ASSD among patients with ILD, we analyzed the association of inflammatory rashes with ASSD in the lung cohort, which was composed of cases and controls with lung involvement.

Finally, we employed multivariable Ridge regression to estimate each variable's weight for ASSD diagnosis prediction. Ridge regression is a regularization method employed in classification tasks, which is able to regularize coefficient magnitude in the presence of multicollinearity. We selected variables incorporated into the multivariable models based on the results of 1) univariable analysis and 2) multivariable penalized regression models run within each domain, as well as 3) clinical input from steering committee members. Linear coefficients obtained were scaled into 0% to 100% to calculate the weights. Ninety-five percent bootstrap confidence intervals (95% CIs) for the weights were built on 1,000 samples from the data set using the bias-corrected and accelerated method. We ran two separate multivariable models with and without anti-ARS autoantibodies, considering that their strong association with ASSD diagnosis could overshadow the effect of other variables.

In the univariable analysis, cases or controls with missing data for each variable were excluded from the analysis for the variable. As for the multivariable regression, we imputed missing data employing random forest models. For this purpose, we used the *rfImpute* function belonging to the randomForest R package. Results are shown as odds ratios (ORs) or 100% weights with 95% CIs. A two-sided *P* value of less than 0.05 was considered statistically significant. All statistical analyses were conducted by a statistician (DR) using R version 4.2.2. (R Foundation for Statistical Computing). The data underlying the findings reported herein are available on a reasonable request from the corresponding author.

RESULTS

The CLASS database. A total of 2,035 ASSD cases and 2,140 controls were submitted by the local investigators. The diagnosis of ASSD and control SARDs/ILD was confirmed in 1,952 and 2,097 records, respectively. For the present study, 948 ASSD cases and 1,077 controls were randomly selected from the list of verified reports (Supplementary Figure S1). The mean age at diagnosis, sex distribution, and disease duration were comparable between patients with ASSD and controls (Table 1). The predominant diagnoses among the controls were DM (28.3%), RA (11.7%), systemic sclerosis (10.8%), polymyositis (8.4%), and interstitial pneumonia with autoimmune features (without anti-ARS autoantibodies) (7.6%).

Joint domain. We observed a significant association between joint involvement overall and ASSD diagnosis. Specifically, 57.3% of patients with ASSD had joint involvement compared with 44.0% of controls (OR 1.71 [95% CI 1.43–2.05], $P < 0.001$) (Figure 2 and Supplementary Table S6). Breaking down the types of joint involvement, isolated arthralgia was not a distinguishing feature for ASSD (11.8% cases vs 12.8% controls, OR 0.91 [95% CI 0.70–1.19], $P = 0.508$). In contrast, arthritis was significantly associated with ASSD diagnosis (45.2% cases vs 31.0% controls, OR 1.84 [95% CI 1.53–2.21], $P < 0.001$). Symmetric polyarthritis was also significantly associated with ASSD, whereas the OR was numerically lower than that for arthritis (34.9% cases vs 28.1% controls, OR 1.38 [95% CI 1.13–1.67], $P = 0.001$).

Table 1. Demographics and clinical characteristics of patients with ASSD and controls included in the univariable analysis*

Characteristics	Patients (n = 948)	Controls (n = 1,077)
Age at diagnosis, mean ± SD, y	60 ± 14	58 ± 17
Female, n (%)	666 (70.3)	762 (70.8)
Ethnicity, n (%)		
Hispanic or Latino	272 (28.7)	324 (30.1)
Not Hispanic or Latino	615 (64.9)	696 (64.6)
Unknown/not reported	61 (6.4)	57 (5.3)
Race, n (%)		
American Indian/Alaska Native	58 (6.1)	46 (4.3)
Asian	150 (15.8)	189 (17.5)
Native Hawaiian or other Pacific Islanders	2 (0.2)	0
Black or African American	78 (8.2)	36 (3.3)
White	562 (59.3)	712 (66.1)
Others	15 (1.6)	16 (1.5)
Unknown/not reported	83 (8.8)	78 (7.2)
Disease duration, median (IQR), y	0.5 (0.2–2.2)	0.5 (0.1–1.8)
Clinical diagnosis of controls, n (%)		
Dermatomyositis	–	305 (28.3)
Rheumatoid arthritis	–	126 (11.7)
Systemic sclerosis	–	116 (10.8)
Polymyositis	–	91 (8.4)
Interstitial pneumonia with autoimmune features[a]	–	82 (7.6)
Sjögren disease	–	61 (5.7)
Systemic lupus erythematosus	–	57 (5.3)
Inclusion body myositis	–	40 (3.7)
Scleromyositis	–	40 (3.7)
Immune-mediated necrotizing myopathy	–	39 (3.6)

* ASSD, anti-synthetase syndrome; IQR, interquartile range.
[a] Not with anti-synthetase antibodies.

Muscle domain. Muscle involvement overall was significantly associated with the diagnosis of ASSD (69.5% cases vs 55.2% controls, OR 1.85 [95% CI 1.54–2.22], $P < 0.001$) (Figure 2 and Supplementary Table S7). Among the different items related to muscle involvement, a significant association with ASSD diagnosis was observed for diffuse myalgia (43.9% cases vs 36.4% controls, OR 1.37 [95% CI 1.14–1.65], $P = 0.001$) and muscle enzyme elevation (54.5% cases vs 44.0% controls, OR 1.52 [95% CI 1.27–1.82], $P < 0.001$), whereas neither muscle weakness (49.1% cases vs 48.3% controls) nor dysphagia (14.9% cases vs 21.9% controls) was associated with ASSD.

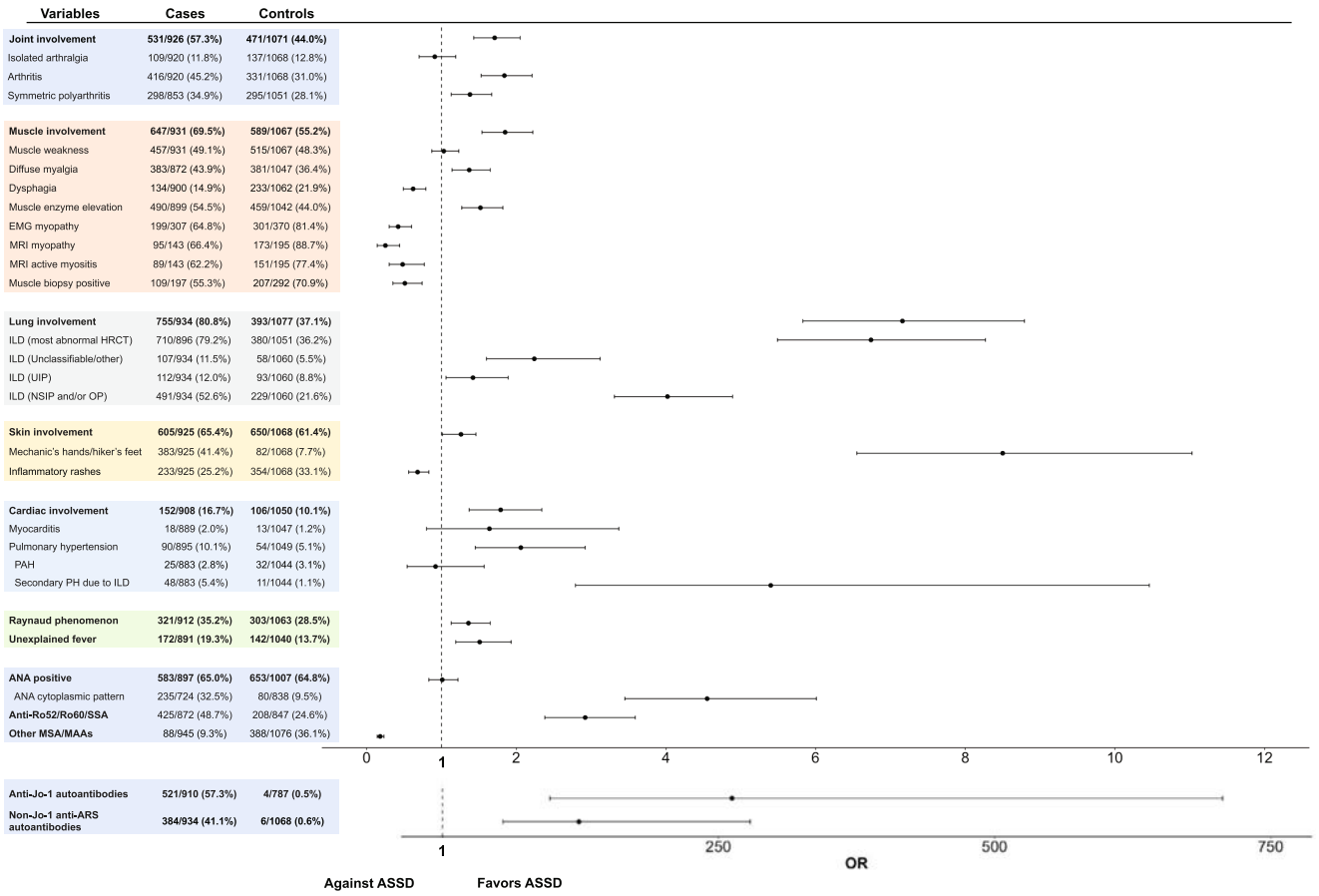

Figure 2. Prevalence of variables in cases and controls and the association of each variable with ASSD diagnosis. ANA, antinuclear autoantibody; ARS, aminoacyl-transfer RNA synthetase; ASSD, anti-synthetase syndrome; EMG, electromyography; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; MAA, myositis-associated autoantibody; MRI, magnetic resonance imaging; MSA, myositis-specific autoantibody; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; OR, odds ratio; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; UIP, usual interstitial pneumonia. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43038/abstract.

Electromyography (EMG) was performed in 54.8% of patients and 68.1% of controls. EMG findings consistent with myopathy were negatively associated with ASSD (64.8% cases vs 81.4% controls of EMG performed, OR 0.42 [95% CI 0.30–0.60], $P < 0.001$). Only 24.2% of patients and 34.5% of controls underwent muscle magnetic resonance imaging (MRI). Interestingly, MRI findings consistent with myopathy were also negatively associated with ASSD diagnosis (66.4% cases vs 88.7% controls of MRI performed, OR 0.25 [95% CI 0.14–0.44], $P < 0.001$). None of the individual MRI findings, including muscle edema (76.9% cases vs 71.8% controls of MRI performed) and fascial edema (21.7% cases vs 19.5% controls of MRI performed), were associated with ASSD. Muscle biopsy was performed in 22.3% and 27.7% of patients and controls, respectively. Muscle biopsy findings suggestive of myopathy overall were negatively associated with ASSD (55.3% cases vs 70.9% controls of muscle biopsy performed, OR 0.51 [95% CI 0.35–0.74], $P = 0.001$). Individual muscle biopsy findings were not significantly associated or negatively associated with ASSD.

Lung domain. Lung involvement was strongly associated with the diagnosis of ASSD (80.8% cases vs 37.1% controls, OR 7.16 [95% CI 5.84–8.81], $P < 0.001$) (Figure 2 and Supplementary Table S8). Nearly all patients (97.2%) and controls (97.7%) with lung involvement underwent high-resolution computed tomography (HRCT). HRCT findings compatible with ILD were significantly associated with ASSD (79.2% cases vs 36.2% controls, OR 6.74 [95% CI 5.49–8.27], $P < 0.001$).

Regarding distinct HRCT ILD patterns, predominant nonspecific interstitial pneumonia (NSIP) and/or organizing pneumonia (OP) pattern was the most prevalent and associated with ASSD diagnosis (52.6% cases vs 21.6% controls, OR 4.02 [95% CI 3.31–4.89], $P < 0.001$). Of note, predominant usual interstitial pneumonia (UIP) pattern (12.0% cases vs 8.8% controls, OR 1.42 [95% CI 1.06–1.89], $P < 0.001$) and unknown/unclassifiable/other patterns (11.5% cases vs 5.5% controls, OR 2.24 [95% CI 1.60–3.12], $P < 0.001$) were less frequent but still significantly associated with ASSD diagnosis. The results remained consistent between the first and the most abnormal HRCT except

for predominant UIP pattern, which was associated with ASSD only in the most abnormal HRCT.

Skin domain. Overall, skin involvement was slightly associated with ASSD diagnosis (65.4% cases vs 60.9% controls, OR 1.26 [95% CI 1.01–1.46], $P = 0.036$) (Figure 2 and Supplementary Table S9). We observed a robust association of mechanic's hands/hiker's feet with ASSD (41.4% cases vs 7.7% controls, OR 8.50 [95% CI 6.55–11.03], $P < 0.001$), whereas the prevalence of hiker's feet was low in both groups (1.6% cases vs 0.8% controls). In contrast, inflammatory rashes were negatively associated with ASSD (25.2% cases vs 33.1% controls, OR 0.68 [95% CI 0.56–0.83], $P < 0.001$). Individual rashes and other skin manifestations either showed a negative association with ASSD or did not provide a clear distinction between ASSD and controls. Skin biopsy was performed only in 56/605 (9.3%) of patients with ASSD and 102/650 (15.7%) of controls with skin involvement.

Cardiac domain. Cardiac involvement overall was associated with the diagnosis of ASSD (16.7% cases vs 10.1% controls, OR 1.79 [95% CI 1.37–2.34], $P < 0.001$) (Figure 2 and Supplementary Table S10). Both patients and controls exhibited a low prevalence of myocarditis (2.0% cases vs 1.2% controls), hindering comparative analysis between patients with ASSD and controls. Pulmonary hypertension (PH) overall was associated with ASSD (10.1% cases vs 5.1% controls, OR 2.06 [95% CI 1.45–2.92], $P < 0.001$). Breaking down the types of PH, secondary PH due to ILD (World Health Organization [WHO] group 3) was associated with ASSD (5.4% cases vs 1.1% controls, OR 5.40 [95% CI 2.79–10.46], $P < 0.001$), whereas pulmonary arterial hypertension (PAH) (WHO group 1) was not (2.8% cases vs 3.1% controls, OR 0.92 [95% CI 0.54–1.57], $P = 0.763$).

Other clinical variables. For the remaining clinical manifestations, Raynaud phenomenon (35.2% cases vs 28.5% controls, OR 1.36 [95% CI 1.13–1.65], $P = 0.001$) and unexplained fever (19.3% cases vs 13.7% controls, OR 1.51 [95% CI 1.19–1.93], $P = 0.001$) were significantly associated with ASSD (Figure 2 and Supplementary Table S11). Dry eyes were slightly associated with ASSD (17.1% cases vs 13.7% controls, OR 1.30 [95% CI 1.01–1.67], $P = 0.038$), but dry mouth was not (17.3% cases vs 14.1% controls, OR 1.27 [95% CI 0.99–1.63], $P = 0.054$).

Serological domain. Antinuclear antibody (ANA) positivity overall did not differentiate ASSD from controls (65.0% cases vs 64.8% controls, OR 1.01 [95% CI 0.83–1.22], $P = 0.946$) (Figure 2 and Supplementary Table S12). ANAs with cytoplasmic pattern were significantly associated with ASSD diagnosis (32.5% cases vs 9.5% controls, OR 4.55 [95% CI 3.45–6.01], $P < 0.001$). As expected, the presence of anti–Jo-1 autoantibodies (57.3% cases vs 0.5% controls), as well as non–Jo-1 anti-ARS autoantibodies (41.1% cases vs 0.6% controls), was strongly associated with ASSD diagnosis (OR 262.17 [95% CI 97.30–706.45], $P < 0.001$ for Jo-1; OR 123.58 [95% CI 54.81–278.61], $P < 0.001$ for non–Jo-1). For non–Jo-1 anti-ARS autoantibodies, the results were consistent regardless of whether the testing method was immunoprecipitation (IP) or not (Supplementary Table S13).

The presence of either anti-Ro52/Ro60 or anti-Sjögren's-syndrome-related antigen A (anti-Ro/SSA) autoantibodies demonstrated a significant association with ASSD (48.7% cases vs 24.6% controls, OR 2.92 [95% CI 2.38–3.59], $P < 0.001$). Analyzing each autoantibody individually upheld the significant relationship between anti-Ro52 autoantibodies and ASSD (51.1% cases vs 23.1% controls, OR 3.48 [95% CI 2.78–4.35], $P < 0.001$), whereas anti-Ro60 autoantibodies (15.3% cases vs 12.2% controls, OR 1.30 [95% CI 0.96–1.77], $P = 0.095$) and anti-Ro/SSA autoantibodies (34.2% cases vs 31.6% controls, OR 1.13 [95% CI 0.49–2.61], $P = 0.777$) were not associated with the diagnosis of ASSD. In contrast, the presence of any other MSAs/MAAs was negatively associated with ASSD diagnosis (9.3% cases vs 36.1% controls, OR 0.18 [95% CI 0.14–0.23], $P < 0.001$). The presence of individual MSAs/MAAs or other autoantibodies such as rheumatoid factor, anti–cyclic citrullinated peptide, anti–double-stranded DNA, anti-Sm autoantibodies, myeloperoxidase–antineutrophil cytoplasmic antibodies (ANCAs), and proteinase 3–ANCAs either did not show a significant association with ASSD diagnosis or had a negative correlation.

Subgroup analyses in cohorts including patients and controls with specific organ involvement. We conducted subgroup analyses in four cohorts focusing on patients and controls having specific organ involvement, ie, joint, muscle, lung, or skin involvement. Clinical diagnoses of controls included in each cohort are presented in Supplementary Table S14.

In all cohorts, arthritis was correlated with ASSD diagnosis, whereas isolated arthralgia was not (Table 2). Muscle weakness, which did not have an association with ASSD in the entire cohort, was associated with ASSD in the subgroups focusing on joint (OR 2.02 [95% CI 1.57–2.61], $P < 0.001$) or lung involvement (OR 2.30 [95% CI 1.77–2.99], $P < 0.001$), whereas it was inversely associated with ASSD in the muscle cohort (OR 0.35 [95% CI 0.26–0.46], $P < 0.001$).

ILD with predominant NSIP and/or OP pattern maintained its strong association with ASSD across all subgroups. Meanwhile, predominant UIP pattern was not associated with ASSD in the joint cohort (OR 1.26 [95% CI 0.85–1.89], $P = 0.253$) and was negatively associated with ASSD in the lung cohort (OR 0.56 [95% CI 0.41–0.76], $P < 0.001$). Inflammatory rashes did not show a significant association with ASSD even in the joint (OR 0.86 [95% CI 0.65–1.14], $P = 0.296$) or the lung cohort (OR 1.04 [95% CI 0.78–1.38], $P = 0.797$), and they were inversely associated with ASSD in the muscle cohort (OR 0.42 [95% CI

Table 2. Subgroup analyses in cohorts including patients with ASSD and controls with specific organ involvement*

	All cohort: case, n = 948; control, n = 1,077		Joint cohort: case, n = 531; control, n = 471		Muscle cohort: case, n = 647; control: n = 589		Lung cohort: case, n = 755; control, n = 393		Skin cohort: case, n = 605; control, n = 650	
	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*
Joint domain	1.71 (1.43–2.05)	<0.001	–	–	3.25 (2.57–4.11)	<0.001	1.26 (0.98–1.61)	0.066	2.05 (1.63–2.57)	<0.001
Isolated arthralgia	0.91 (0.70–1.19)	0.508	0.63 (0.47–0.85)	0.002	1.16 (0.83–1.62)	0.380	0.94 (0.65–1.38)	0.765	0.99 (0.72–1.37)	0.959
Arthritis	1.84 (1.53–2.21)	<0.001	1.58 (1.18–2.11)	0.002	3.30 (2.58–4.22)	<0.001	1.30 (1.01–1.68)	0.040	1.53 (1.20–1.95)	0.001
Symmetric polyarthritis	1.38 (1.13–1.67)	0.001	0.98 (0.75–1.29)	0.913	2.30 (1.78–2.98)	<0.001	0.98 (0.75–1.27)	0.858	1.65 (1.29–2.12)	<0.001
Muscle domain	1.85 (1.54–2.22)	<0.001	4.31 (3.27–5.69)	<0.001	–	–	3.62 (2.80–4.69)	<0.001	1.56 (1.22–1.98)	<0.001
Muscle weakness	1.03 (0.87–1.23)	0.714	2.02 (1.57–2.61)	<0.001	0.35 (0.26–0.46)	<0.001	2.30 (1.77–2.99)	<0.001	0.93 (0.74–1.16)	0.535
Diffuse myalgia	1.37 (1.14–1.65)	0.001	2.08 (1.60–2.71)	<0.001	0.92 (0.72–1.18)	0.513	2.61 (1.95–3.50)	<0.001	1.03 (0.82–1.29)	0.807
Muscle enzyme elevation related to myositis	1.52 (1.27–1.82)	<0.001	3.71 (2.84–4.86)	<0.001	0.90 (0.67–1.20)	0.460	3.02 (2.30–3.97)	<0.001	1.40 (1.12–1.76)	0.004
EMG findings suggestive of myopathy	0.42 (0.30–0.60)	<0.001	0.72 (0.43–1.20)	0.213	0.42 (0.30–0.60)	<0.001	0.52 (0.28–0.99)	0.046	0.43 (0.28–0.66)	<0.001
Muscle MRI findings consistent with myopathy	0.25 (0.14–0.44)	<0.001	0.46 (0.19–1.13)	0.091	0.25 (0.14–0.44)	<0.001	0.30 (0.12–0.76)	0.012	0.29 (0.15–0.57)	<0.001
Muscle biopsy findings suggestive of myopathy	0.51 (0.35–0.74)	0.001	0.96 (0.53–1.72)	0.884	0.51 (0.35–0.74)	0.001	0.64 (0.33–1.23)	0.178	0.62 (0.39–0.99)	0.046
Lung domain	7.15 (5.84, 8.81)	<0.001	4.63 (3.56, 6.19)	<0.001	10.77 (8.28–14.10)	<0.001	–	–	7.61 (5.88–9.89)	<0.001
Unknown/ unclassifiable/ other ILD patterns alone[b]	2.24 (1.60–3.12)	<0.001	2.71 (1.66–4.44)	<0.001	3.83 (2.34–6.28)	<0.001	0.95 (0.68–1.35)	0.788	2.36 (1.53–3.66)	<0.001
Predominant UIP pattern[b]	1.42 (1.06–1.89)	0.019	1.26 (0.85–1.89)	0.253	3.90 (2.26–6.72)	<0.001	0.56 (0.41–0.76)	<0.001	2.93 (1.89–4.54)	<0.001
Predominant NSIP and/or OP pattern[b]	4.02 (3.31–4.89)	<0.001	2.76 (2.11–3.61)	<0.001	5.51 (4.20–7.25)	<0.001	1.33 (1.04–1.71)	0.025	3.57 (2.80–4.54)	<0.001
Skin domain	1.26 (1.01–1.46)	0.036	1.50 (1.15–1.95)	0.003	0.96 (0.75–1.22)	0.744	1.36 (1.06–1.75)	0.016	–	–
Mechanic's hands/ hiker's feet	8.50 (6.55–11.03)	<0.001	8.46 (5.90–12.14)	<0.001	7.70 (5.58–10.64)	<0.001	6.26 (4.42–8.87)	<0.001	11.95 (8.99–15.88)	<0.001
Inflammatory rashes[c]	0.68 (0.56–0.83)	<0.001	0.86 (0.65–1.14)	0.296	0.42 (0.33–0.54)	<0.001	1.04 (0.78–1.38)	0.797	0.52 (0.42–0.66)	<0.001
Cardiac domain	1.79 (1.37–2.34)	<0.001	2.10 (1.44–3.07)	<0.001	1.95 (1.40–2.72)	<0.001	1.38 (0.97–1.97)	0.074	1.87 (1.35–2.60)	<0.001
Myocarditis	1.64 (0.80–3.37)	0.176	3.02 (0.98–9.34)	0.055	1.18 (0.55–2.55)	0.669	2.18 (0.61–7.79)	0.228	0.88 (0.34–2.25)	0.791
PH	2.06 (1.45–2.92)	<0.001	1.94 (1.19–3.16)	0.008	2.55 (1.59–4.07)	<0.001	1.42 (0.92–2.17)	0.112	2.08 (1.36–3.18)	0.001
Pulmonary arterial hypertension (group 1)	0.92 (0.54–1.57)	0.763	0.72 (0.34–1.50)	0.377	0.90 (0.46–1.76)	0.757	0.91 (0.45–1.82)	0.783	0.72 (0.39–1.35)	0.311

(Continued)

Table 2. (*Cont'd*)

	All cohort: case, n = 948; control, n = 1,077		Joint cohort: case, n = 531; control, n = 471		Muscle cohort: case, n = 647; control: n = 589		Lung cohort: case, n = 755; control, n = 393		Skin cohort: case, n = 605; control, n = 650	
	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*
Secondary PH due to ILD (group 3)	5.40 (2.79–10.46)	<0.001	5.39 (2.06–14.12)	0.001	12.18 (3.73–39.81)	<0.001	2.39 (1.23–4.67)	0.011	14.36 (4.40–46.92)	<0.001
Other clinical variables										
Raynaud phenomenon	1.36 (1.13–1.65)	0.001	1.35 (1.04–1.75)	0.025	1.72 (1.34–2.19)	<0.001	1.03 (0.80–1.33)	0.820	1.34 (1.07–1.67)	0.011
Unexplained fever	1.51 (1.19–1.93)	0.001	1.52 (1.11–2.08)	0.009	1.18 (0.88–1.57)	0.274	1.83 (1.29–2.60)	0.001	1.25 (0.94–1.65)	0.123
Serological domain										
ANAs with cytoplasmic pattern	4.55 (3.45–6.01)	<0.001	4.43 (3.01, 6.53)	<0.001	4.49 (3.12, 6.47)	<0.001	4.44 (2.97, 6.65)	<0.001	4.44 (3.10, 6.35)	<0.001
Anti–Jo-1 autoantibodies	262.17 (97.30–706.45)	<0.001	698.77 (97.26–5,020.35)	<0.001	315.87 (100.34–994.35)	<0.001	NA[d]	NA[d]	172.02 (63.46–466.28)	<0.001
Non–Jo-1 anti-ARS autoantibodies	123.58 (54.81–278.61)	<0.001	203.64 (28.39–1,460.87)	<0.001	96.13 (30.56–302.33)	<0.001	91.62 (29.15–288.00)	<0.001	142.79 (45.40–449.09)	<0.001
Anti-Ro52/Ro60 or anti-Ro/SSA autoantibodies	2.92 (2.38–3.59)	<0.001	3.28 (2.43–4.43)	<0.001	3.71 (2.85–4.84)	<0.001	2.79 (2.09–3.73)	<0.001	3.07 (2.38–3.95)	<0.001
Other MSAs/MAAs[e]	0.18 (0.14–0.23)	<0.001	0.21 (0.15–0.30)	<0.001	0.10 (0.07–0.13)	<0.001	0.24 (0.17–0.32)	<0.001	0.12 (0.09–0.16)	<0.001

* ANA, antinuclear antibody; ARS, aminoacyl-transfer RNA synthetase; ASSD, anti-synthetase syndrome; EMG, electromyography; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; MAA, myositis-associated autoantibody; MRI, magnetic resonance imaging; MSA, myositis-specific autoantibody; NA, not applicable; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; OR, odds ratio; PH, pulmonary hypertension; UIP, usual interstitial pneumonia; 95% CI, 95% confidence interval.

[a] Comparators were those without each specific variable analyzed.

[b] Most abnormal HRCT pattern.

[c] Gottron signs/papules, heliotrope rash, V-sign, Shawl sign, and malar rash.

[d] OR and *P* value could not be calculated because the prevalence in controls was zero.

[e] Anti–Mi-2, anti-TIF1-γ, anti-MDA5, anti-SAE, anti-NXP2/MJ, anti-SRP, anti-HMGCR, anti-PM-Scl, anti-U1RNP, and anti-Ku autoantibodies.

0.33–0.54], $P < 0.001$). The presence of ANAs with cytoplasmic pattern, anti-ARS autoantibodies, anti-Ro52/60 or anti-Ro/SSA autoantibodies, and other MSAs/MAAs showed uniform performance across all subgroups, in line with the results obtained in the entire cohort.

Multivariable analysis. We performed multivariable Ridge regression to estimate the weight of each clinical or serological variable for ASSD diagnosis prediction, incorporating covariates based on the results of univariable analysis and intradomain penalized multivariable regression, as well as clinical judgment (Table 3). According to the input from the steering committee, diffuse myalgia was considered positive only in the presence of muscle enzyme elevation. As for ILD, we analyzed whether predominant NSIP and/or OP patterns had additional weight. We did not incorporate secondary PH due to ILD into the multivariable model because it would cause significant multicollinearity with ILD.

In the multivariable model with anti-ARS autoantibodies, the highest estimated weights were for anti-ARS autoantibodies (% weight 39.3 [95% CI 35.2–46.5] for anti–Jo-1 positive by any methods or non–Jo-1 anti-ARS positive by IP; %weight 38.7 [95% CI 34.6–44.8] for non–Jo-1 anti-ARS positive by non-IP methods), followed by mechanic's hands/hiker's feet (%weight 11.8 [95% CI 10.0–13.6]), ILD (%weight 11.1 [95% CI 9.9–13.0]; additional %weight 4.1 [95% CI 2.2–5.2] for predominant NSIP and/or OP patterns), ANAs with cytoplasmic pattern (%weight 7.2 [95% CI 5.4–8.6]), muscle enzyme elevation (%weight 7.2 [95% CI 5.7–8.3]), anti-Ro52/60 or anti-Ro/SSA autoantibodies (%weight 6.7 [95% CI 5.1–7.8]), arthritis (%weight 4.2 [95% CI 2.6–5.3]), and unexplained fever (%weight 3.4 [95% CI 1.2–4.9]). Of note, muscle weakness had a significant weight for ASSD diagnosis prediction (%weight 2.9 [95% CI 0.6–4.2]), whereas diffuse myalgia did not provide significant additional weight to muscle enzyme elevation (additional %weight 1.2 [95% CI 0.0–2.6]). In the model without anti-ARS autoantibodies, the weight for each variable increased substantially, which also identified Raynaud phenomenon as another variable with a significant weight (% weight 2.8 [95% CI 0.1–4.5]).

Table 3. The estimated weights of clinical and serological variables for ASSD diagnosis prediction by multivariable Ridge regression*

	Before imputation		After imputation		%Weight (95% CI)	
Variables	Cases, n = 948	Controls, n = 1,077	Cases, n = 948	Controls, n = 1,077	Model with anti-ARS	Model without anti-ARS
Isolated arthralgia	109/920 (11.8)	137/1,068 (12.8)	109/948 (11.5)	137/1,077 (12.7)	1.0 (0.0–3.0)	2.4 (0.0–4.6)
Arthritis	416/920 (45.2)	331/1,068 (31.0)	431/948 (45.5)	331/1,077 (30.7)	4.2 (2.6–5.3)	7.1 (5.4–8.8)
Muscle weakness	457/931 (49.1)	515/1,067 (48.3)	463/948 (48.8)	515/1,077 (47.8)	2.9 (0.6–4.2)	3.1 (0.7–4.8)
Muscle enzyme elevation related to muscle disease	490/899 (54.5)	459/1,042 (44.0)	520/948 (54.9)	464/1,077 (43.1)	7.2 (5.7–8.3)	11.2 (9.3–13.2)
Diffuse myalgia (additional)	312/445 (70.1)	312/449 (69.5)	343/520 (66.0)	329/464 (70.9)	1.2 (0.0–2.6)	0.0 (0.0–0.0)
EMG or MRI findings consistent with myopathy	282/948 (29.8)	406/1,077 (37.7)	284/948 (30.0)	406/1,077 (37.7)	0.4 (0.0–2.2)	1.5 (0.0–3.5)
Muscle biopsy findings suggestive of myositis	101/882 (11.5)	169/1,054 (16.0)	102/948 (10.8)	169/1,077 (15.7)	0.0 (0.0–0.0)	0.6 (0.0–3.0)
ILD confirmed by HRCT	755/934 (80.1)	393/1,060 (37.1)	769/948 (81.1)	403/1,077 (37.4)	11.1 (9.9–13.0)	16.3 (14.5–19.5)
Predominant NSIP and/or OP patterns (additional)	504/755 (66.8)	244/393 (62.1)	517/769 (67.2)	246/403 (61.0)	4.1 (2.2–5.2)	6.8 (4.7–8.4)
Mechanic's hands or hiker's feet	383/925 (41.4)	82/1,068 (7.7)	400/948 (42.2)	82/1,077 (7.6)	11.8 (10.0–13.6)	18.2 (16.3–21.3)
Raynaud phenomenon	321/912 (35.2)	303/1,063 (28.5)	338/948 (35.7)	303/1,077 (28.1)	1.9 (0.0–3.1)	2.8 (0.1–4.5)
Unexplained fever	172/891 (19.3)	142/1,040 (13.7)	212/948 (22.4)	142/1,077 (13.2)	3.4 (1.2–4.9)	5.6 (3.2–7.5)
Anti–Jo-1 positive by any methods or non–Jo-1 anti-ARS positive by IP	602/945 (63.7)	4/1,076 (0.4)	602/948 (63.5)	4/1,077 (0.4)	39.3 (35.2–45.6)	–
Non–Jo-1 anti-ARS positive by non-IP methods	284/945 (30.1)	6/1,076 (0.6)	284/948 (30.0)	6/1,077 (0.6)	38.7 (34.6–44.8)	–
ANAs with cytoplasmic pattern	235/724 (32.5)	80/838 (9.5)	272/948 (28.7)	80/1,077 (7.4)	7.2 (5.4–8.6)	14.1 (12.3–17.1)
Anti-Ro52/Ro60 or anti-Ro/SSA autoantibodies	425/872 (48.7)	208/847 (24.6)	473/948 (49.9)	208/1,077 (19.3)	6.7 (5.1–7.8)	12.7 (11.1–14.8)

* Values are the number/total number (%) unless otherwise specified. ANA, antinuclear antibody; ARS, aminoacyl-transfer RNA synthetases; ASSD, anti-synthetase syndrome; EMG, electromyography; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; IP, immunoprecipitation; MRI, magnetic resonance imaging; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; 95% CI, 95% confidence interval.

DISCUSSION

The current study used a large, multicenter database including patients with ASSD and mimicking conditions. We identified several clinical and serological factors associated with ASSD diagnosis based on univariable and multivariable analysis, including arthritis, muscle involvement including muscle weakness and muscle enzyme elevation, ILD, mechanic's hands, secondary PH due to ILD, Raynaud phenomenon, unexplained fever, ANAs with cytoplasmic pattern, anti-Ro52 autoantibodies, and as expected, Jo-1 or non–Jo-1 anti-ARS autoantibodies. In contrast, dysphagia, EMG/MRI/muscle biopsy findings suggestive of myopathy, inflammatory rashes, myocarditis, and PAH were not associated with ASSD diagnosis. In some cases, these variables were even inversely associated with ASSD, likely due to the higher frequency of those findings in the control group. Our findings offer a comprehensive set of variables as well as their weights, aimed at establishing data-driven classification criteria for ASSD.

Regarding joint involvement, isolated arthralgia was not a defining feature of ASSD. Notably, the OR for symmetric polyarthritis was numerically lower compared with that for arthritis overall, underscoring the phenotypic heterogeneity of arthritis in ASSD. This heterogeneity appears to be affected by the timing of joint involvement onset during the disease course. Patients who present with arthritis at the initial stages of ASSD commonly have symmetric polyarthritis (70%),[11] whereas patients who develop "de novo" arthritis during their clinical course are more likely to exhibit asymmetric oligoarthritis.[25] Our analysis is limited here because we did not collect specific data for oligoarthritis; however, given this heterogeneity, it becomes evident that the variable "arthritis" should not be restricted to symmetric polyarthritis.

Univariable analyses demonstrated that, within the muscle domain, a particular focus was not on muscle weakness, but rather on diffuse myalgia and muscle enzyme elevation as factors significantly associated with an ASSD diagnosis. Although ASSD is traditionally classified under the umbrella of IIMs, it is important to recognize that muscle weakness is not ubiquitously reported in this patient group. For instance, 25% in the Pittsburgh cohort[9] and 20% in the AENEAS cohort[7] were amyopathic and remained so even after the median follow-up periods of longer than three years. Moreover, patients with specific anti-ARS autoantibodies, including anti-PL-12, OJ, and KS autoantibodies, were reported to maintain an amyopathic profile through their disease trajectory.[26] Our findings thus corroborate that muscle involvement is not universally prevalent in ASSD and, if present, may exhibit a milder phenotype with myalgia and/or muscle enzyme elevation. With that said, subgroup analyses within the joint or ILD cohort revealed an association between muscle weakness and ASSD diagnosis, and importantly, the multivariable regression identified muscle weakness as a variable with a significant weight for ASSD diagnosis prediction; therefore, muscle weakness should be considered as a variable in future classification criteria.

Only 20% to 60% of patients or controls in our database underwent EMG, muscle MRI, or muscle biopsy, restricting our ability to comprehensively evaluate their diagnostic utility in distinguishing ASSD from its mimickers. Nonetheless, findings suggestive of myopathy from these modalities either failed to differentiate ASSD from controls or, paradoxically, were inversely correlated with the diagnosis of ASSD, even in subgroup analyses focusing on joint or lung involvement. The high prevalence of DM in the control groups (17.6%–45.7%) could explain these counterintuitive associations. The limited number of patients who underwent EMG/muscle MRI/muscle biopsy demonstrates that these modalities are not commonly assessed in patients with ASSD in daily practice and warrants further efforts to unravel the characteristics and diagnostic utility of EMG/muscle MRI/muscle biopsy findings in ASSD.

In our cohort, ~80% of patients with ASSD had ILD diagnosed via HRCT. In patients with ASSD, ILD typically shows a unique HRCT pattern with overlapping NSIP and OP.[27] In the present study, predominant NSIP/OP pattern accounted for 69.2% of ILD in ASSD and was in a robust association with ASSD, which provided a significant additional weight in the multivariable regression model. Meanwhile, UIP pattern was associated with ASSD only in the worst HRCT available, but not in the first HRCT. Interestingly, we also observed a significant association of unclassifiable/unknown/other patterns with ASSD. These findings underline the phenotypic heterogeneity of ILD within the ASSD cohort, suggesting that various ILD patterns, including UIP and other unclassifiable patterns, should be considered when constructing future ASSD classification criteria.

Regarding cutaneous involvement, mechanic's hands showed a strong association with ASSD. Although inflammatory rashes were either not useful or negatively associated with ASSD even in the joint, muscle, or lung cohort, the prominence of mechanic's hands accentuates its potential for specificity in ASSD classification. As for other skin features, such as hiker's feet, their low occurrence in our data set merits further investigation given their recent recognition.[28] The lack of association of inflammatory rashes with ASSD could be partly attributed to the fact that DM diagnosis accounted for 28.3% of all controls used in the analysis. Around 20% of patients with ASSD present with inflammatory rashes, which are well-recognized clinical features of ASSD.[9] It remains controversial whether those cases should be classified as 1) ASSD-DM overlap, 2) ASSD with inflammatory rashes, or 3) DM with anti-ARS antibodies.[29,30] Although a recent study reported a significant overlap in the pathophysiology of DM-like skin lesions in ASSD and DM,[31] further studies are necessary to elucidate the potential pathophysiologic differences in patients with "pure" ASSD—ie, those without inflammatory rashes—and patients with ASSD with inflammatory rashes.

Additional clinical manifestations associated with ASSD included Raynaud phenomenon and unexplained fever, consistent with previous publications.[7,12] PH overall was associated

with ASSD; however, this merits cautious interpretation because the relationship appears primarily driven by secondary PH due to ILD (WHO group 3), rather than PAH (WHO group 1). In a multicenter cohort in France, only 8% of patients with ASSD were diagnosed with precapillary PH by right heart catheterization.[32] Recently, a new definition of PH has been proposed and is widely accepted.[33,34] Lowering mean pulmonary arterial pressure and pulmonary vascular resistance threshold for defining precapillary PH should increase the prevalence of both group 1 and group 3 PH in ASSD. With that said, considering the low prevalence and the negative result from the present analysis, PAH may not be considered in future classification criteria.

Our analysis revealed a high level of association for both Jo-1 and non–Jo-1 anti-ARS autoantibodies, indicating that local diagnostic practices may rely heavily on these markers. The extremely high ORs for anti-ARS autoantibodies might have been affected by selection bias; local investigators were unlikely to submit ASSD cases without anti-ARS autoantibodies or controls with anti-ARS autoantibodies because ASSD is commonly recognized as a serological subset of IIMs. This strong association could overshadow the impact of other variables as observed in the two multivariable models with and without anti-ARS autoantibodies. Other autoantibodies, such as ANAs with cytoplasmic pattern and anti-Ro52 autoantibodies, were significantly associated with the diagnosis of ASSD. This highlights the potential utility of these autoantibodies in the classification criteria, particularly in settings where access to non–Jo-1 anti-ARS autoantibody detection may be limited and when the precision of alternative detection methods, such as line immunoassay (LIA), remains uncertain.[18,35,36]

The strengths of this study lie in its expansive, international scope of real-world data, allowing us to mitigate selection bias that is common in smaller cohort studies. However, we must acknowledge several limitations. First, the reliability of autoantibody data may be compromised because of variations in assay methods across participating centers, most of which employed non-IP techniques. To mitigate this, we are conducting central IP, enzyme-linked immunosorbent assay, and/or LIA on the majority of both case and control patient sera.[36] Second, ASSD or non-ASSD (controls) was defined solely on the clinical diagnosis of participating physicians. Since the disease concept of ASSD has not been established yet, the clinical diagnosis of ASSD or non-ASSD could differ depending on the investigators, specialties, or regions. Also, any case-control study or criteria development is heavily dependent on the mix of controls used for comparison, where any single control type may lead to skewed results. We believe that our data ascertained from 92 centers across five continents likely represent real-world data.

In conclusion, univariable and multivariable analyses of the CLASS database identified several key variables associated with ASSD diagnosis. Our results provide insights into the key clinical features of ASSD, which can help clinicians as well as lay the groundwork for the development of data-driven classification criteria for ASSD. The CLASS project team is planning to simplify and/or provide minor modifications of the weights or variables based on feedback from the steering committee to propose candidate classification criteria. The steering committee will discuss the criteria in terms of face validity, feasibility, ease of use, etc, to reach the final consensus, and the final classification criteria will be tested on the validation data set.

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AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Drs Aggarwal and Cavagna confirm that all authors have provided the final approval of the version to be published, and take responsibility for the affirmations regarding article submission (e.g., not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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LETTER

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Osteoarthritis: Magnetic resonance imaging definitions and disease progression: comment on the article by Chang et al

To the Editor:

We read with interest the recent paper by Chang and colleagues[1] suggesting a similar performance of recent magnetic resonance imaging (MRI)–based definitions of osteoarthritis (OA) of the knee based on whether those definitions predicted the later development of radiographic disease or symptomatic and radiographic disease. Their conclusions were that knees that met either definition were at increased risk of knee OA, but that most knees that met these definitions failed to develop later radiographic or symptomatic OA. This paper represents a thoughtful analysis of the OA Initiative (OAI) data, but there are problems with this "predict later OA" approach.

Risk factors could predict later OA but should not necessarily be part of a definition of disease. For example, obesity is a powerful risk factor for later knee OA, but one would not include it in a definition of OA. A definition of knee OA that included obesity would predict later OA better than a definition not including obesity. Their definition A from Hunter et al,[2] which performed slightly better than definition B,[3] consists of a set of formulas to define OA that include bone marrow lesions (in the tibiofemoral but not patellofemoral joint) and meniscal damage along with other features. Meniscal damage and bone marrow lesions are well-documented risk factors for later OA and, by themselves, would predict later OA regardless of whether they should be used to define OA. Their inclusion in definition A may explain why it was slightly better at predicting later OA.

There are other concerns about the definition of Hunter and colleagues. Because, in part, of the inclusion of these features, this definition allows for OA to be defined without any visible cartilage damage. This would contrast with biologic approaches to OA, in which cartilage damage is a signature pathologic feature. Another concern is that definitions of patellofemoral OA differ from definitions of disease in the tibiofemoral joint.

Lastly, the failure of these two definitions to show progression to later OA has little to do with their validity but is probably because of OA's "state of inertia,"[4] whereby most knees with OA remain in their pathologic state and do not progress. Only a minority of knees show continuing progression. This state was recently corroborated in the Multicenter OA Study,[5] and Chang et al confirm that it is present in the OAI.

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Reply

To the Editor:

We appreciate Dr Felson's thoughtful comments on our study.[1] He raises an interesting point regarding the distinction between risk factors and disease definitions. Our objective was to determine whether the MRI-based criteria[2,3] developed to define knee OA are associated with the future development of radiographic and symptomatic disease. This investigation helps us better understand the relevance and utility of these definitions in individuals at higher risk for knee OA, particularly in the context of guiding recruitment for clinical trials targeting early-stage OA or identifying candidates for preventive strategies.

It is important to clarify that our goal was not to consider the strengths and weaknesses of each of the two MRI-based definitions, which have been discussed previously.[3] Rather, our focus was on evaluating whether these existing MRI

definitions identify individuals who will develop clinically significant OA over time.

We agree with Dr Felson that the development and progression of knee OA is often in a "state of inertia."[4] As we discuss in the manuscript, the relatively low incidence of radiographic knee OA over 11 years in our study sample underscores the challenge of tracking a disease that progresses very slowly. Although extending the follow-up period could offer additional insights, finding ways to identify individuals who will go on to develop clinically significant disease remains crucial.[5,6] Exploring alternative imaging-based algorithms or algorithms that incorporate MRI features, symptoms, and physical examination findings, and leveraging machine learning and artificial intelligence may be fruitful approaches for future analyses.

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Incorrect about International Classification of Diseases coding for patients with systemic lupus erythematosus: comment on the article by Haukeland et al

To the Editor:

I read with interest the article by Haukeland et al[1] in which the authors found the use of International Classification of Diseases (ICD) coding only identified 45% of patients for whom (electronic) chart review by a clinician resulted in a systemic lupus erythematosus (SLE) diagnosis based on the presence of otherwise unexplained multiorgan disease and typical immunologic findings. The correct application of ICD codes is quite complex and requires significant insight and experience with coding rules. ICD coding has been found to be more reliable in regions such as Australia, where trained in-house clinical coders who communicate with physicians in case of uncertainty provide the ICD codes to the administrative databases for health authorities.[2,3] My understanding is that in Norway, health personnel (including trainees and non-specialists) are required to provide ICD codes when discharging patients, even though they lack the required training. If this is correct, then it would be hard to agree with the authors' broad statement that ICD coding is insufficient to identify patients with SLE in administrative databases. This may apply to Norway only. I wonder if the authors' multiorgan diagnostic approach would have captured patients with lupus nephritis confirmed by incident-isolated biopsy without other organ manifestations and what the "typical immunologic findings" are should they be applied.

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Reply

To the Editor:

We thank Dr Nossent for his interest in our study "Declining Incidence of Systemic Lupus Erythematosus in Norway 1999–2017: Data From a Population Cohort Identified by International Classification of Diseases, 10th Revision Code and Verified by Classification."[1] We agree with Dr Nossent's comment that correct application of International Classification of Diseases, 10th Revision (ICD-10), codes for Systemic Lupus Erythematosus (SLE) is complex and requires skills. His view that trained in-house clinical coders will improve ICD-10 coding accuracy for patients with SLE may well be right, but data are largely missing. Therefore, we welcome studies assessing SLE coding accuracy in health systems that allocate resources to in-house clinical coders.

In our study, we were able to confirm SLE diagnosis in 1,558 out of 3,488 individuals (45%) who were registered with an ICD-10 SLE discharge code (M32.1, M32.8, M32.9) in Southeast Norway from 1999 to 2017 through structured medical record review. Dr Nossent asks whether this rather low accuracy reflects that ICD-10 coding in Norway, in some instances, is done by less experienced doctors. We do believe that the available evidence from population-based SLE studies around the world speaks against this possibility. Admittedly, the number of studies that has gone through the intensive effort of medical record review is limited, but in the studies that exist, the accuracy rates in identifying patients through ICD-10 coding are within the same range; a US population-based study reports a 49% accuracy rate for one or more ICD-10 codes identifying SLE.[2] Similarly, a 2013 systematic review reports an accuracy of ICD-10 codes for identifying SLE in the general population to be slightly higher than our study (50%–60%).[3]

Further supporting the notion that the challenges of SLE coding are universal rather than specific to Norway, a transdiagnosis study found that SLE was the systemic autoimmune disease with the highest rate of patients inaccurately coded by the ICD-10.[4] Finally, because 90% of all patients with SLE in Norway are diagnosed and treated by hospital-based rheumatologists, the ICD-10 coding is predominantly done by clinicians familiar with SLE.[5] There is no easy solution to the coding challenges for diagnosing SLE, but we do believe that it would be a step forward if studies applying ICD-10 code–based definitions for patients with SLE were obliged to report on the accuracy of their definition.

Regarding Dr Nossent's question on typical immunologic findings, our study defines this as at least one immunologic disturbance compatible with SLE, typically in the form of antinuclear antibodies (ANAs). In a recent article on classification of the Nor-SLE cohort, we provide a detailed description of patients who were ANA negative with new-onset SLE.[6] Regarding isolated lupus nephritis, we have two comments. First, the ICD-10 searches included all nephrology departments in Southeast Norway, excluding the possibility of missing patients with SLE observed exclusively by nephrologists. Second, among the 34% of the patient cohort with lupus nephritis, all had at least one other clinical or immunologic finding confirmed by medical record review. In all, we consider our study to be an important contribution to the ongoing discussion on use of administrative data in SLE research, and we thank Dr Nossent for his insightful comments on this matter.

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Role of autoantibodies in lupus nephritis: comment on the article by Fava et al

To the Editor:

We commend Fava et al for their insightful study on the role of anti-C1q and anti–double-stranded (dsDNA) autoantibodies in lupus nephritis (LN).[1] The investigation into these antibodies as noninvasive biomarkers is a significant step forward. However, some aspects warrant further exploration to strengthen the study's conclusions.

First, the study highlights a significant association between elevated anti-C1q and anti-dsDNA antibody levels and proliferative LN. These findings suggest that these antibodies may play a critical role in LN pathology. However, the study does not delve into the specific pathogenic mechanisms of anti-C1q antibodies. Given C1q's role in initiating the classical complement pathway, these antibodies may interfere with the clearance of apoptotic cells, leading to increased exposure of autoantigens and immune complex formation.[2] Further research is needed to explore these mechanisms across different LN subtypes and to understand how complement activation and immune complex deposition contribute to glomerular inflammation.

Furthermore, the authors report a significant reduction in antibody levels correlating with treatment response. After 12 months of treatment, autoantibody concentrations in patients with proliferative LN with a complete treatment response was declined. This may reflect not only reduced disease activity but also the effectiveness of the treatment. However, the study does not clearly distinguish whether the reduction in antibody levels is due to immunosuppression or actual disease improvement. If the decrease is primarily treatment-induced, the predictive value of these antibodies as independent biomarkers may be limited. Moreover, the study suggests that patients with multiple positive autoantibodies, such as anti-Sm and anti-RNP, have poorer outcomes, particularly in nonproliferative LN, indicating a need for further investigation into how different autoantibody combinations affect disease progression.[3]

Additionally, the study shows that patients with proliferative LN have higher levels of these autoantibodies compared to those with membranous LN. This suggests that proliferative LN may rely more on antibody-mediated immune responses, whereas membranous LN might involve different immunological pathways. Understanding these differences is crucial for tailoring treatment strategies. The lower antibody levels in membranous LN may indicate a greater role for T cell–mediated responses or other non–antibody-dependent mechanisms, which warrants further exploration.

Finally, challenges remain in translating these findings into clinical practice. Variability in testing methods across laboratories could affect clinical decision-making. Standardizing these assays and validating them in diverse populations is essential.

In summary, the study offers promising insights, but a deeper understanding of the underlying biology and practical application of these biomarkers is needed to fully realize their clinical potential.

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Reply

To the Editor:

We thank Ran et al for their comments on our work investigating the association of autoantibodies with lupus nephritis (LN) histological features and treatment response. As the primary aim of our study was to assess the clinical relevance of autoantibodies as biomarkers,[1] defining additional autoantibody

mechanisms was beyond the scope of this paper. However, as speculated in our manuscript discussion, we agree that anti-C1q may inhibit apoptotic cell uptake, potentially increasing autoantigen exposure and immune complex deposition.[2] Although significant work has occurred in epitope mapping and animal models,[2–4] we agree that additional mechanistic work in large cohorts of diverse patients with systemic lupus erythematosus would be helpful. In addition, we agree that our findings may suggest that membranous LN involves non–antibody-dependent mechanisms or autoantibodies with specificities that were not explored in our study, as highlighted in the discussion of our manuscript.

Although we appreciate the insights of Ran et al, we have a different perspective on a couple of points. Specifically, our primary focus was on the predictive value of baseline autoantibody levels rather than changes over time. Therefore, whether the changes in autoantibody levels were driven by immunosuppression or actual disease improvement does not diminish the clinical utility of our findings. Furthermore, all patients with clinically actionable results were treated with immunosuppression, as is the standard of care. Because treatment was not protocolized, patients received different regimens according to their history at the discretion of their physician. It may be impossible to evaluate whether decreases in autoantibodies are from immunosuppression or treatment-associated disease improvement via different modes of action. This may need further investigation in clinical trials with access to deep phenotyping and repeat biopsies or in animal models.

In this study, multiple positive autoantibodies are associated with proliferative disease, but we did not observe differences in the number of positive autoantibodies based on treatment response in patients with either proliferative or membranous LN. We did see that patients with a complete response were more likely to decrease their anti-Sm/anti–nuclear RNP antibody responses by 6 to 12 months after biopsy. Given the heterogeneity of autoantibody expression in patients with LN, we agree that further investigation into the role of specific autoantibody combinations in disease progression is important to the field.

Finally, we acknowledge that variability in testing methods across laboratories presents a challenge in translating these biomarkers into clinical practice. However, our findings underscore the clinical need for standardized, commercially available assays for anti-C1q to facilitate their broader clinical use across nations.

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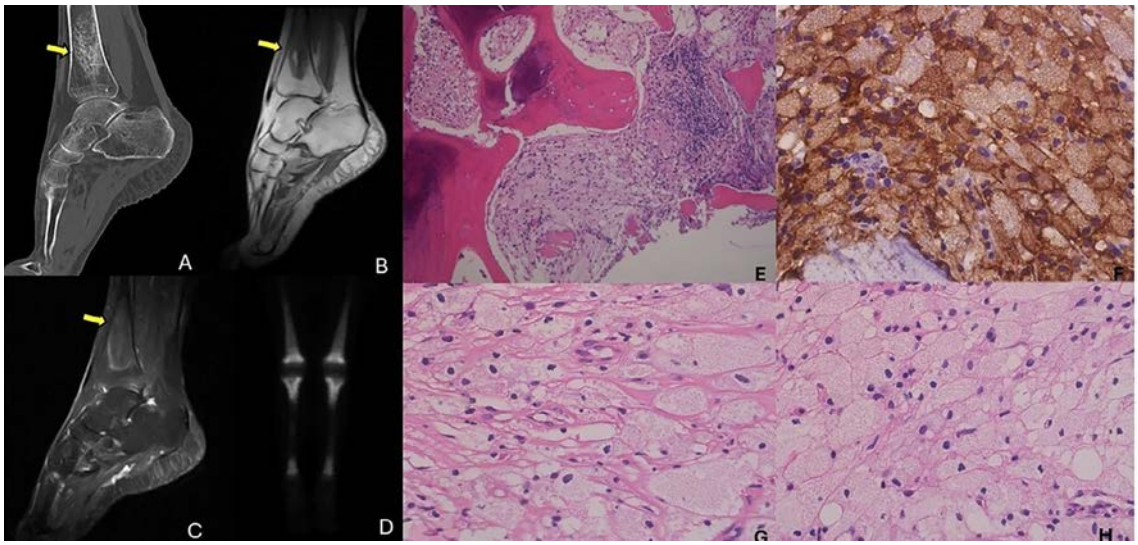
Clinical Images: A rare and misleading condition: isolated skeletal involvement of Erdheim–Chester disease



The patient, a 60-year-old woman with osteoporosis, complained of pain in the right ankle, which spread to the perimalleolar region, and then the left ankle. No fractures were detected on the radiograph; magnetic resonance (MR) imaging showed bone edema in the cuboid–calcaneal bone heads and the left distal metadiaphyseal tibia. The patient received nonsteroidal anti-inflammatory drugs, analgesics, and bisphosphonates for suspected complex regional pain syndrome, without benefit. Given the persistent pain at the tibias bilaterally, computed tomography (CT) was performed, showing (A) sclerosis of the trabecular bone in the distal third of the tibial diaphysis; (B and C) sagittal T1-weighted and STIR MR images showed extensive spongiosa edema in the fourth and fifth segments of both tibial diaphyses, well demarcated by the continuous trabecular bone, with a 7-cm craniocaudal extension; (D) the skeletal scintigraphy confirmed the radiotracer uptake at the upper and lower tibial diaphyses. Given the atypical bone lesions and the unresponsiveness to the treatments administered, a bone biopsy was performed. (E, G, and H) Histologic examination revealed intertrabecular fibrosis and infiltrates of foamy histiocytes, which were (F) diffusely positive for CD163, aspects compatible with Erdheim–Chester disease, a non-Langerhans cell histiocytosis that provokes an abnormal aggregation of histocytes in several organs (cardiovascular and central nervous system, retroperitoneum), including long bones.[1] BRAF (V600E) mutation was detected (via Droplet Digital Polymerase Chain Reaction, allele frequency [AF] 0.06%), and histologic findings supported the diagnosis by excluding other potential mimickers.[2] A CT scan of the abdomen was negative for pathologic findings; the patient was then advised to start the BRAF inhibitor vemurafenib.

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