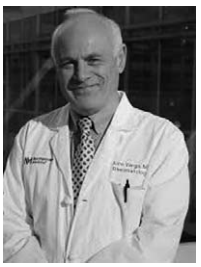
# Editorial introductions

*Current Opinion in Rheumatology* was launched in 1989. It is one of a successful series of review journals whose unique format is designed to provide a systematic and critical assessment of the literature as presented in the many primary journals. The field of Rheumatology is divided into 15 sections that are reviewed once a year. Each section is assigned a Section Editor, a leading authority in the area, who identifies the most important topics at that time. Here we are pleased to introduce the Journal's Section Editors for this issue.

## SECTION EDITORS

### John Varga



John Varga MD is the John and Nancy Hughes Distinguished Professor at Northwestern University's Feinberg School of Medicine in Chicago, Illinois, USA.

Coming to the United States as a refugee, Dr Varga attended Columbia University, and obtained his medical degree from New York University. Following Rheumatology Fellowship in Boston, USA, he pursued post-doctoral research training with Professor Sergio Jimenez at the University of Pennsylvania, USA. In 2004, he joined the faculty at Feinberg School of Medicine, USA, where he founded and directs the integrated Scleroderma Program. His research focuses on the pathogenesis and treatment of scleroderma and fibrosis, and bridges clinical and laboratory-based investigation.

Dr Varga has mentored over 20 trainees, several of whom are now independent academic investigators. He is the author of more than 350 original articles, 40 book chapters and four books. His research has been continuously funded by the National Institutes of Health. He is an elected member of the Association of American Physicians, and is Master of the American College of Rheumatology.

### Anna Ghirardello

Anna Ghirardello, DSc, PhD, graduated in Biological Sciences from the University of Padova, Italy where she also qualified in Clinical Pathology. She received her PhD degree and post-doctoral fellowship at the University of Padova. She is Biologist at the University Hospital of Padova. Her main fields of interest include the pathogenesis and laboratory diagnostics of connective tissue diseases, focused primarily on systemic lupus erythematosus and idiopathic inflammatory myopathies. Dr Ghirardello is a member of the Italian Society of Rheumatology (SIR), Council member of the Italian Interdisciplinary Forum for the Research in Autoimmune Diseases (FIRMA), and member of the EuroMyositis Registry. She has authored over 120 ISI publications.

### Andrea Doria

Andrea Doria is Professor of Rheumatology and Head of the Unit of Connective Tissue Disease and Rare Rheumatic Diseases, Division of Rheumatology, Department of Medicine, University of Padua, Italy. He is Head of the Rheumatology Unit, University of Padua and Director of Rheumatology postgraduate medical school.

Professor Doria received his medical degree and qualification in Rheumatology from the University of Padua. He was Council member of the Italian College of Rheumatology (CRO) between 1999 and 2005 and a Council member of the Italian Society of Rheumatology (SIR) from 2007 to 2010 and from 2013 until now. He is also a member of American College of Rheumatology (ACR).

Professor Doria has organised over ten international conferences on autoimmunity and was involved as ''expert'' in the EUropean League Against Rheumatism (EULAR) Standing Committee for the development of clinical and therapeutic recommendations: (1) EULAR recommendations for the management of systemic lupus erythematosus (SLE)—Assessment of the SLE patient (2008–2009); (2) EULAR recommendations for the management of SLE Part II—Neuropsychiatric disease

(2008–2009); (3) Joint EULAR and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis (2012). Professor Doria is a member of the Lupus Academy Steering Committee and co-Chaired the 4th Annual Meeting held in Rome 27th February to 1st March 2015. He was the chair of the 10th European Lupus Meeting, held in Venice (Italy) 5–8th October 2016.

Professor Doria is on the Editorial Boards of several rheumatology and immunology journals, including Lupus, Autoimmunity, Clinical and Experimental Rheumatology, Autoimmunity Reviews, Journal of Autoimmunity, Experimental Biology and Medicine, Rheumatology Reports, Journal Autoimmunity Highlights and Reumatismo (the official journal of Italian Society of Rheumatology).

He has authored over 250 ISI publications on SLE and other connective tissue diseases. These include clinical studies describing new manifestations or subgroups of autoimmune disorders, prognostic risk factors, diagnostic tests and therapeutic interventions, as well as immunochemical studies that evaluate autoantibodies, epitopes and complementary epitopes of autoantigens. In addition, he has authored and co-authored three books, over 90 book chapters and conference proceedings, and over 500 abstracts for national and international conferences.

Professor Doria has long-standing experience of the clinical management of patients with connective tissue diseases. The Unit in which he works is a tertiary referral rheumatology centre, within Italy, for the diagnosis and management of patients affected with systemic connective diseases. In addition, he has expertise in the management and follow-up of pregnant patients with systemic rheumatic diseases. Professor Doria has also trained over 30 students in Rheumatology.

## Mariele Gatto

Dr Mariele Gatto, MD, is Rheumatologist and currently attending her last year of PhD course in Clinical and Experimental Sciences at Padova University, Italy. Dr Gatto performs both clinical activity and laboratory research at Padova University, with a major focus on development and treatment of systemic lupus erythematosus (SLE) and other connective tissue diseases. So far, her *Cursus Studiorum* was carried out between Padova University and other foreign institutions where Dr Gatto could acquire and improve research skills, particularly at Zabludowicz Center for Autoimmune Diseases in Tel Aviv, Israel and at Charité Hospital in Berlin, Germany, with a major focus on B cells in lupus.

Dr Gatto is actively involved in patient recruitment and follow-up within randomized controlled trials, investigating novel therapeutics in SLE, inflammatory myositis and Sjögren syndrome, as well as in training of younger fellows and students at Padova Medical School.

Dr. Gatto has attended several national and international meetings and symposia as speaker and was awarded so far with four prizes (CORA young researcher award 2015; prize of the Italian Society of Rheumatology 2016; CORA award 2019; DIMAR 2019 award at Medicine Department of Padova university) for best abstract presentation.

She is author or co-author of 58 publications available in PubMed.

## Mariana Kaplan

Dr Mariana Kaplan is Senior Investigator and Chief of the Systemic Autoimmunity Branch at the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, USA.

Dr Kaplan's research has focused on identifying mechanisms of organ damage and premature atherosclerosis in systemic autoimmunity. Her laboratory focuses on identifying how innate immunity promotes loss of tolerance and end-organ damage in SLE, rheumatoid arthritis and other systemic autoimmune diseases. Dr Kaplan also has an interest in identifying novel therapeutic targets to prevent premature vascular damage in systemic autoimmune diseases, using a bench to bedside approach. She has been principal investigator in various investigator-initiated clinical trials assessing new treatment approaches in autoimmunity. Dr Kaplan has served in numerous advisory and committee roles with the American College of Rheumatology, Rheumatology Research Foundation, American Association of Immunologists and Lupus Foundation of America and has been a member of the editorial boards of many immunology and rheumatology journals. She is a member of the American Society for Clinical Investigation and

the Association of American Physicians and a recipient of the Henry Kunkel Young Investigator Award and the Edmund L. Dubois Memorial Lectureship, both from the American College of Rheumatology. She has also been recipient of the Evelyn Hess Award from the Lupus Foundation of America for her significant contributions to the understanding of systemic lupus erythematosus, and the Charles L. Christian award for her research impact in understanding lupus.

# Detection and classification of systemic sclerosis-related interstitial lung disease: a review

*Daniel J. DeMizio and Elana J. Bernstein*

**Purpose of review**
Systemic sclerosis (SSc) is a heterogeneous disease with a variable disease course. Interstitial lung disease (ILD) is one of the leading causes of morbidity and mortality in patients with SSc. The present review highlights recent advances in the classification, diagnosis, and early detection of SSc-associated ILD (SSc-ILD).

**Recent findings**
Risk stratification through measurement of disease extent on high-resolution computed tomography (HRCT) of the chest, longitudinal declines in pulmonary function tests (PFTs), and mortality prediction models have formed the basis for classifying clinically significant ILD. HRCT may be preferred over PFTs for screening, as PFTs lack sensitivity and have a high false-negative rate. Novel imaging modalities and biomarkers hold promise as adjunct methods for assessing the presence and severity of SSc-ILD, and predicting risk for progressive disease. Further validation is required prior to their use in clinical settings.

**Summary**
Classification of SSc-ILD has shifted to a personalized approach that considers an individual patient's probability of progressive disease through identification of risk factors, measurement of disease extent on HRCT, longitudinal declines in PFTs, and mortality prediction models. There remains an unmet need to develop screening guidelines for SSc-ILD.

**Keywords**
classification, high-resolution computed tomography, interstitial lung disease, screening, systemic sclerosis

## INTRODUCTION

Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by immune dysregulation, vasculopathy, and inflammation resulting in excessive fibrosis of the skin and internal organs [1,2]. Interstitial lung disease (ILD) is one of the most common manifestations of SSc, affecting approximately 40–60% of patients within this population. SSc-associated ILD (SSc-ILD) is the leading cause of hospitalization, morbidity, and mortality in patients with SSc, accounting for approximately 35% of SSc-related deaths [3–6]. The 2013 American College of Rheumatology/European Legion Against Rheumatism (ACR/EULAR) classification criteria defines SSc-ILD as, 'pulmonary fibrosis seen on high-resolution computed tomography (HRCT) or chest radiograph, pronounced in the basilar portions of the lungs, or occurrence of ''Velcro'' crackles on auscultation, not because of another cause such as congestive heart failure' [7]. Major risk factors for the development of SSc-ILD identified from observational studies include diffuse cutaneous SSc, African American race, older age at disease onset, shorter disease duration, presence of anti-Scl-70 antibodies, and the absence of anticentromere antibodies [8–10].

As of this writing, there are no FDA-approved treatments for SSc-ILD. Current management approaches follow either a strategy of close monitoring of symptoms and pulmonary function tests (PFTs), or a regimen of immunosuppression with close follow-up of symptoms and PFTs. First-line therapy for treatment of SSc-ILD is mycophenolate mofetil (MMF), which was shown to have similar efficacy to and less toxicity than cyclophosphamide in Scleroderma Lung Study (SLS) II [11]. In the recently published SENSCIS trial, patients with SSc-ILD who were taking nintedanib had a lower

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## KEY POINTS

- Classification of SSc-ILD can occur in various dimensions. Current trends suggest a more personalized assessment of an individual patient's pattern of disease, extent of disease, risk factors, and longitudinal assessment of lung function in the context of their radiographic and/or histopathologic pattern of disease. Such classification and risk stratification can help to guide treatment.
- There are no clinical practice guidelines for ILD screening in SSc. Significant global practice variation still exists.
- HRCT is currently the gold standard for the detection of ILD. Although useful, PFTs lack sensitivity for detection of ILD. All patients with newly diagnosed SSc should receive a baseline HRCT to evaluate for underlying ILD.
- Lung ultrasound is a noninvasive, radiation-free technique with high sensitivity and specificity for the screening and diagnosis of SSc-ILD. Lack of standardization and length of time for a scan, however, are major barriers for adoption.
- SP-D, KL-6, and CCL18 have emerged as promising candidate biomarkers for the diagnosis of SSc-ILD, assessment of disease severity, and predictors for progressive disease, respectively. Numerous additional biomarkers are under investigation.

annual rate of forced vital capacity (FVC) decline than those taking placebo (difference, 41 ml per year; 95% confidence interval [CI], 2.9–79.0; *P*-value = 0.04) [12]. Notably, 48% of patients in the trial were taking MMF concomitantly with nintedanib or placebo. At this time, it remains unknown where nintedanib will fit into the treatment algorithm of SSc-ILD. It may become the first FDA-approved medication for the treatment of SSc-ILD. Research into newer therapies, such as pirfenidone [13], is ongoing, yet morbidity and mortality from SSc-ILD remain high. Therefore, it is critical that we investigate methods to screen, classify, and risk stratify patients with SSc-ILD, so we can detect disease early and identify those at high risk of progression. Research into early detection and treatment of SSc-ILD may eventually enable prevention of progressive disease. In the present review, we aim to summarize fundamental preexisting literature and provide insight into recent advances in the classification, diagnosis, and early detection of SSc-ILD.

## CLASSIFICATION OF SYSTEMIC SCLEROSIS-ASSOCIATED INTERSTITIAL LUNG DISEASE

There are several ways to classify SSc-ILD: by histopathology, radiographic pattern, radiographic extent of disease, and likelihood of progression (Fig. 1).

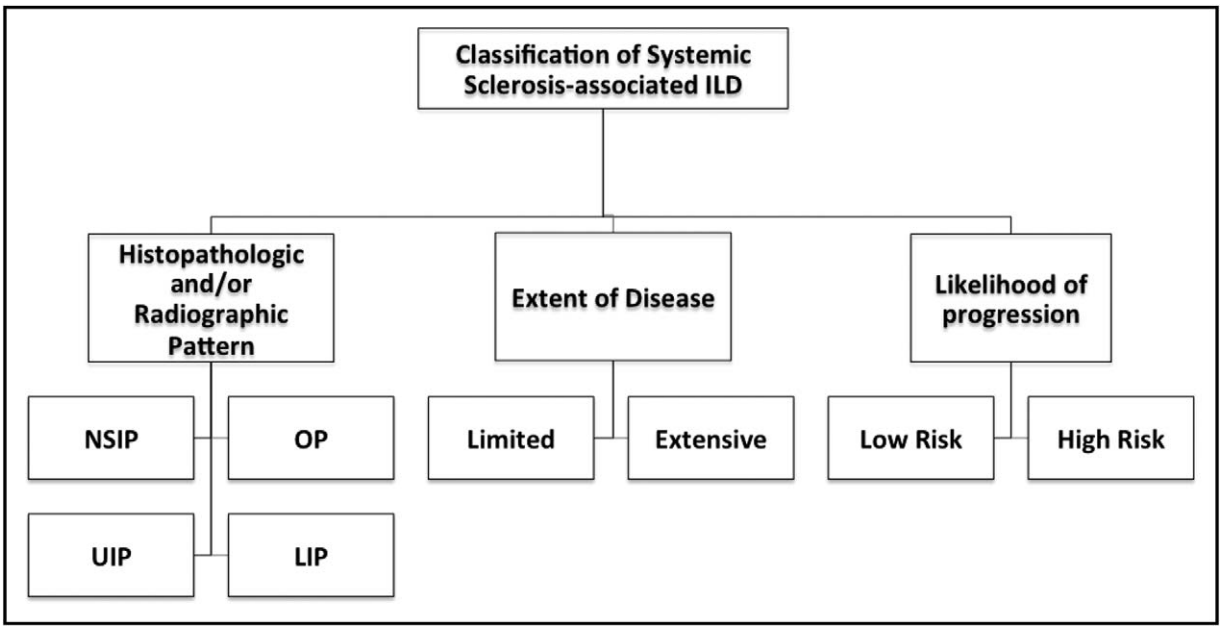


**FIGURE 1.** Classification of systemic sclerosis-associated ILD. Our approach to the classification of SSc-ILD based on review of available data. SSc-ILD can be classified by histopathology, radiographic pattern of disease, radiographic extent of disease, and likelihood of progression. Factors that help determine likelihood of progression include FVC trajectory, disease subtype, autoantibody status, and demographics. FVC, forced vital capacity; LIP, lymphoid interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; UIP, usual interstitial pneumonia.

Histopathologically, SSc-ILD is characterized by early pulmonary infiltration of inflammatory cells into the lung parenchyma with resultant fibrosis and can be classified into specific patterns of disease including nonspecific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), organizing pneumonia, and lymphoid interstitial pneumonia [14,15]. The most common radiographic pattern on HRCT scan of the chest is NSIP, present in approximately 65% of cases and characterized by ground glass opacities in a primarily peripheral distribution with subpleural and basilar predominance. This contrasts with the UIP pattern, present in approximately 25% of cases, characterized by disrupted lung architecture, dense areas of patchy fibrosis, and honeycombing in a primarily subpleural distribution [14]. Lung biopsy is usually not required to confirm the diagnosis of SSc-ILD unless other diagnoses such as malignancy or infection are suspected, as patterns can be determined through HRCT alone with a high degree of reliability [15].

Although these radiographic and histopathologic classifications are useful, and there is a trend for shorter survival in patients with a UIP pattern compared to those with an NSIP pattern [14], the prognosis of patients with SSc-ILD is quite variable and is more closely linked to both disease extent at baseline and progressive functional decline [14,16,17]. Given this variability, it is important not only to characterize SSc-ILD by histopathologic or radiographic pattern, but also to quantify disease extent and classify patients according to their individual risk of progression. In an era of increasingly personalized medicine, further advances in composite clinical screening algorithms and better characterization of predictive markers for progressive disease promise improvements in our overall management of SSc-ILD.

In 2008, Goh *et al.* [18] developed a classification system to stage the extent of ILD in SSc. Using a combination of HRCT and PFTs, they classified patients into limited and extensive disease categories. Extensive disease was defined as more than 20% lung involvement on HRCT, or 10–30% ILD involvement on HRCT and FVC less than 70% predicted. Limited disease was defined as or less 10% ILD involvement on HRCT, or 10–30% lung involvement on HRCT and an FVC at least 70% predicted [18]. This staging system has been validated as a predictor of mortality, and SSc-ILD patients with extensive disease have an approximately three-fold increased risk of clinical decline (defined as need for supplemental oxygen or lung transplantation) and death compared to those with limited disease [18–20].

Patients with SSc-ILD can also be stratified by their likelihood of progression. There is marked variability in the clinical course of disease: some patients have a slowly progressive decline in, or even stability of, FVC, whereas others experience a rapidly progressive course, leading to lung transplantation or death, despite treatment [21]. Group-based trajectory modeling based on retrospective review of longitudinal FVC values from 254 patients with SSc has identified seven distinct FVC trajectories: very low baseline FVC with slow decline (5.5% of patients), very low baseline FVC with improvement (13.8% of patients), low baseline FVC with fast decline (9.5% of patients), low baseline FVC that remained stable (19.7% of patients), low-normal baseline FVC with improvement (31.1% of patients), normal baseline FVC with improvement (16.1% of patients), and normal baseline FVC that remained stable (4.3% of patients) [22]. Similarly, the findings of Goh *et al.* [18] have since been extrapolated to show that dynamic changes in imaging studies and PFTs hold prognostic value and can be utilized to help predict risk of progressive disease [21,23,24]. One-year declines in FVC and diffusion capacity for carbon monoxide (DLCO), for example, have been shown to predict survival in patients with extensive disease, with a decrease in FVC by more than 10% and/or a decrease in DLCO by more than 15% during 1 year associating with poorer prognosis [21,24]. These parameters have since been incorporated into the outcome measures in rheumatology (OMERACT) definition of progression of connective tissue disease-associated ILD ([25]. More recently, Volkmann *et al.* [26▪▪] developed a mortality prediction model through *post hoc* Cox regression analyses of patients from SLS I and II. They showed that significant declines in FVC ($\geq$10%) and DLCO ($\geq$15%) over 24 months were the most robust predictors of long-term survival, even when adjusting for treatment arm and baseline disease severity [26▪▪]. Additional risk factors for worse prognosis of SSc-ILD included elevated baseline plasma C-reactive protein (CRP) levels, gastroesophageal reflex disease, pulmonary arterial hypertension, older age, African American race, and male sex [26▪▪,27,28].

Akin to approaches reported in the idiopathic pulmonary fibrosis (IPF) literature [29–31], several prediction models have been developed to risk stratify patients with SSc-ILD using clinical variables available at the time of a patient's initial office visit [32▪,33▪]. The $SpO_2$ and Arthritis (SPAR) model is one such tool, designed to predict ILD progression, that was developed using two independent prospective cohorts of patients who met 2013 ACR/EULAR Classification Criteria for SSc and had mild ILD

(<20% lung involvement) assessed by HRCT at baseline [32▪]. ILD progression was defined as a relative decrease in FVC by at least 15% or a decline in FVC by at least 10% combined with a decrease in DLCO by at least 15% at 1-year follow-up. In their multivariate analyses, declines in $SpO_2$ after 6-min walk test and arthritis (defined as one or more tender and swollen joints as judged by a treating physician) were identified as independent predictors of ILD progression in both cohorts, with an optimal $SpO_2$ cut-off value of 94% by ROC analysis [32▪]. The smoking history, age, and DLCO (SADL) model is another validated risk prediction model for all-cause mortality in SSc-ILD developed using two independent prospective cohorts of patients meeting 2013 ACR/EULAR Criteria for ILD. The SADL model uses a patient's smoking history, age, and DLCO to classify him or her into a low-risk, moderate-risk, or high mortality risk group at 3 years from ILD diagnosis [33▪]. Although further validation is required, such models are easily used and can potentially guide clinicians in their management decisions.

Although classification of SSc-ILD by radiographic pattern continues to hold important treatment and survival implications, we believe a personalized medicine approach that accounts for an individual's radiographic pattern and extent, FVC trajectory, autoantibody status, disease subtype, and demographic variables, is critical to determining the likelihood of progression and informing treatment decisions.

## SCREENING AND DIAGNOSIS OF SYSTEMIC SCLEROSIS

Diagnosing SSc-ILD at an early stage can be challenging, as it may develop insidiously and patients may have asymptomatic, 'subclinical' ILD. The most common early clinical manifestations of SSc-ILD include exertional dyspnea and nonproductive cough, both of which are nonspecific [34]. Given that patients may be asymptomatic or have nonspecific symptoms, and that ILD is both highly prevalent and the leading cause of death in SSc, universal screening of patients with SSc for ILD is critical. According to reports summarizing the proceedings from the ACR and Association of Physicians of Great Britain and Ireland Connective Tissue Disease (CTD)-ILD Summit, the development of a screening system with the dual objective of identifying early-stage disease and identifying those at greatest risk for progression and functional decline is a major unmet need within the field [35]. The two most widely applied methodologies for ILD screening are PFTs and HRCT.

PFTs are a valuable noninvasive method to assess severity of ILD and monitor disease course. Although PFTs are widely utilized to screen patients with SSc for ILD, they lack sensitivity and have a high false-negative rate for the detection of ILD [36]. In a single-center prospective cohort study, Suliman *et al.* [36] showed that among 64 patients with ILD on HRCT, approximately 62.5% had a normal FVC, defined as at least 80% predicted. The sensitivity of an FVC less than 80% predicted for detection of SSc-ILD was only 38%; this increased only to 72% when the following parameters were combined: FVC less than 80% predicted or ΔFVC at least 10% or total lung capacity less than 80% predicted or DLCO less than 70% predicted and forced expiratory volume in 1s/FVC greater than 0.7. Showalter *et al.* [37] performed a similar study of 265 patients meeting 2013 ACR/EULAR Classification Criteria for SSc to identify the sensitivity, specificity, and negative predictive value of PFTs for the presence of SSc-ILD on HRCT, and to determine optimal FVC and DLCO thresholds for the presence of SSc-ILD on HRCT. An FVC less than 80% predicted (sensitivity 69%, specificity 73%) and DLCO less than 62% predicted (sensitivity 60%, specificity 70%) were identified as the optimal thresholds to define ILD; however, all FVC and DLCO threshold combinations had a negative predictive value of less than 0.7. Collectively, these data suggest a high risk of missing SSc-ILD if relying solely on PFTs. Moreover, ILD is a radiographic (and/or histopathologic) diagnosis, thus imaging studies are required for diagnosis.

HRCT of the chest is the gold standard for detection of ILD and enables assessment of the radiographic pattern and extent of disease [35,38]. Launay *et al.* [39] showed the utility of baseline HRCT, as 34 of 40 (85%) patients with SSc with normal HRCT at baseline still had normal HRCT at a mean follow-up of 5 years. Thus, these baseline HRCTs had both diagnostic and prognostic value. Routine use of screening HRCT in SSc may identify asymptomatic individuals with subclinical ILD at high risk for developing clinically significant ILD, just as the presence of subclinical ILD on baseline cardiac computed tomography (CT) scans predicts development of clinically significant ILD in community-dwelling adults [40]. High attenuation areas (HAA), defined as the percentage of imaged lung with CT attenuation values between −600 and −250 Hounsfield units, are a novel CT-based quantitative biomarker of subclinical ILD that have strong construct validity as a biomarker of subclinical lung inflammation and extracellular matrix remodeling, processes that precede ILD, in community-dwelling adults [40,41]. In community-dwelling

adults enrolled in the Multi-Ethnic Study of Atherosclerosis, greater HAA is associated with reduced FVC, reduced exercise capacity, elevated serum levels of matrix metalloproteinase-7 (MMP-7) and interleukin-6 (IL-6), the development of interstitial lung abnormalities (ILA, a qualitative visually-identified subclinical ILD phenotype on CT) on CT at 10-year follow-up, and an increased risk of developing clinically evident ILD and ILD-specific mortality at 12-year follow-up [41]. Thus, research is needed into whether quantification of HAA on screening HRCTs in patients with SSc can identify individuals at high risk of developing clinically evident ILD and ILD-specific mortality. Ultimately, identification of subclinical ILD on screening HRCTs in patients with SSc may permit a 'window of opportunity' for intervention.

However, there remains substantial practice variation in rheumatologists' use of HRCT to screen for ILD in their patients with SSc. In a survey of 676 ACR member rheumatologists in New York, New Jersey, Pennsylvania, and Connecticut, and 356 SSc experts worldwide, only 51% of the general rheumatologist respondents and 66% of the SSc expert respondents reported routinely performing screening HRCTs in their patients with SSc [42▪▪]. Moreover, there was significant global practice variation among SSc experts in their HRCT ordering practices: screening HRCT was ordered by 100% (7 of 7) of SSc experts in Latin America, 80% (4 of 5) in Asia, 79% (45 of 57) in Europe, 60% (28 of 47) in the United States, 33% (2 of 6) in Canada, and 0% (0 of 5) in Australia [42▪▪]. Further, there was little agreement regarding indications for HRCT among rheumatologists who do not routinely order screening HRCTs in their patients with SSc. In our practice, we routinely order HRCTs to screen for ILD in all newly diagnosed patients with SSc.

Given that PFTs lack sensitivity for detection of SSc-ILD, that HRCT is the gold standard for diagnosis of ILD, and that there is significant variation in both general rheumatologists' and SSc expert rheumatologists' use of HRCTs to screen for ILD in SSc, there is an urgent need to develop screening guidelines for the detection of ILD in SSc.

## NOVEL METHODS FOR SCREENING AND CLASSIFICATION

### Novel imaging techniques

Given the lack of any clear screening guidelines and gaps in our ability to prognosticate patients adequately, research into novel imaging is an intense area of focus. Over the past 15 years, lung ultrasound (LUS) has emerged as an attractive non-invasive, radiation-free technique with high sensitivity and specificity for the diagnosis of ILD [43,22,45]. Assessment for pleural irregularities and increased number of B-lines, discrete vertical hyperechoic reverberations arising from pleural lines, serves as the basis for LUS assessment for ILD. An increased number of B-lines is associated with thickening of the lung parenchyma and is suggestive of ILD [43]. Previous studies identified a greater number of B-lines in patients with ILD than in those without ILD on HRCT, with a concordance rate of 83% [46,47]. A recent meta-analysis of 11 studies analyzing LUS for diagnosis of CTD-ILD yielded a pooled sensitivity and specificity of 85.9 and 83.9%, respectively [44]. Current limitations of LUS for ILD screening in SSc include lack of standardization (e.g. number of lung zones or intercostal spaces to examine, and which probe to use), operator skill dependence, possible confounding because of skin fibrosis, and the total length of time required for the procedure [43].

Several promising exploratory methods of ILD detection are being investigated. Lung ultrasound surface wave elastography (LUSWE) is a new ultrasound application that measures the elasticity of superficial lung tissue. Zhang *et al.* [48] found significant increases in the speed of ultrasound wave propagation through the more fibrotic lung surfaces of patients with SSc-ILD compared to healthy controls, which could be useful for screening patients with SSc for ILD, although it remains unclear how this technology fares in detecting changes of ILD other than fibrosis (e.g. ground glass opacifications) [48]. Research into additional novel approaches such as magnetic resonance imaging (MRI) and molecular imaging are ongoing and show the ability to detect SSc-ILD with high accuracy without the use of ionizing radiation [49–51]. In 2018, for example, Gargani *et al.* [50] evaluated the utility of lung MRI in 32 patients with SSc who underwent concurrent cardiac MRI with dedicated lung scanning (with T1 and STIR imaging) and chest HRCT. Mean T1/STIR times and HRCT semiquantitative scoring were calculated for each patient. The authors showed that mean STIR was moderately correlated with HRCT scores ($r = 0.52$; $P < 0.01$). Similarly, in a proof of concept study, Schniering *et al.* [51] successfully targeted integrin αvβ3 (alpha-v-beta-3) and somatostatin receptor 2 (SSTR2), two proteins upregulated in ILD lungs, to illustrate the role of nuclear imaging to visualize ILD in animal models and patients with known ILD. Their study shows the potential for screening to occur on a molecular level through the precise targeting of proteins associated with fibrotic lung parenchyma.

### Circulating biomarkers

In recent years, much research has gone into investigating the clinical utility of biomarkers for the diagnosis of ILD and assessment of disease severity and progression [52–53]. Although the precise utility of biomarkers remains investigational and no single serologic marker has been fully validated, numerous potential biomarkers identified in the IPF literature have been subsequently investigated in SSc-ILD [e.g. MMP-7, surfactant protein-D (SP-D), Krebs von den Lungen-6 (KL-6), and C-C motif chemokine ligand 18 (CCL18)] [53–56]. In addition, novel potential biomarkers continue to be explored, such as antibodies against chemokine receptors CXCR3 and CXCR4, two G-protein-coupled receptors implicated in the pathogenesis of pulmonary fibrosis via mediation of cell migration [57]. Significantly higher levels of CXCR3 and CXCR4 antibodies were found in patients with SSc-ILD compared to controls, and levels of these antibodies were shown to correlate inversely with FVC and DLCO in patients with SSc. Somewhat paradoxically, however, patients with SSc-ILD with more progressive disease tended to have lower CXCR3 and CXCR4 antibody titers compared to those with more stable disease [57].

Although many of these biomarkers hold promise, there have been conflicting results regarding their sensitivity, specificity, and predictive power [56,58▪▪]. In a combined cohort study of 427 Norwegian and French patients meeting 2013 ACR/EULAR Classification Criteria for SSc, four promising ILD biomarkers [SP-D, KL-6, CCL18, and soluble OX40 ligand (OX40L)] were analyzed for their ability to diagnose and predict progression of SSc-ILD [58▪▪]. Serum levels of KL-6 were significantly inversely correlated with FVC ($r = -0.317$; $P < 0.001$) and DLCO ($r = -0.335$; $P < 0.001$) and positively correlated with the extent of fibrosis on HRCT ($r = 0.551$; $P < 0.001$). Similarly, KL-6 [odds ratio (OR) = 2.41; 95% CI, 1.43–4.07] and SP-D (OR = 3.15; 95% CI, 1.81–5.48; $P < 0.001$) were significantly associated with the presence of lung fibrosis in multivariable analysis adjusting for age and SSc disease duration, defined as the time between first non-Raynaud's symptom and blood sample collection [58▪▪]. These findings corroborate previous studies of KL-6 in SSc-ILD, but are inconsistent with previous smaller analyses of SP-D [59–62]. In a longitudinal analysis, CCL18 was an independent predictor of more than 10% decrement in FVC over a mean follow-up period of 3.2 years (hazard ratio = 2.90; 95% CI, 1.25–6.73; $P = 0.014$) and the development of de-novo extensive disease per Goh criteria (hazard ratio = 3.71; 95% CI, 1.02–13.52; $P = 0.048$) [58▪▪]. These results support previous smaller studies that showed elevated CCL18 levels were predictive of significant declines in FVC [63–66]. In sum, these findings suggest potential roles for SP-D as a diagnostic biomarker of SSc-ILD, KL-6 as a biomarker of lung fibrosis severity, and CCL18 as a biomarker of progressive SSc-ILD [58▪▪]. Despite the potential for their use in the diagnosis, classification, and risk stratification of SSc-ILD, however, further validation and standardization is ultimately required before such biomarkers should be utilized in clinical practice.

## CONCLUSION

ILD is a common manifestation of SSc and the leading cause of mortality in this patient population. Although radiographic and histopathologic classifications help to define general disease patterns, stark variations in clinical course limits the utility of assessing SSc-ILD on the basis of pattern alone. Recent work suggests a paradigm shift has occurred, with patients classified primarily according to their probability of severe, progressive disease through identification of risk factors, measurement of disease extent on HRCT, longitudinal declines in FVC, and mortality prediction models. Although no clinical practice guidelines for ILD screening in SSc exist, we recommend screening with HRCT and PFT in all patients with SSc. Biomarkers, lung ultrasound, and novel imaging modalities serve as promising adjunctive or alternative means of screening and diagnosis. Further validation is required before they should be used in clinical practice.

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### Conflicts of interest

*There are no conflicts of interest.*

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

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# Gastrointestinal involvement in systemic sclerosis: an update

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**Purpose of review**
This review provides important updates in systemic sclerosis (SSc)-related gastrointestinal disease, specifically focusing on the most recent literature.

**Recent findings**
In the past year, several studies were published that present interesting insights into SSc and gastrointestinal disease. Studies focusing on newly identified risk factors, novel approaches to diagnosis and assessment of disease activity, survival and quality of life demonstrate progress in our understanding of this challenging area. Additional data on specific SSc gastrointestinal-related topics, such as the link between gastrointestinal and pulmonary disease, nutrition, and the microbiome, are also now available.

**Summary**
SSc gastrointestinal disease is heterogeneous in its clinical presentation, which presents a challenge in diagnosis and management. In the past year, several studies have evaluated risk factors and clinical features associated with specific gastrointestinal complications in SSc. Objective gastrointestinal testing may help to identify specific SSc gastrointestinal subgroups and provide diagnostic accuracy to guide targeted therapies. Survival in very early SSc is affected by the severity of gastrointestinal involvement. Other important gastrointestinal subsets, including patients with esophageal disease and interstitial lung disease, should carefully be considered when developing a management plan for this patient population.

**Keywords**
diagnosis, dysmotility, gastrointestinal, management, systemic sclerosis

## INTRODUCTION

Gastrointestinal involvement is the most common internal organ complication of systemic sclerosis (SSc), affecting over 90% of patients. Gastrointestinal clinical manifestations in SSc are variable, as regions from the esophagus to the anorectum may be affected. This clinical heterogeneity often leads to several key challenges for the treating physician. These include: identifying patients at high risk for progressive severe gastrointestinal disease; determining whether immune-mediated disease activity or existing damage is causing symptoms; determining whether early initiation of pro-motility agents or other gastrointestinal medications may prevent complications; and determining whether there is a role for immunosuppression in preventing gastrointestinal complications. Gastrointestinal complications in SSc also significantly impact healthcare costs and quality of life [1,2,3▪▪].

In the past year, several manuscripts were published that begin to address some of these areas of critical need in SSc gastrointestinal disease. Here the data and clinically relevant information are provided, which may help physicians diagnose and manage this complicated group of patients.

## METHODS

The following initial search terms (01/01/2018-04/01/2019) were used in PubMed: 'scleroderma gastrointestinal' (denominator = 20); 'SSc gastrointestinal' (denominator = 28), 'SSc gastrointestinal treatment' (denominator = 18), 'SSc GERD' (denominator = 6), 'SSc gastroparesis' (denominator = 0), 'SSc gastric antral vascular ectasia' (denominator = 1), 'SSc

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## KEY POINTS

- Gastrointestinal disease in SSc significantly increases healthcare costs and patient's quality of life.
- Several clinical and demographic features are associated with patients at high risk of developing severe gastrointestinal complications in SSc.
- Objective testing in SSc gastrointestinal disease is important in understanding the underlying problems in patients with more severe or refractory disease, and may ultimately be useful in distinguishing between disease activity and damage.
- Although dietary modification and probiotics may play a role in the management of SSc gastrointestinal disease, more data is needed to determine benefit and to identify an optimal approach.
- Further studies focused on the benefits of early interventions with promotility agents and the application of novel treatments in SSc gastrointestinal disease will be important.

SIBO' (denominator = 3), 'SSc bowel' (denominator = 29), 'SSc colon' (denominator = 2), 'fecal incontinence SSc' (denominator = 2) and 'SSc nutrition' (denominator = 12). The search was specific for human studies. There were 35 original research articles of interest identified based on their relevance to SSc gastrointestinal disease. Case reports were excluded from this review.

## RESULTS

### Clinical, serologic and genetic risk factors associated with gastrointestinal complications in systemic sclerosis

#### Clinical and demographic risk factors

Clinical features and other risk factors associated with severe gastrointestinal dysmotility in SSc were recently reported in a retrospective cohort study (Table 1) [4▪▪]. Patients with severe gastrointestinal disease requiring total parenteral nutrition, and patients with mild gastrointestinal disease were compared, and it was determined that severe gastrointestinal disease was significantly associated with male sex [odds ratio (OR) 2.47, 95% confidence interval (CI) 1.34–4.56], myopathy (OR 5.53, 95% CI 2.82–10.82), and sicca symptoms (OR 2.40, 95% CI 1.30–4.42), even after adjusting for potential confounders. Patients with higher skin scores were also found to have an increased risk of significant gastrointestinal dysmotility [5], and a disease duration of greater than 5 years was associated with a high risk for developing small intestinal bacterial overgrowth (SIBO), (OR 9.38; 95% CI 1.09–80.47) [6].

The incidence, predictors and outcomes associated with severe gastrointestinal disease in SSc were also explored by a group in Canada. Patients with early disease (disease duration <2 years) were included, and severe gastrointestinal disease was defined as a Medsger GI severity score of at least 3 (pseudo-obstruction with or without malabsorption) and/or at least 10% weight loss in association with the use of antibiotics for bacterial overgrowth or esophageal stricture [7▪]. The probability of developing severe gastrointestinal disease was estimated at 9.1% at 2 years and 16% at 4 years. Severe gastrointestinal disease was associated with myositis (OR 4.68, 95% CI 1.65–13.24), telangiectasia's (OR 2.45, 95% CI 1.19–5.04) and a higher modified Rodnan skin score (OR 1.03, 95% CI 1.01–1.07) in the adjusted model. Severe gastrointestinal disease was also associated with a more than two-fold increase in the risk of death (hazard ratio 2.27, 95% CI 1.27–4.09) and worse health-related quality of life [Short Form Health Survey physical ($\beta = -2.37$, $P = 0.02$) and mental ($\beta = -2.86$, $P = 0.01$) component summary scores].

The clinical and demographic factors associated with pseudo-obstruction in SSc was also reported in a longitudinal cohort study using the Johns Hopkins Scleroderma Center Database. Patients with a modified Medsger GI score of 3, who had evidence of pseudo-obstruction as confirmed by chart review, were compared with patients with no history of pseudo-obstruction (Medsger GI <3) [8▪]. Factors predictive of pseudo-obstruction were older age (hazard ratio 1.02; 95% CI 1.00–1.04), male sex (hazard ratio 1.75; 95% CI 1.42–2.43), diffuse cutaneous disease (hazard ratio 2.52; 95% CI 1.59–3.99), myopathy (hazard ratio 1.83, 95% CI 1.09–3.08) and opioid exposure (hazard ratio 2.38; 95% CI 1.50–3.78). Autoantibodies to RNA polymerase-3 were negatively associated with pseudo-obstruction (hazard ratio 0.34; 95% CI 0.17–0.66). Avoidance of opioids whenever possible in high-risk patients may reduce pseudo-obstruction events in SSc.

These studies all provide evidence that male sex, skeletal muscle involvement, and higher modified Rodnan skin scores and/or diffuse cutaneous disease were associated with more severe gastrointestinal disease. Studies aimed at understanding the association between the gastrointestinal motility (perhaps smooth muscle atrophy), SSc skeletal myopathy, and severe cutaneous disease may provide insights into disease mechanism.

**Table 1.** Summary of clinical and serologic features associated with significant gastrointestinal disease

| Gastrointestinal outcomes | Associated variables | Point estimates or other measures | PubMed number |
|---|---|---|---|
| Severe vs. mild gastrointestinal disease | Male sex | OR 2.47 (95% CI 1.34–4.56) | PMID: 29193842 |
| (Medsger GI = 4 vs. Medsger GI ≤1) | Myopathy | OR 5.53 (95% CI 2.82–10.82) | |
| | Sicca symptoms | OR 2.40 (95% CI 1.30–4.42) | |
| Severe vs. mild-to-moderate GI disease | Myositis | OR 4.68 (95% CI 1.65–13.24) | PMID: 30517716 |
| (Medsger GI ≥3 and/or ≥10% weight loss vs. Medsger GI <3) | Telangiectasia | OR 2.45 (95% CI 1.19–5.04) | |
| | Higher modified Rodnan skin scores | OR 1.03 (95% CI 1.01–1.07) | |
| | Death | HR 2.27 (95% CI 1.27–4.09) | |
| | Worse health-related QOL | $\beta = -2.37$ (SE 1.04) | |
| Pseudo-obstruction vs. none | Older age | HR 1.02; 95% CI 1.00–1.04 | PMID: in press |
| (Medsger GI = 3 vs. Medsger GI <3) | Male sex | HR 1.75; 95% CI 1.42–2.43 | |
| | Diffuse cutaneous disease | HR 2.52; 95% CI 1.59–3.99 | |
| | Myopathy | HR 1.83, 95% CI 1.09–3.08 | |
| | Opioid use | HR 2.38; 95% CI 1.50–3.78 | |
| Moderate to severe vs. mild GI disease | More symptoms of autonomic dysfunction | | |
| (Medsger GI ≥2 vs. Medsger GI <2) | Orthostatic intolerance | Median 10.0 vs. 0; $P = 0.006$ | PMID: 29907667 |
| | Secretomotor dysfunction | Median 6.4 vs. 4.3; $P = 0.03$ | |
| | Anti-RNPC3 antibody positive | OR 3.8 (95% CI 1.0–14.3) | PMID: 30242973 |
| | Anti-RNA polymerase 3 antibodies, subunit RPC155 vs. both RPC155 and RPA194 subunits | 51% vs. 26%; $P = 0.043$ | PMID: 30888702 |
| Higher UCLA GIT 2.0 scores | Carriers of the C-allele compared with the G-allele of the *IL-6* gene | 85% vs. 50%; $P < 0.05$ | PMID: 29948346 |
| | UCLA GIT 2.0 bloating and distention scores | $1.4 \pm 0.9$ vs. $0.78 \pm 0.8$; $P = 0.05$ | |

GIT, gastrointestinal tract; HR, hazard ratio; Medsger GI, Medsger GI severity score; OR, odds ratio; PMID, PubMed ID; QOL, quality of life; UCLA GIT 2.0, UCLA GI tract 2.0 patient reported outcome survey where higher scores mean more severe symptoms.

## Symptoms of autonomic dysfunction

As the autonomic nervous system plays an important role in controlling gastrointestinal motility, it is interesting that symptoms of autonomic dysfunction were found to associate with gastrointestinal severity in SSc [9]. SSc patients ($n = 104$) were recruited during routine clinical visits and asked to complete the COMPASS-31 questionnaire, a validated tool to assess symptoms of autonomic dysfunction. Patients with more severe gastrointestinal disease had significantly higher scores across several COMPASS-31 subdomains, including orthostatic intolerance and secretomotor dysfunction. There was also a dose–response relationship between gastrointestinal disease severity and autonomic symptom burden, suggesting that autonomic dysfunction may play an important role in SSc gastrointestinal dysmotility. Interestingly, another

study found that SSc gastrointestinal patients with autonomic symptoms had more emotional distress on the GIT 2.0 survey than patients with minimal autonomic symptoms. This suggests that autonomic dysfunction in SSc may not only affect gastrointestinal motility, but may also play a role emotional distress [10].

### Autoantibodies

The presence of specific autoantibodies are reported to identify high-risk SSc gastrointestinal patients [11▪]. A large SSc cohort study evaluated the association between anti-RNPC3 antibodies and SSc gastrointestinal disease. Anti-RNPC3 antibodies were associated with moderate-to-severe gastrointestinal disease, defined by Medsger gastrointestinal score at least 2 (OR 3.8, 95% CI 1.0–14.3), even after adjusting for relevant covariates. An association between severe gastrointestinal disease and antibodies targeting only the RPC155 unit of RNA polymerase-3 rather than both protein subunits, RPC155 and RPA194 (51 vs. 26%; $P = 0.043$), was also reported [12], suggesting that specific autoantigen subunits targeted in SSc can identify patients with a high risk for severe gastrointestinal disease.

### Genetic factors

Given the observation that polymorphisms in the *IL-6* gene are important in the susceptibility to SSc, the clinical manifestations associated with the 174C/G of the *IL-6* gene polymorphism were evaluated in SSc patients ($n = 102$) and healthy controls ($n = 93$) [13]. Carriers of the C-allele compared to the G-allele of the *IL-6* gene, showed higher UCLA GIT 2.0 total scores (0.85 vs. 0.5, $P < 0.05$) and higher bloating/distension scores ($1.4 \pm 0.9$ vs. $0.78 \pm 0.8$, $P = 0.05$), suggesting that the *IL-6* gene variant -174C/G is associated with greater risk for severe gastrointestinal symptoms. The study did not explore objective measures of gastrointestinal function in that no objective gastrointestinal imaging was reported.

## Updates in systemic sclerosis gastrointestinal disease: diagnostic testing

### Assessment of gastroesophageal reflux disease

The degree of esophageal exposure to gastric acid, despite proton pump inhibitor (PPI) therapy, was reported in patients with SSc [14▪]. Investigators performed a case-controlled retrospective analysis, including 38 SSc and 38 non-SSc patients who underwent esophageal pH testing matched for PPI formulation and dose, hiatal hernia size, age, and sex. SSc patients had significantly longer acid exposure times, longer median bolus clearance and lower nocturnal impedance values, suggesting that adjunctive therapies, such as a prokinetic drug is needed for more adequate GERD control.

As distal esophageal baseline impedance levels reflect the esophageal mucosal integrity in reflux disease, investigators also sought to determine whether baseline impedance levels identify esophageal involvement in SSc [15]. Approximately 100 patients with nonerosive reflux disease (NERD) or SSc, and 50 healthy controls were prospectively evaluated. Median baseline impedance values were lower in both SSc and NERD patients compared with healthy controls ($P < 0.01$). Measurement of baseline impedance may be used as an objective marker of SSc esophageal involvement.

### Assessment of esophageal motility

The clinical diagnosis contributing to absent esophageal contractility (defined by high-resolution esophageal manometry) was determined in a cohort of 207 patients [16]. Systemic autoimmune rheumatic diseases were identified in 81% ($n = 169$) of cases, including 64% ($n = 132$) with SSc. The remaining patients (20%; $n = 38$) had nonrheumatic diseases, demonstrating that the absence of esophageal contractility is not specific for SSc.

The association between esophageal dysmotility and abnormal gastric transit was assessed using esophageal high-resolution manometry (HRM) and liquid and solid gastric emptying scintigraphy (GES) data. Gastrointestinal patient data ($n = 482$), which included a subset of SSc patients ($n = 33$) were reviewed and analyzed. Patients with esophageal dysmotility were more likely to be older (OR 1.013), have abnormal gastric transit (OR 2.14), SSc (OR 6.29) and dysphagia (OR 2.63), whereas patients with abnormal gastric transit were more likely to have esophageal dysmotility (OR 2.11), autonomic dysfunction (OR 2.37) and other findings. The strong association between esophageal dysmotility and abnormal gastric transit provides evidence that a common pathogenic mechanism, such as autonomic dysfunction, may be present in both conditions [17▪].

## Evaluating gastrointestinal disease activity in systemic sclerosis with a novel tool

A novel 18F PET-MRI with T1 MOLLI mapping was utilized to screen 16 SSc patients and five healthy controls for inflammation and fibrosis in the bowel [18]. A significant increase in mean T1 values in the large ($P < 0.001$) and small bowel ($P = 0.02$) were identified between cases and controls, and the

percentage of nonfibrotic and noninflammed tissue was significantly lower in SSc patients than controls for the large bowel ($P = 0.03$). However, none of these findings correlated with the GIT total or subdomain scores. The clinical relevance of the abnormalities observed by this tool is therefore not yet defined.

## Treatment of gastrointestinal complications in systemic sclerosis

### Dietary modification in systemic sclerosis gastrointestinal disease

The evidence to support dietary modification in the management of SSc gastrointestinal symptoms was examined by a systematic literature review [19]. Though some improvements in patient-reported outcome assessment of gastrointestinal symptoms were identified after either the initiation of probiotic therapy or the initiation of a low-FODMAP diet, the overall level of evidence was weak and could not fully support dietary modification for treatment of gastrointestinal involvement in SSc.

Risk factors associated with malnutrition in SSc were examined in a French population [20]. SSc patients who had vitamin C, Se and/or thiamine levels checked during a 5-year period were included. Overt malnutrition was present in 14 (17%) patients, and deficiencies in Se (35%), vitamin C (31%) and/or thiamine (6%). Malnourished patients had significantly lower hemoglobin (10.6 vs. 12.9 g/dl, $P < .0001$) and vitamin C levels (3.6 vs. 10.6 mg/l, $P = 0.003$). Vitamin C deficiency also associated with esophagitis or Barrett's mucosa (OR 4.05, 95% CI 1.27–13.54; $P = 0.02$), modified Rodnan skin score 14 or less (OR 0.33, 95% CI 0.11–1; $P = 0.05$) and pulmonary artery hypertension (27 vs. 0%; $P = 0.0006$). This suggests that vitamin C testing may identify important complications in malnourished SSc patients. The potential determinants of malnutrition [2015 European Society of Clinical Nutrition and Metabolism (ESPEN)] was explored in a large SSc cohort [21], where outpatients ($n = 141$) were enrolled and body composition was analyzed by densitometry. Patients with malnutrition (9.2%) had a significantly lower forced vital capacity (FVC) and more overall severe disease.

### Probiotics in systemic sclerosis gastrointestinal disease

A randomized placebo-controlled trial examined the effects of probiotics in SSc. Patients with moderate-to-severe total scores on the UCLA GIT 2.0 ($n = 73$) were included and followed over 8 weeks [22]. The primary endpoint was improvement in the total GIT score. Although there was no difference in GIT scores between groups, the trial did not enrich for patients with distention and bloating, which is the indication for which probiotics have previously been found to be helpful in SSc [23]. As the total GIT score is a composite of a variety of gastrointestinal symptoms, this small heterogeneous population may have been underpowered to detect the benefit of probiotics between important gastrointestinal subgroups.

### Medications

Several studies in the past year evaluated the medical treatment of gastrointestinal complications in SSc. A systematic review and meta-analysis examined the treatment of SSc-related SIBO [24]. Treatment outcomes included symptomatic relief or SIBO eradication. Studies were generally of low quality and uncontrolled. The effectiveness of octreotide, individual antibiotics or antibiotic combinations were examined. They concluded that antibiotics may eradicate SIBO in some patients, but that there is a paucity of data reporting the effectiveness of either prokinetics (e.g. octreotide) or probiotics in SSc.

Another study evaluated the side effects, medication adherence, and dose ranges for pyridostigmine in SSc patients with refractory gastrointestinal symptoms. Pyridostigmine is not labelled for use in scleroderma gastrointestinal disease. Patients were defined as responders if they remained on pyridostigmine for at least 4 weeks with documented clinical benefit. Of 31 patients treated with pyridostigmine for at least 4 weeks, 51.6% reported symptomatic improvement. Constipation was the most commonly improved symptom based on prevalence prior to therapy, and diarrhea was the most common adverse event. Pyridostigmine may hold promise in SSc gastrointestinal disease, particularly, in patients with refractory constipation, though controlled studies need to be done [25].

### Minimally invasive surgery

The treatment of refractory GERD in patients with SSc with laparoscopic Roux-en-Y gastric bypasses (RYGB) was evaluated at Cleveland Clinic [26]. SSc patients undergoing fundoplication ($n = 7$) or RYGB ($n = 7$) for the treatment of GERD (2004–2016) were identified. Of the patients who had assessment of their GERD symptoms at follow-up, all five patients in the RYGB group and only three (50%) patients in the fundoplication group reported symptom improvement, and no mortality occurred during the 30-day follow-up. Although more studies are needed, laparoscopic RYGB may be a well-tolerated and effective alternative to fundoplication in a subset of SSc patients with severe esophageal dysmotility.

## Impact of gastrointestinal complications on healthcare costs and quality of life in systemic sclerosis

Rates of SSc-related adult hospitalizations in the United States and factors associated with in-hospital mortality, longer length of stay and higher hospital costs was studied using the National Inpatient Sample (2012–2013) [1]. From the gastrointestinal standpoint, acute bowel obstruction and aspiration (OR >2.0 with $P < 0.0001$ for both) both predicted higher cost of hospitalization. This emphasizes the importance of defining risk factors for this subgroup and initiating specific interventions to reduce risk in such patients.

The correlation between health-related quality of life and the oropharyngeal manifestations of SSc was assessed in a systematic review [27]. Oropharyngeal manifestations of SSc (i.e. maximal mouth opening, Mouth Handicap in SSc Scale) were significantly associated with an impaired quality of life. However, overall there was a low level of evidence among the included studies.

The impact of malnutrition on quality of life (QoL) was studied in 129 patients with SSc who were screened with the Malnutrition Universal Screening Tool [28]. All patients completed the Short Form 36 Questionnaire and the Scleroderma Health Assessment Questionnaire (SHAQ). The prevalence of malnutrition was 10.9%. All QoL scores (except bodily pain and self-reported health) were significantly impaired in malnourished patients, and the SHAQ, which assesses disease-specific QoL, was significantly higher in the malnourished patients. The authors proposed that standardized nutritional screening should be conducted in SSc to identify the risk of malnutrition and enable the initiation of multimodal treatment.

Functional disability and its predictors in SSc (EUSTAR) were assessed using patients from the prospective DeSScipher cohort with a completed SHAQ [29], and the effects of disability-related factors were analyzed. High SHAQ scores were associated with the presence of gastrointestinal symptoms (esophageal, gastric or intestinal) in the multivariable model suggesting that SSc patients perceive gastrointestinal complications as significant contributors to their level of disability.

The association between poor sleep and GERD was also evaluated in patients with SSc ($n = 287$) who completed the UCLA GIT 2.0, the Pittsburgh sleep quality index (PSQI), the fatigue severity scale (FSS) and the multidimensional gastrointestinal symptom severity index (GSSI) [30]. Poor sleep quality was identified in 194 (68%) patients and associated with significantly higher GIT Reflux scores ($P < 0.001$), and moderate/severe heartburn on GISSI ($P < 0.001$). The association between GERD symptoms and poor sleep remained in the multivariable model (OR 2.53, 95% CI 1.52–4.25; $P < 0.001$).

## Updates in the microbiome and *Helicobacter pylori* in systemic sclerosis

One systematic review and metanalysis characterized the association between gastric *Helicobacte pylori* infection and SSc [31]. Eight observational studies ($n = 1446$ subjects) were examined and in the pooled results there was an increased prevalence of *H. pylori* infection in SSc patients compared to healthy controls (OR 2.10, 95%CI 1.57–2.82). Prospective studies exploring the timing of SSc onset and *H. pylori* infections would be helpful in further understanding this interesting association.

Fecal microbiota and the plasma metabolome were characterized in SSc patients (n = 59) and healthy controls (HCs) (n = 28) [32▪]. A model of nine bacteria was capable of differentiating HCs from SSc patients. It was determined that SSc gut microbiota have fewer protective butyrate-producing bacteria and more pro-inflammatory noxious genera, especially Desulfovibrio compared to HC's. Interestingly, a multivariate model with 17 metabolite intermediates clearly distinguished cases from controls. This is consistent with prior studies suggesting that SSc intestinal microbiota are characterized by pro-inflammatory alterations that may promote intestinal damage and influence amino acid metabolism.

## The association between esophageal disease and interstitial lung disease in systemic sclerosis

Several cross-sectional international studies during the past year have evaluated the association between esophageal dysfunction and interstitial lung disease (ILD) in SSc. Even after adjusting for potential confounders, the studies from Greece, Japan and Italy, all confirmed an association between esophageal involvement and interstitial lung disease in SSc [33–35]. A longitudinal study examined whether a causal relationship between esophageal disease and ILD severity, ILD progression and mortality exists [36▪]. HRCT scans from 145 SSc-ILD patients were scored for fibrosis, esophageal diameter, and the presence of a hiatal hernia. Interestingly, for every 1 cm increase in esophageal diameter, a 1.8% higher fibrosis score and 5.5% lower forced vital capacity was identified ($P \leq 0.001$). Patients with a hiatal hernia had a higher fibrosis score ($P = 0.001$). Though esophageal diameter predicted worsening fibrosis score over the subsequent year ($P = 0.02$), this was not significant when adjusting for the baseline fibrosis

score ($P = 0.16$). The investigators ultimately determined that whereas esophageal diameter and hiatal hernia are independently associated with SSc-ILD severity and mortality, they are not associated with ILD progression. It was, therefore, concluded that it is unlikely that esophageal disease is a significant cause of SSc ILD progression.

### Scleroderma Clinical Trials Consortium Gastrointestinal Working Group

The Scleroderma Clinical Trials Consortium continues to support collaborative research in SSc gastrointestinal disease with the aim of to improving clinical trials and observational studies [37▪,38▪]. Ongoing international gastrointestinal-focused studies are now further exploring the role of the microbiome in SSc gastrointestinal complications using an international patient population.

## CONCLUSION

The risk stratification, diagnostic approach, outcomes, and management of SSc gastrointestinal disease remain active areas of research. Although there has been progress in the past year in each of these areas, there remains much work to be done. Developing further insights into disease mechanism, biomarkers of disease activity, and standardized, data-driven approaches to diagnosis and management remain high priorities in this field.

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### Conflicts of interest

*There are no conflicts of interest.*

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# Role of type I interferons and innate immunity in systemic sclerosis: unbalanced activities on distinct cell types?

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**Purpose of review**
The role of type I IFNs (IFN-I) in the promotion of autoimmunity has been well established. However, its role in the skin fibrosis of systemic sclerosis (SSc) is less clear. IFN-I can participate to tissue repair, and, here, we will consider the extent to which IFN-I's role in SSc skin fibrosis may reflect in part IFN-I functions during wound healing.

**Recent findings**
Studies are beginning to delineate whether IFN-I has a protective or pathogenic role and how IFN-I affects tissue biology. Recent support for a pathogenic role came from a study depleting plasmacytoid dendritic cells during bleomycin-induced skin fibrosis. The depletion reduced the bleomycin-induced IFN-I-stimulated transcripts and both prevented and reversed fibrosis. Additionally, two recent articles, one identifying SSc endothelial cell injury markers and one showing repressed IFN signaling in SSc keratinocytes, suggest the possibility of unbalanced IFN-I activities on distinct cells types.

**Summary**
Recent results support a pathogenic role for IFN-I in skin fibrosis, and recent studies along with others suggest a scenario whereby SSc skin damage results from too much IFN-I-activity driving vasculopathy in combination with too little IFN-I-mediated epidermal integrity and antifibrotic fibroblast phenotype.

**Keywords**
lupus, scleroderma, skin, type I interferon, wound healing

## INTRODUCTION

The contribution of innate immunity to the pathogenesis of systemic sclerosis (SSc) has been well established both using patients' samples and using multiple mouse models. However, the precise role of innate cells, and in particular macrophages and plasmacytoid dendritic cells (pDCs), in disease and how they regulate the subsequent adaptive response in SSc patients is still unclear. One of the mediators of innate immune activation, type I IFN (IFN-I), has been associated with the development of fibrosis but also with many other autoimmune diseases that are not fibrotic. We will focus on the role that IFN-I can play and will consider the extent to which IFN-I's role in SSc skin fibrosis may reflect in part IFN-I functions in tissue repair.

## IMPACT OF TYPE I INTERFERONS IN IMMUNITY AND DISEASES

The key role of IFN-I in immunity is to participate in the antiviral response and the pleiotropic activity of IFN-I has been well described. The IFN-I are comprised of 17 different proteins including 13 IFN-α subtypes, IFN-β, IFN-ω, IFN-κ, and IFN-ε. The need for such a large number of proteins, all of which share the same receptor, in immunity is unclear and could result from evolutionary pressure on the IFN pathway. It is also possible that these are differentially expressed by different cells, immune or not, and are associated with distinct functions of specific cell types [1].

The contribution by IFN-I to autoimmunity has first been described in systemic lupus erythematosus (SLE) but has since been reported in many other autoimmune diseases, including SSc [2], because of

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## KEY POINTS

- IFN-I can both promote wound healing by promoting epithelial barrier integrity and disrupt wound healing by inhibiting proliferation or survival of endothelial cells, and its activities may be dose-dependent and context-dependent.
- Recent results support a pathogenic role for IFN-I in skin fibrosis.
- Recent results also show endothelial injury that could be IFN-I-mediated and suppressed IFN-I-stimulated genes in SSc keratinocytes.
- We speculate that the skin damage of SSc skin fibrosis may reflect, in part, excessive IFN-I effects on endothelial combined with too little on keratinocytes and fibroblasts, resulting in vascular damage, disruption of the epidermal integrity, and profibrotic fibroblasts.

the presence in blood cells of highly expressed genes that are regulated by IFN-I and form the so-called IFN-signature. IFN-I can be produced by most cells in the body and multiple receptors sensing pathogens can induce its production. During an immune response, pathogens are recognized by multiple layers of sensors that will trigger an inflammatory response. However, in autoimmunity, it is likely that the inability to control the response to nucleic acids plays a central role [3], even though the identity of the sensors and the cell types involved is still debated. Recent studies are pointing to a key role for the nucleic acid sensing toll-like receptor (TLR)7, TLR8, and TLR9 in promoting autoimmunity with a particular role for TLR8 in controlling the development of fibrosis [4▪▪]. Although the activation of pDCs through TLR7 and TLR9 has been well documented in lupus [5–8], the expression of TLR8 by pDCs of SSc patients and the functional consequences on disease using a mouse model of skin fibrosis was unexpected and stresses not only the key role played by nucleic acid recognition in autoimmunity but also the induction of fibrosis.

One of the mediators of nucleic acid recognition and innate cell stimulation is IFN-I. The inflammatory response that follows tissue injury and that is required for tissue repair can, in some cases lead to fibrosis. In particular, macrophages and the nature of their activation can sway the balance between tissue repair and fibrosis [9]. In addition, IFN-I is associated with fibrosis in SSc patients [10,11], but also with many nonfibrotic autoimmune diseases, such as SLE [12–15]. How IFN-I may contribute to SSc skin fibrosis is unclear. Here we will briefly discuss the roles of IFN-I in SLE, wound healing, and SSc, and then highlight recent findings that suggest a role for pDCs and unbalanced IFNI-I activities on skin stromal cells in fibrosis.

## TYPE I INTERFERONS IN LUPUS

The first observation that IFN-I may contribute to the pathogenesis of SLE were described about 40 years ago [16,17]. Patients can now be characterized by measuring the levels of IFN-inducible genes, which are chronically elevated and can correlate with some of the clinical features of patients [12–15]. The cells responsible for the high presence of IFN-I are believed to be the pDCs, although it is likely that other cells may participate to the overall IFN response over time in patients [8,18]. The role played by pDCs and IFN-I is complex as the key findings rely on measuring interferon-stimulated genes (ISGs) in the blood, not tissues. Blood constitute a heterogeneous population of cells and the dependence of ISGs on certain IFN is still unclear and whether specific ISGs can be linked to specific symptoms is still undefined. Recent findings show that depleting or attenuating pDCs can prevent disease in lupus-prone mice [19,20] and can reduces the IFN-I response in the skin of SLE patients with encouraging signs of clinical efficacy in the skin [21▪▪]. These data suggest that IFN-I produced by pDCs may have a critical role in generating lesions in the skin whereas its contribution to the overall disease is less clear. IFN-I can also be produced by other cells as well such like epithelial cells [22], which adds complexity to our understanding of how IFN-I impacts the disease. Cutaneous diseases including lichen planus, dermatomyositis, lichen sclerosis, and cutaneous GVHD that share the common pathological feature named ‘interface dermatitis’ [23] all have IFN-signature in the skin but are not associated primarily with fibrosis. These findings may reflect the different tissue microenvironmental contexts of each disease.

Although SLE is not generally considered a fibrosing disease, Putterman and colleagues recently published that nonresponder lupus nephritis patients can have a high IFN-I signature and a fibrotic signature in kidney tubular epithelial cells [24▪]. The fibrotic signature was not associated with frank, histological fibrosis, suggesting that these signatures are pointing to an early event and is a harbinger of things to come. The results suggest the possibility of a functional interaction between IFN-I and fibrosis, especially at early stages, and support a potential role for type I IFN in driving fibrosis.

## TISSUE REPAIR

Type I IFN can promote wound healing in the skin [25,26]. Repair of epidermal tissues, such as skin and

gut involves epithelial closure to reform a protective barrier, and the protective role of IFN-I in wound healing in part reflects its contributions to epithelial barrier formation or maintenance. In a tape stripping injury model whereby tape is applied and then pulled off the skin a number of times, IFN-α, likely from pDCs [27], was upregulated within a day after injury, and mice deficient in the IFN alpha/beta receptor (IFNAR) were less able to induce a protective keratinocyte activation response [25]. Similarly, mice lacking TLR3 failed to close skin wounds [28], and, in the gut, IFNAR has a similar function in promoting barrier integrity of the intestinal epithelium [29]. Consistent with these observations, overexpression of IFN-I promoted, via myeloid cells, epithelial proliferation, and repair [26]. Together, the data point to a protective role for IFN-I in promoting epithelial integrity during wound healing.

How exactly IFN-I promotes wound healing and epithelial closure is less clear. With tape stripping, IFNAR-/- mice showed reduced IL-6, IL-17, and IL-22 responses, suggesting that a lack of inflammatory responses contribute to reduced wound healing [25,27]. However, the importance of the reduced inflammatory cytokines was not directly tested. IFN-I also had a protective role upon skin injury with subacute ultraviolet radiation (UVR) [30], as IFNAR-/- mice showed greater skin inflammation compared with controls. Although these studies were apparently at odds with each other with regard to the effect on skin inflammation, IFNAR clearly had a beneficial role in both models. Elkon and colleagues proposed that differences in levels, source, and inducers of IFN (higher, by pDCs, and dependent on TLR7 and TLR9 for tape stripping, whereas UVR produced lower levels of IFN in the absence of pDCs and the IFN was dependent on TLR3 and STING) could potentially account for differences in inflammation. Potentially, some of the differences could be because of direct dermal damage by UVR but not tape stripping, that leads to inflammation that is controlled by IFNAR, perhaps via effects on epidermal integrity. IFNAR can also act on myeloid cells to promote their ability to stimulate healing. IFNAR on myeloid cells has been shown to promote keratinocyte proliferation [26], and IFNAR will modulate pDC expression of cytokines, potentially contributing to the inflammatory cytokines that were lost in IFNAR-/- mice with tape stripping [25].

In addition to a role for IFNAR in epithelial barrier function, IFN-I can affect immunity at the skin level, which could have implications for wound healing. For example, IFN-I produced by keratinocytes activates dendritic cells in the skin [31]. This causes them to be better antigen-presenting cells and presumably to migrate toward the draining lymph node to activate the immune system. IFNAR can also activate accumulation of effector T cells in a tissue by activating macrophages [32] and can initiate tissue inflammation by promoting macrophage necroptosis [33]. Regulatory T cells, which have tissue repair properties [34], are also directly regulated by IFNAR to promote their development and function [35]. Cumulatively, the data suggest that IFNAR can promote wound healing by acting on local cells to repair tissue and also by stimulating immune cells to control infectious agents.

In some settings involving an excess of cytokine, however, IFN-I can also impede wound healing. For example, injection of IFN-α into wound edges delayed wound closure [36]. This was associated with an antiproliferative effect of IFN-α on the capillaries that normally grow into the wound, as well as on fibroblasts and keratinocytes. These results are in line with the sensitivity of endothelial cells to IFN-α *in vitro* [37], an ehrlichial infection model whereby infection-induced IFN-I signaling on nonhematopoietic cells is detrimental to the host [38] and a skin viral infection model that was associated with lymphatic dysfunction [39]. Whether excessive IFN combined with an unrepaired wound (versus excessive IFN in the absence of a wound) leads eventually to fibrosis would be interesting to investigate.

The idea that IFN-I effects are context-dependent is supported in considering the role of IFN-I in bacterial infection secondary to viral infections [40]. Here, the timing of type I IFN seems to be a critical parameter that determines its exact effect. IFN expressed at high levels prior to secondary infection can suppress the host response, including neutrophil recruitment, Th17 generation, and expression of antibacterial peptides, leaving the host less able to control the secondary infection. This is similar to the role of IFN-I in suppressing a protective Th1 response with *Mycobacterium tuberculosis* and *Mycobacterium leprae*. On the other hand, IFN at 14 days after the start of viral infection reduces the risk of secondary infections. The reasons for a protective role later after a viral infection is unclear, but it is interesting to speculate that IFN-I -mediated remodeling of the affected tissue or of the secondary lymphoid organs where immune responses are initially generated after the initial infection contributed to better protection.

In wound healing then, IFN-I can have different functions in different contexts and has effects in addition to effects on immunity. Overall, IFN-I is protective in promoting epidermal barrier integrity during would healing, but excessive IFN-I can disrupt vascular function.

## TYPE I INTERFERONS IN SYSTEMIC SCLEROSIS

In SSc, there is an association between IFN-I and disease. An IFN signature is found in the blood of about 50% of SSc patients, and it is apparent even early in disease [41–43]. Importantly, an IFN signature is also found in skin, again in association with early disease [44–46]. Polymorphisms of IFN regulatory genes *IRF-4,5,7,8* and *STAT4* have been found to be associated with SSc [41], further suggesting the possibility of a pathogenic role for IFN-I. As in SLE, there is also anecdotal evidence that IFN-I used for treating other diseases was associated with development of SSc [41]. Intriguingly, anti-IFNAR (Anifrolumab) in phase I trials led to suppression of IFN signature and TGFβ signaling in SSc skin [47] and further trials to assess clinical efficacy could help us understand the extent to which IFN-I contributes to pathogenesis.

A pathogenic role for IFN-I in skin fibrosis was further supported by two recent studies. Delaney *et al.* [49] treated a graft-versus-host disease (GVHD) model that is characterized by skin inflammation and fibrosis and used as an SSc model [48] with anti-IFNAR. The GVHD model showed upregulated IFN-I stimulated gene expression in the skin and anti-IFNAR reduced the IFN-I signature as well as fibrosis development, suggesting that IFN-I drove fibrosis development. In 2018, Ah Kioon *et al.* [4▪▪] examined the role of pDCs in scleroderma that can express very large quantities of IFN-I. They found that pDCs accumulate in SSc skin and express higher levels of IFN-α than cells from healthy controls spontaneously in culture. Although the pDCs also expressed additional cytokines, such as CXCL4, pDC depletion reversed the upregulated IFN-associated genes seen in the bleomycin-induced skin fibrosis model and improved fibrosis. Both these studies in different models [4▪▪,49], then, supported the idea that IFN-I overall promotes skin fibrosis, presumably via a local mechanism.

How IFN-I affects skin biology during fibrosis is critical for insight into the role of IFN-I, but understanding is currently limited. Interferon alpha expression in human scleroderma skin has been attributed to pDCs or other types of immune cells that are located near blood vessels and associated with capillary rarefaction [50]. This suggests a scenario whereby high levels of IFN-I and its antiangiogenic effects on endothelial cells may contribute to the pathogenesis of scleroderma skin fibrosis. The Delaney *et al.* [49] study treating the GVHD model with anti-IFNAR also showed that the reduced fibrosis development was associated with prevention of endothelial cell injury, supporting the idea that IFN-I -mediated vascular injury contributed to fibrosis. Single-cell RNA sequencing of SSc skin has not reported the finding of an IFN-I signature per se in endothelial cells [51▪▪], but endothelial cells were found to express higher levels of vWF [51▪▪], a marker of endothelial cell damage, and vWF has been shown to have downregulated anifrolomab in SLE patients [52]. Potentially, then, excessive IFN-I contributes to the endothelial cell damage in scleroderma skin.

In contrast, however, short-term primary cultures of keratinocytes from SSc skin have shown repressed IFN-I signaling when compared with transcriptome of healthy control keratinocytes [53▪▪]. This echoes the repressed IFN-I signaling found in cultured SSc skin and lung fibroblasts [46,54]. Whether the repression in keratinocytes and, potentially, fibroblasts reflects the in-vivo fibroblast state or is a result of culturing remains to be further sorted out. Interesting, recent work by Browning and colleagues showing some increased VCAM-1 expression by perivascular but not other fibroblasts in SSc skin [55], which could potentially reflect increased IFN-I signaling [49,56]. This study points to the heterogeneity of fibroblasts in all tissues that is becoming better understood [57,58▪–61▪,62] and demonstrates the potential for specific regulation of a subset of cells that may not be detected in bulk sequencing of a particular cell type or that may be lost upon culturing. In the study of scleroderma, the results from single-cell RNA sequencing analyses of fibroblasts and keratinocytes directly from SSc skin will likely be illuminating.

Despite the likely heterogeneity there is in any given cell type, however, the surprising results of Kahlenberg and colleagues raise the interesting possibility that the high levels of IFN-I in the skin in SSc may not signal to all cell types in a balanced way; some cell types, such as keratinocytes and fibroblasts, may be 'nonresponders' whereas others, such as endothelial cells, may be very sensitive to IFN-I [53▪▪]. Interestingly, Varga and colleagues have shown that IFN-β induces an antifibrotic phenotype in cultured fibroblasts, raising the possibility that reduced IFN-I signaling in SSc fibroblasts may be pathogenic [46,63]. Similarly, given the role of IFN-I in promoting epidermal barrier integrity, repressed IFN-I responses in keratinoctyes in SSc has the potential to disrupt barrier integrity, thus contributing to the wound healing phenotype of the epidermis [64] and skin damage in SSc. Potentially, the high levels of IFN-I in SSc is meant to be a protective response to a perceived 'wound', but the unbalanced response to IFN-I results in damage to the skin, thereby contributing to the fibrotic phenotype.

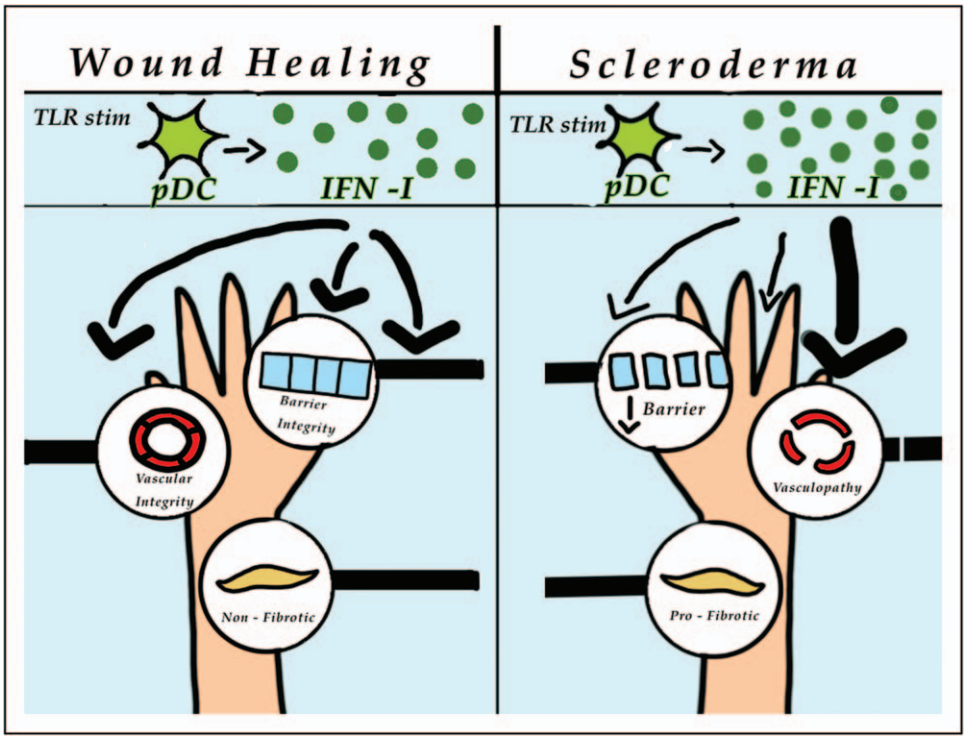


**FIGURE 1.** Model of type I interferon activities on skin stromal cells during wound healing and in scleroderma. Left panel: during wound healing, IFN-I from toll-like receptor (TLR)-stimulated pDCs acts on the epithelium to promote skin barrier integrity, the vasculature to promote normal function, and fibroblasts to maintain an antifibrotic phenotype. Right panel: in scleroderma skin fibrosis, the excess IFN-I has an imbalanced effect on different stromal compartments. The vasculature is sensitive to the IFN-I, leading to vasculopathy, whereas the epidermis and fibroblasts have repressed IFN-I signaling, leading to disrupted barrier function and a profibrotic phenotype, respectively. IFN-1, type I interferon.

## CONCLUSION

Although high IFN-I levels are associated with SSc and recent data support the idea that high IFN-I is overall pathogenic in SSc skin fibrosis, we propose here that understanding how IFN-I is pathogenic may require us to consider that an imbalance in the non-hematopoietic 'stromal' response to IFN-I may be a contributing factor. Potentially, the sensitive endothelial cell response to IFN-I is driving a vasculopathy whereas the repressed responses in keratinocytes and fibroblasts drives epidermal dysfunction and a profibrotic fibroblast phenotype (Fig. 1). In an attempt to repair the 'wound' that is not healing, IFN-I levels are driven up, further exacerbating the skin damage. It is also possible that the stromal responses reflect also indirect responses whereby IFN-I acts on myeloid and other immune cells to mediate the distinct endothelial, keratinocyte, and fibroblast responses. Here, more detailed understanding of the effects of IFN-I on the stromal elements in SSc patients or scleroderma models and of the direct and indirect IFN-I-responding cells will help us better understand how IFN-I should be therapeutically targeted in SSc skin fibrosis, and potentially in wound healing and other autoimmune and inflammatory conditions.

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### Conflicts of interest

*F.J.B. has been acting as a consultant for Astra Zeneca, Jansen, Biogen, and EMD Serono but otherwise has no other conflicts. T.T.L. has no conflicts of interest.*

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# T and B lymphocytes in fibrosis and systemic sclerosis

*Shiv Pillai*

**Purpose of review**
To summarize recent advances in the understanding of the pathogenesis of autoimmune fibrotic diseases. These diseases include IgG4-related disease, systemic sclerosis and lupus nephritis.

**Recent findings**
Recent studies indicate that a poorly studied subset of helper T cells, cytotoxic CD4+ T cells and subpopulations of disease-specific activated B cells infiltrate inflamed tissues and collaborate to induce tissue fibrosis in autoimmune fibrotic diseases. Cycles of apoptosis induced by antigen-specific cytotoxic CD4+ T cells followed by macrophage-mediated clearing of apoptotic cells and finally tissue remodeling driven by cytokines released by these auto-antigen-specific activated T and B cells may contribute to the activation of fibroblasts and myofibroblasts and the laying down of collagen. In scleroderma, this process likely involves the apoptosis of endothelial cells and other neighboring cells and the subsequent remodeling of the tissue.

**Summary**
Self-reactive cytotoxic CD4+ T cells infiltrate tissues where they may be nurtured by activated auto-reactive B cells, induce apoptosis, secrete cytokines and thus drive autoimmune fibrosis.

**Keywords**
cytotoxic CD4+ T cells, fibrosis, IgG4-related disease, systemic sclerosis

## INTRODUCTION

Autoimmune fibrotic diseases that are of broad interest to rheumatologists include IgG4-related disease (IgG4-RD), lupus nephritis and scleroderma or systemic sclerosis (SSc). SSc is a poorly understood fibrotic disease, that is, particularly refractory to treatment and whose clinical presentation varies considerably. The disease is characterized by a vasculopathy that involves small blood vessels and inflammation and fibrosis in the neighboring interstitial tissue. These features of vasculopathy, interstitial inflammation and fibrosis are observed in both limited and diffuse SSc, although there is considerable patient-to-patient heterogeneity both in terms of clinical presentation and disease progression [1,2]. In general, patients respond poorly to most immunosuppressive therapies but autologous hematopoietic stem cell transplantation results in a halt in disease progression and is the only current path to a cure [3▪▪]. Although our knowledge regarding the pathogenesis of this disease remains extremely limited, the response to hematopoietic stem cell transplantation represents the strongest evidence that the underlying disease mechanisms are likely immunological. With the advent of the clinical use of an antibody to CD117 as an alternative to whole body radiation, autologous hematopoietic stem cell transplantation may gain wider acceptance as a viable therapeutic approach in the near future [4▪].

The discovery of a range of distinctive autoantibodies in SSc patients has largely been the basis for viewing this disease as an autoimmune disorder, but the self-antigen or antigens that drive the pathologic immune processes in SSc remain to be identified. There is no evidence to suggest that the most clinically useful autoantibodies mainly directed against nuclear and cytosolic antigens (including the centromeric CENP proteins, topoisomerase I and RNA polymerase III among others), are of direct causal significance in the disease [5]. Antiendothelial cell antibodies that recognize cell surface proteins, such as the angiotensin

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## KEY POINTS

- In autoimmune fibrotic diseases, immune cell-mediated apoptosis is likely to be a key event.
- In these disorders, auto-antigen-specific CD4+ cytotoxic T cells clonally expand, secrete cytokines, such as IL-1β, infiltrate tissues and mediate apoptosis of specific target cells.
- In systemic sclerosis, the target cells for CD4+ cytotoxic T cells may be endothelial cells.
- Activated B cells and T cells likely collaborate in tissue sites to orchestrate postapoptotic tissue remodeling and fibrosis.

II type I receptor and the endothelin-1 type A receptor, might be responsible for the activation of endothelial cells and also for the apoptotic death of endothelial cells mediated by antibody-dependent cellular cytotoxicity [6,7]. It is interesting to note that at least some specific auto-antigens, in particular, CENP-A, CENP-B and the interferon-inducible 16 protein (IFI-16), the latter a known cytosolic DNA sensor, are expressed at particularly high levels in endothelial cells [8▪]. Whether or not peptides from these antigens are presented on human leukocyte antigen (HLA) molecules for T-cell recognition, and thus contribute to T-cell-mediated endothelial cell apoptosis remains to be established.

Lymphocytic infiltration of disease tissues has been observed in the earlier stages of disease. Once interstitial fibrosis sets in, infiltrates are difficult to find in the later stages of the disease. Understanding immune mechanisms that contribute to the pathogenesis of SSc requires a focus on the qualitative and quantitative analysis of the lymphocytic infiltrate in the earliest stages of the disease in untreated patients.

From an adaptive immune perspective, autoimmune fibrosis, whether one considers systemic sclerosis, IgG4-RD or lupus nephritis, probably involves yet to be identified triggers that lead to a break in T-cell tolerance. We argue that the induction of CD4+ cytotoxic T cells, that presumably recognize self-peptides presented by HLA class II molecules on specific host cells, results in the killing of these host cells as well as the release by these activated T cells of inflammatory pro-fibrotic cytokines. It is likely that CD8+ T cells against distinct HLA class I-restricted epitopes from the same or different proteins also contribute to apoptosis and fibrosis, though currently there is less known about the CD8+ T-cell lineage in autoimmunity and fibrosis. Apoptotic cell death is almost immediately followed by clearance of the dying cells by largely M2c-type macrophages. Apoptosis likely occurs contemporaneously with cytokine-driven activation of fibroblasts and myofibroblasts and the laying down of collagen that 'fills in' the once cellular regions of the inflamed and damaged organ with fibrous material. In some fibrotic disease states, activated B cells likely contribute to the process of fibrosis in a number of ways that may include the presentation of antigen to pathogenic T cells and the release of pro-fibrotic molecules. A likely role for B cells in the pathogenesis in systemic sclerosis is considered below.

## KOCH'S POSTULATES FOR HUMAN DISEASE AND THE NEED FOR QUANTITATION OF TISSUE IMMUNE INFILTRATES

One of the challenges in human immunology is the difficulty in obtaining scientific proof for postulated disease mechanisms. There is no real equivalent to Koch's postulates to validate disease mechanisms for any complex multigene human disorder. This limitation stems largely from the lack of truly representative animal models for most human diseases. However, some clinical trials that are targeted at specific molecules can help provide mechanistic clarity about the relevance of certain cell types or immune processes in disease. Remission of disease when one blocks a specific molecule or eliminates a specific circulating cell type represents one of the few ways in which disease mechanisms can be confirmed in human patients.

In inflammatory diseases, the adaptive immune response is typically initiated in a draining lymph node and the 'action' then shifts to the disease lesions, and this process may only be indirectly and incompletely captured in the circulation. Sampling of the blood of a patient with active disease can provide information about lymphocytes that have acquired the ability to recirculate but is unlikely to capture gross or even nuanced alterations in tissue-resident or even short-lived tissue-infiltrating effector lymphocytes. There are, therefore, obvious limitations to conclusions that have been drawn primarily from the study of circulating B and T cells in disease. Even when disease lesions have been interrogated, technical limitations have limited the usefulness of the information obtained. This is primarily because a very limited view of the immune complexity of disease lesions has typically been elucidated and furthermore, the information generated has generally been nonquantitative.

In systemic sclerosis, many assumptions regarding immune mechanisms of relevance to the pathogenesis of the diseases have been made based on the limited and nonquantitative examination of

human tissues often using a single color to analyze one protein in one type of immune cell at a time. Other assumptions have been made by extrapolating findings from rodent models of disease, which often have very limited similarity to specific human disorders. The development of novel techniques like single-cell RNA-sequencing, multicolor flow cytometry and mass cytometry have allowed the interrogation of many cell types in disease tissues [9].

The use of approaches that lead to the restoration of antigenicity in fixed tissue sections, and staining with a range of antibodies each with distinct fluorescent, nucleic-acid or metal-based labels in order to identify many different proteins simultaneously and/or sequentially in a tissue allows the identification of many different immune cells in a given biopsy [10,11]. Combining these approaches with automated slide-scanning devices to allow the accurate quantitation of larger swaths of tissue has begun to provide quantitative information on different T and B-cell subsets in human lesions. Another recently developed approach that shows great potential is Slide-seq, wherein transcriptomic information is obtained spatially across a tissue slide allowing single cell transcriptomic information to be resolved in spatial terms [12]. The use of cell distance mapping has also allowed the analysis of cell–cell interactions in disease tissues. Some of these approaches have been applied to the study of IgG4-RD and are currently in use in SSc as well [13▪▪,14,15]. It is, therefore, likely that a much clearer picture about T and B cells in SSc is certain to emerge in the coming year as all these approaches allow the field to delve even deeper.

## TYPE 2 IMMUNITY AND FIBROSIS

T cells were first described in SSc lesions a few decades ago [16,17] and evidence has also long existed for oligoclonal expansions of T cells in skin lesions in SSc [18]. It has been assumed that this disease is one of type 2 immunity. About two decades ago, at a time when limited knowledge existed about CD4+ T-cell subsets and quantitative approaches to tissue interrogation not even been conceived of, $T_{H2}$ cells were first identified in skin biopsies of systemic sclerosis [19]. Type 2 immunity has long been linked to fibrosis but this view has been based largely on data, which has accrued from only a small number of clinical examples that might not be relevant in the context of SSc. Type 2 immunity is of causal relevance in a subset or 'endotype' of patients with bronchial asthma, a disease in which fibrosis is a regular feature, and these patients tend to have eosinophilia and respond to corticosteroids. Type 2 immunity is undoubtedly relevant in two rodent models of lung fibrosis, secondary to injury induced by bleomycin or by silica. Type 2 immune responses may involve some attenuation of T regulatory cell function, an inductive role for TSLP and IL-33 made by epithelial cells, the activation of innate lymphoid type 2 (ILC2) cells and Th2 cells, the release by T cells of cytokines, such as IL-4, IL-5, IL-13 and IL-9 among others and the activation of M2 type macrophages, basophils, mast cells and eosinophils. TGF-β produced by many cells including eosinophils may then contribute to the activation of fibroblasts and myofibroblasts and the secretion of collagen. However, systematic quantitation of $T_{H2}$ cells in tissues of IgG4-RD patients has failed to reveal the accumulation of these cells [13▪▪,14] and no report that involves any form of quantitative and comprehensive analyses of T-cell subsets in tissue sites has revealed an accumulation of $T_{H2}$ cells in SSc.

## CD4+ CYTOTOXIC T CELLS MAY INDUCE APOPTOSIS AND SECRETE INFLAMMATORY CYTOKINES IN THE CONTEXT OF AUTOIMMUNE FIBROTIC DISORDERS

In IgG4-RD we have observed the clonal expansion of cytotoxic CD4+ T cells (CD4+ CTLs) in the blood and the accumulation of these CD4+ CTLs along with activated B cells and plasmablasts in disease lesions [13▪▪,14,15,20]. These CD4+ CTLs are presumably self-reactive and may be specific for peptides from overexpressed self-proteins. They may not only induce the apoptotic death of host cells but also secrete pro-fibrotic cytokines, in particular TGF-β and IL-1β. These cells are drawn into specific tissue sites and along with activated B cells, plasmablasts and macrophages presumably activate fibroblasts and myofibroblasts leading to the excessive release of collagen and the fibrotic remodeling of the tissue. In one of these studies [13▪▪], we showed an increase in circulating CD4+CTLs in both limited and diffuse SSc but quantitative multicolor tissue studies in SSc, as described in IgG4-RD, are yet to be reported.

In IgG4-RD, apart from the fact that that $T_{H2}$ cells are not prominent in inflamed tissues, the previous descriptions of CD163 expressing macrophages in this disease could reflect the presence of M2c macrophages that are known to clear apoptotic cells [21]. The use of quantitative studies in disease, therefore, reveals that in some autoimmune fibrotic diseases, fibrosis may be driven in part by apoptosis induced by CD4+CTLs and the cytokines they secrete, but other immune cells may also contribute to the disease process.

CD4+ CTLs are heterogenous CD4+ T cells that express granzymes and perforin. We described a novel marker on these cells called SLAMF7 and showed that these cells are cytolytic and can express, process and secrete IL-1β (without undergoing pyroptosis) and TGF-β1 [13▪▪]. Earlier assumptions that CD4+ CTLs cells may be senescent cells did not take into account the heterogeneity of these cells and the presence of subsets [22]. A particular subset of CD4+ CTLs that is clonally expanded and that we have linked to disease is metabolically active. Although our studies have linked CD4+ CTLs to disease, the CD4+CTL pool in most people does contain cells that are cytomegalovirus (CMV)-specific and the antigen specificity of CD4+ CTLs in the setting of autoimmune disease has not been established. There have been recent reports describing what are likely to be similar cells that have developed in different milieus, in rheumatoid arthritis and in systemic lupus erythematosus [23,24]. There is a growing interest today in CD4+ CTLs largely in the context of antiviral protection and from the standpoint of immune responses in cancer, but recognition of their contributions to inflammatory fibrotic diseases is a recent phenomenon, encouraging the autoimmune disease field to look beyond Th1 and Th17 CD4+ T-cell subsets.

When considering immune processes linked to fibrosis, these data have led us to consider two broad categories of fibrotic disorders driven by two different sets of mechanisms that converge on some common events. It is possible that there are many other similarities between these two processes or that there may be other subcategories of pathogenic mechanisms in fibrosis. In 'allergic fibrosis' as seen in bronchial asthma and schistosomiasis, the driving process is a type 2 immune response, and $T_{H2}$ cells and their cytokines especially IL-5 and IL-13 contribute to the activation in mast cells, basophils and eosinophils as described above, culminating with macrophage, myofibroblast and fibroblast activation and the deposition of collagen. In 'cytolytic fibrosis' in contrast, the key event seems to be the apoptotic elimination of host cells, followed by the inflammatory induction by activated T and B cells of an exaggerated repair process involving macrophages, myofibroblasts and fibroblasts.

From an SSc viewpoint, it is likely that immune mechanisms of pathogenesis can best be studied in the early stages of disease and the skin offers a site that permits relatively early examination of disease lesions. The ability to investigate early may not be available in some diseases, such as idiopathic pulmonary fibrosis. In this latter disorder, some patients have germline genetic variants in genes that are linked to alveolar stability and others in genes that regulate telomere length; both sets of variants may contribute to apoptosis. In many patients, it is possible that apoptosis might be immunologically mediated. However, in patients with idiopathic pulmonary fibrosis, immune infiltrates are not prominent in lesions and the pathogenesis of this disorder remain elusive.

Although autoimmune immune-mediated cytolysis might be linked to cytotoxic CD4+ CTLs, it is also possible that CD8+ T cells mediate autoimmune cytolysis in autoimmune fibrotic diseases. A relative abundance of CD4+ CTLs does not exclude potential pathogenic roles for other CD4+ T-cell subsets, such as $T_{H1}$, $T_{H2}$, $T_{H1}7$ and $T_{FH}$ cells, especially if they prove to be abundant at disease sites.

## TFH CELLS, TH 17 CELLS, CD8+ T CELLS AND SYSTEMIC SCLEROSIS

A number of reports have variously implicated $T_{FH}$ cells, $T_{H17}$ cells, regulatory T cells and CD8+ T cells in SSc [25▪,26–28]. None of these studies have employed quantitative analyses of T-cell subsets in affected tissues. On recent study suggested the IL-21 produced by $T_{FH}$ cells may contribute to fibrosis. In a parallel study, examining fibrosis linked to a graft-versus-host disease model, evidence was provided for a role in fibrosis of IL-21 secreting ICOS+ $T_{FH}$-like cells [25▪]. Apart from a paucity of quantitative information regarding $T_{FH}$ cells in lesions, in general, while B cells are seen in SSc tissues, germinal centers are not prominent, raising the possibility that the cells that have been described as $T_{FH}$ cells may be some version of pre-GC type $T_{FH}$ cells (that are typically extra-follicular) or possibly differently polarized CD4+ T cells that make IL-21. It would be interesting to know whether these cells express SLAMF7 and Granzymes. In IgG4-RD, while germinal centers are prominent, many $T_{FH}$ cells are found outside germinal centers, and these likely contribute to the IgG4 class switch [15]. More granular data on T cells in disease lesions in SSc is sorely required.

For both CD4+ and CD8+ T cells that have broken tolerance, self-antigens induce T-cell activation initially in a draining lymph node followed by re-activation and possibly additional polarization events in the lesion itself. The milieu in the lymph node as well as in the lesional tissue may contribute to more than one CD4+ T-cell subset being induced. However, cytokines that are most dominant during this inductive process may contribute to one population dominating over others. In IgG4-RD, the CD4+ T-cell subset that dominates is the CD4+ CTL subset. This may well be the case in SSc as well. It is likely that there may be some parallel activation of CD8+ T cells as well and that these cells may also potentially contribute to apoptosis and fibrosis.

## HOW STRONG IS THE CASE FOR B CELLS IN SYSTEMIC SCLEROSIS?

In many autoimmune diseases, even those generally believed to be driven primarily by T cells, B-cell depletion using Rituxan has often led to remission and an attenuation of infiltrating tissue T-cell numbers [29]. Although large double-blind studies on Rituxan in SSc have not been described, there are some studies that have reported clinical improvement with Rituxan especially when early diffuse SSc patients have been treated with this B-cell depleting agent [30▪,31▪]. Activated B cells and plasmablasts are a feature of a number of inflammatory diseases and these cells may present antigens to CD4+ T cells, secrete cytokines and possibly also contribute to fibrosis by the secretion of profibrotic molecules. In SSc, activated B cells have been observed in the circulation [32,33], and there is restoration of B-cell homeostasis after autologous hematopoietic stem cell transplantation [34]. By drawing parallels with IgG4-RD and based on a growing recognition that Rituxan may be efficacious in SSc if patients are treated early enough, we predict a synergistic role between activated B cells and CD4+ CTLs in the pathogenesis of systemic sclerosis.

## CONCLUSION

In most autoimmune disorders, we assume that an environmental trigger activates self-reactive immunological effector mechanisms in genetically

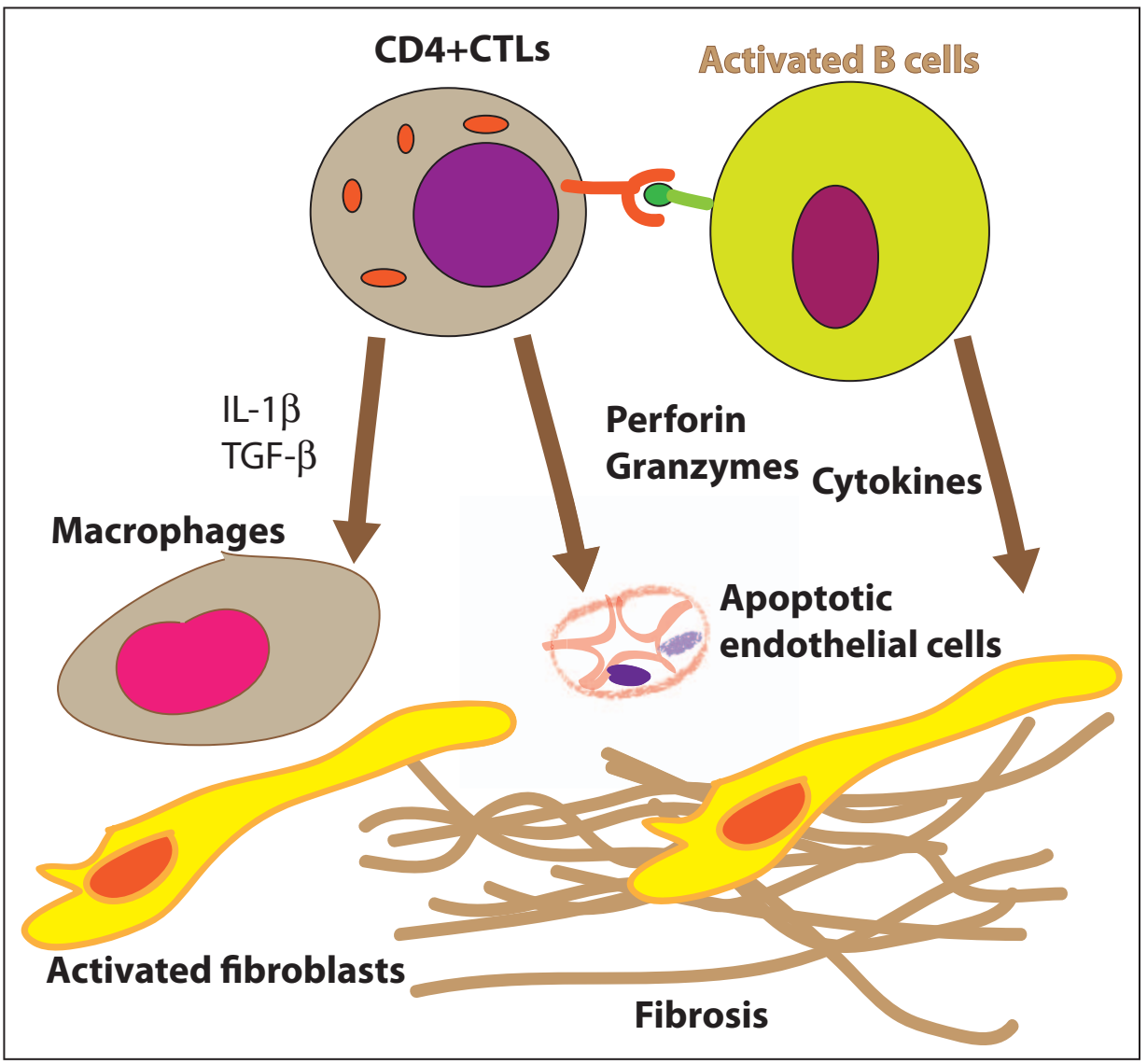


**FIGURE 1.** A model for autoimmune fibrosis focusing on systemic sclerosis. Tissue infiltration by CD4+CTLs and activated B cells allows the latter to likely capture and present self-antigens on HLA class II molecules to the cytotoxic CD4+ T cells. The latter induce apoptosis of target cells (shown here as endothelial cell apoptosis) and the subsequent re-modeling leads to fibrosis. CD4+ CTLs, cytotoxic CD4+ T cells. HLA, Human Leukocyte Antigen.

susceptible individuals. Although the underlying triggers for autoimmune fibrotic disorders, such as IgG4-RD and SSc remain unknown, the effector mechanisms likely involve collaboration between clonally expanded activated B cells and CD4+ CTLs that infiltrate affected tissues. These B cells likely reactivate the CD4+ CTLs at disease sites by presenting antigenic self-peptides to them. Following their re-activation, the T cells then may induce the apoptosis of host targets and also secrete profibrotic cytokines that lead to the activation of fibroblasts and myofibroblasts that lay down the fibrotic collagen matrix that eventually compromises the function of the affected organ (Fig. 1).

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## Conflicts of interest

*S.P. is on the Scientific Advisory Board for Abpro Inc.*

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# Macrophages and cadherins in fibrosis and systemic sclerosis

*Sarah To and Sandeep K. Agarwal*

**Purpose of review**
Macrophages are key players in systemic sclerosis (SSc) and fibrosis. The mechanism by which macrophages regulate fibrogenesis is unclear and understanding the origin and function of macrophages is critical to developing effective therapeutics. Novel targets on macrophages are under investigation and recently, cadherins have emerged as a potential therapeutic target on macrophages. The current review will discuss the importance of macrophages in SSc and fibrosis and summarize recent studies on the role of cadherin-11 (Cdh11) on macrophages and fibrosis.

**Recent findings**
Genome-wide expression studies demonstrate the importance of macrophages in SSc and fibrosis. Although M2 macrophages are associated with fibrosis, the presence of a mixed M1/M2 phenotype in fibrosis has recently been reported. Several studies aiming to identify macrophage subsets involved in fibrogenesis suggest that monocyte-derived alveolar macrophages are key players in the development of murine lung fibrosis. Recent functional studies show that Cdh11 regulates macrophages, fibroblast invasion, and adhesion of macrophages to myofibroblasts.

**Summary**
Macrophages play an important role in SSc and fibrosis. New insights into the mechanisms by which macrophages regulate fibrogenesis have been discovered on the basis of Cdh11 studies and suggest that targeting Cdh11 may be an effective target to treat fibrosis.

**Keywords**
cadherins, fibrosis, macrophages, systemic sclerosis

## INTRODUCTION

Systemic sclerosis (SSc) is the prototypical systemic fibrotic disease. Although the pathogenesis of SSc is incompletely understood, inflammation is an initial process that leads to extracellular matrix (ECM) deposition and fibrosis. Innate immune cells, such as macrophages, are important players in fibrogenesis. The importance of macrophages was described in histologic studies and now microarray studies extend these findings. The current review will discuss the importance of macrophages in SSc and fibrosis.

## MACROPHAGES AND SYSTEMIC SCLEROSIS

The inflammatory infiltrate in SSc skin is characterized by a mononuclear cell infiltrate, with a predominance of macrophages [1,2]. Microarray studies have subsequently highlighted their importance in SSc. Microarray analyses of SSc skin biopsies identified four intrinsic subsets: inflammatory, fibroproliferative, limited and normal-like [3]. A recent study has confirmed these subsets and determined that changes in macrophage gene expression in the inflammatory subset was associated with improvement in skin scores associated with mycophenolate mofetil treatment [4▪]. Additional analyses of common gene expression modules and gene–gene coexpression networks in SSc patients have identified interconnections between macrophages with adaptive immune function and interferons (IFNs) [5]. Finally, a recent study comparing tocilizumab versus placebo, demonstrated that expression of macrophage related genes, including CD14

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## KEY POINTS

- Monocyte-derived alveolar macrophages are key players in the development of murine lung fibrosis.
- Cdh11 plays a role in the pathogenesis of fibrosis in multiple tissues.
- Cdh11 regulates macrophage behavior, fibroblast invasion, and adhesion of macrophages to myofibroblasts.

and interleukin (IL)13RA1, correlated with progression of skin fibrosis in the placebo arm [6▪]. Together, these microarray expression studies highlight a potential role for macrophages in SSc.

Macrophages are often grouped into subsets, which may have important implications for SSc and fibrosis. Classically activated macrophages (M1) develop in the presence of IFN-γ and are considered inflammatory. Alternatively activated macrophages (M2) develop in the presence of IL-4 and IL-13 and may be involved in wound healing. It has been hypothesized that M2 macrophages are profibrotic. Indeed, cells positive for M2 markers, $CD163^+$ and $CD204^+$, are increased in SSc skin and blood compared with control patients [7]. Furthermore, SSc patients have been reported to have higher circulating profibrotic macrophages in their blood [8]. Consistent with the importance of M2 macrophages in SSc, microarray studies also suggest that M2 macrophages are a core molecular process in SSc skin [5].

However, more recent data suggests that M2 polarization of macrophages in SSc may be an oversimplification. Flow cytometric analyses of SSc peripheral blood suggests that M2 markers are indeed increased in SSc patients; however, these cells also expressed some M1 genes such as TLR4 [9▪]. A subsequent study confirmed that SSc patients have a higher percentage of $CD204^+CD163^+CD206^+TLR4^+CD80^+CD86^+$ and $CD14^+CD206^+CD163^+CD204^+TLR4^+CD80^+CD86^+$ peripheral blood cells compared with controls [10▪]. These data care consistent with a mixed M1/M2 macrophage population in SSc, which was associated with SSc-related interstitial lung disease (SSc-ILD) [10▪]. A similar mixed M1/M2 phenotype has been reported in murine lung fibrosis [11▪,12▪,13▪]. Whether these data reflect a lack of complete M2 polarization, the potential of plasticity of macrophages in the M1/M2 paradigm or changes in our interpretation of macrophage populations on the basis of more extensive molecular characterization needs to be determined.

Together, these studies stress the importance of macrophages in SSc pathogenesis and fibrosis. The extent to which macrophages can be used as prognostic factors and/or therapeutic targets in SSc is an area of important and active investigation. Equally important is the advancement in our understanding of the mechanisms by which macrophages regulate the development and/or resolution of fibrosis.

## ORIGINS OF MACROPHAGES IN LUNG FIBROSIS

Tissue fibrosis results from excessive deposition of ECM by myofibroblasts. In the lungs, this starts with injury to alveolar epithelial cells followed by recruitment of immune cells to initiate a cellular response network to repair the damaged tissue. Normal tissue architecture is restored with a well-controlled wound healing response. However, when the wound healing response becomes dysregulated because of repetitive alveolar injury, fibrosis develops. Macrophages play a key role in the inflammation, proliferation, and remodeling phases of tissue repair because of their ability to change functional phenotypes depending on the environmental cues they receive.

Under normal homeostatic conditions, two populations of macrophages are found in the lung; tissue-resident alveolar macrophages (Tr-AMs), which line the inner alveolar surface, and interstitial macrophages, which reside in the lung interstitium. During tissue injury, monocytes are recruited from the bone marrow and differentiate into macrophages. During the early inflammatory phase, a microenvironment of injured epithelial cells and IFN-γ induces the M1 differentiation of resident and recruited macrophages. Following resolution of the inflammation, a cytokine microenvironment of IL-4 and IL-13 promotes M2 differentiation of macrophages to initiate fibrotic remodeling. M2 macrophages secrete various growth factors such as transforming growth factor β1 (TGF-β1) (reviewed in [14]), platelet-derived growth factor [15], and insulin-like growth factor 1 [16] to activate fibroblasts and facilitate tissue repair. When M2 macrophages persist beyond acute repair, the persistence of these growth factors leads to continued myofibroblast activation and fibrosis.

During lung fibrosis, the number of total alveolar macrophages increases [12▪,17]. Multiple murine studies have sought to understand the origins of macrophages that participate in fibrogenesis. Much attention has been given to $Ly6C^{hi}$ 'inflammatory' monocytes, which are recruited to the lung during fibrogenesis [18–20]. However, recently, a subset of Ly6C monocytes termed segregated-nucleus-containing atypical monocytes (SatM) has been identified. SatM are derived from

a novel progenitor population, distinct from the progenitors that give rise to the Ly6C$^{hi}$ monocytes and promote fibrosis [21]. Murine models of lung fibrosis have also demonstrated a role for recruited monocyte-derived alveolar macrophages (Mo-AMs), rather than Tr-AMs or interstitial macrophages in the development of pulmonary fibrosis [12▪,13▪]. Misharin *et al.* utilized a lineage tracing system to demonstrate that alveolar macrophages during lung fibrosis originated from monocyte-derived cells. Furthermore, some of these monocyte-derived cells persist in the lung after the resolution of fibrosis to restore the alveolar macrophage pool [12▪]. Transcriptomic profiling of the Mo-AMs showed high level of expression of fibrosis-related genes, which are downregulated upon differentiation into mature alveolar macrophages. Consistent with this hypothesis, selective genetic deletion of Mo-AMs by depleting caspase-8 and inducing necroptosis through the receptor interacting protein kinase 3 pathway, resulted in amelioration of lung fibrosis. However, selectively deleting Tr-AMs with liposomal clodronate did not affect recruitment of Mo-AMs or the severity of fibrosis. Although these studies suggested that interstitial macrophages are not major contributors to lung fibrosis, interstitial macrophages have higher levels of profibrotic genes than alveolar macrophages [12▪,22] so their involvement in lung fibrosis cannot be completely ruled out.

Additional evidence for the importance of Mo-AMs in the development of lung fibrosis comes from a report by McCubbrey *et al.* [13▪]. Depletion of Mo-AMs by deleting c-FLIP using a previously validated tetracycline-inducible hCD68rtTA system resulted in an amelioration of lung fibrosis. Interestingly, transcriptomic profiling of the Mo-AMs showed high levels of expression of fibrosis-related genes, confirming their potential role in the development of lung fibrosis.

Finally, another macrophage population has been identified using the single-cell RNA sequencing of bleomycin induced lung fibrosis. This novel population of profibrotic macrophages has an intermediate gene expression profile between monocyte-derived interstitial macrophages and alveolar macrophages [23▪]. Deletion of this population of macrophages reduced lung fibrosis. Whether these macrophages are distinct or the same populations defined by different techniques and/or molecular markers, must be better understood.

Together, these studies demonstrate that Mo-AMs are key participants in lung fibrosis. Confirmation of these populations in SSc-ILD and skin fibrosis will be important to translate these interesting findings to patients.

## TARGETING MACROPHAGES IN FIBROSIS

The above studies emphasize that targeting Mo-AM may be beneficial in lung fibrosis. Understanding the function of macrophages is critical to developing therapeutic strategies to target them during fibrosis. Several studies have recently demonstrated that targeting macrophages can reduce skin and lung fibrosis. Using three different models of dermal fibrosis, pharmacological inhibition of phosphodiesterase 4 (PDE4) was effective in reducing tissue fibrosis [24]. The effect of PDE4 inhibition was at least in part mediated through modulation of macrophages, specifically a reduction in M2 macrophages. Similarly, targeting the adenosine pathway through blockade of the $A_{2B}$ adenosine receptor decreases skin fibrosis and these changes were associated with a reduction in M2 macrophages [25]. Finally, nintedanib, an inhibitor of multiple kinases and profibrotic growth factor receptors, attenuated skin and lung fibrosis in the Fra2 transgenic mouse model of fibrosis [26]. These changes were also associated with a decrease in M2 macrophages. The potential benefits of nintedanib are also noted in patients with SSc-ILD, but it is not known if these effects are mediated through modulation of M2 macrophages or other key profibrotic cells [27]. Together, these studies demonstrate that targeting of M2 macrophages may be effective in reducing fibrosis.

Targeting of M1 macrophages may also be a therapeutic option in fibrosis. During the early stages of the bleomycin-induced fibrosis mode, the expression of a gene called response gene to complement 32 (RGC32) was observed in murine macrophages [28▪]. Genetic deletion of RGC32 protected mice from lung and skin fibrosis. Furthermore, elegant bone marrow chimera experiments demonstrated that RGC32 modulation of fibrosis was mediated through the macrophages. Finally in-vitro studies demonstrated that RGC32 was important for M1 macrophage differentiation. Therefore, targeting M1 macrophages, at least early in the disease process, may be a viable therapeutic option as well.

These studies are important as they demonstrate a role for macrophages in the development of skin and lung fibrosis. They also support the potential for targeting macrophages in patients with fibrosis, including SSc. Identification of novel targets on macrophages is necessary for the treatment of fibrosis. Accordingly, as will be discussed below, cadherins have recently emerged as a potential therapeutic target.

## CADHERIN-11 AND FIBROSIS

Cadherins are a family of transmembrane adhesion molecules that mediate calcium-dependent homophilic cell-to-cell interactions by binding to the

same cadherin on adjacent cells to form adherens junctions (reviewed in [29–32]). Cadherin-11 (Cdh11) is a type II classical cadherin initially identified in osteoblasts [33] and subsequently shown to be expressed in lung, kidney, and brain [33–35]. Cdh11 expression in breast [36], prostate [37], and pancreatic cancer cells [38] is associated with a more mesenchymal and metastatic phenotype. Cdh11 is also expressed on synovial fibroblasts and plays an important role in inflammatory arthritis, potentially through regulation of invasion and production of IL-6 and matrix metalloproteinases [39–42]. These studies support a role for Cdh11 in multiple diseases which share molecular pathways involved in SSc and fibrosis.

Multiple studies support Cdh11 as a mediator of fibrosis. Cdh11 expression is increased in skin and lungs of patients with SSc and idiopathic pulmonary fibrosis and fibrotic tissue in multiple mouse models of fibrosis [43–46]. Recently, an increase in Cdh11 mRNA has also been detected on peripheral blood cells from SSc patients; however, the specific cell was not characterized [47]. Confirming a role for Cdh11 in skin and lung fibrosis are studies where Cdh11-deficient mice demonstrate reduced fibrosis in the bleomycin models of skin and lung fibrosis [45,46]. Furthermore, inhibition of Cdh11 using monoclonal antibodies attenuates lung and skin fibrosis in multiple mouse models [45,46,48], suggesting that Cdh11 may serve as a potential therapeutic target in fibrotic diseases.

Recent studies have also shown a key role for Cdh11 in the development of liver and kidney fibrosis [49▪,50]. Given shared molecular pathways of liver and kidney fibrosis with skin and lung fibrosis, these studies are relevant to SSc. Using carbon tetrachloride as a model for liver fibrosis, fibrotic livers demonstrated increased Cdh11 expression which localized to injured hepatocytes, hepatic stellate cells, and macrophages. Cdh11-deficient mice had reduced liver fibrosis, collagen deposition, α-smooth muscle actin accumulation, and IL-6 and TGF-β1 production, demonstrating a role for Cdh11 in liver fibrosis. Finally, increased levels of Cdh11 protein has also been detected in urine from patients with kidney fibrosis [50]. However, its mechanistic role in the development of kidney fibrosis has not been reported. Together, these studies support an important role for Cdh11 in the development of fibrosis in multiple tissues and with potential implications for SSc.

## Mechanisms of cadherin-11-mediated fibrosis

In addition to the expression of Cdh11 on fibroblasts, Cdh11 expression has been observed on injured type II alveolar epithelial cells in the lung [46] and injured hepatocytes in the liver [49▪]. These studies have also demonstrated expression of Cdh11 on alveolar macrophages in the fibrotic lung [46], dermal macrophages in SSc skin [45], and macrophages in fibrotic liver [49▪]. Finally, Cdh11 expression has been confirmed in synovial macrophages from temporal mandibular joints [51] and alveolar macrophages [52▪▪]. These studies suggest that Cdh11 may regulate multiple steps in the fibrotic process through fibroblasts, epithelial cells, and macrophages.

Cdh11 likely regulates the fibroblast and mesenchymal cells during fibrogenesis. Indeed, Cdh11 is important in mesenchymal stem cell maintenance and differentiation [53]. Recently, Cdh11 was shown to regulate fibroblast migration and invasion through the mesenchymal-specific transcription factor FOXF1 [54▪]. Black *et al.* [54▪] showed using human IPF genomics data, human lung biopsies, and transgenic mice with myofibroblast-specific deletion of FOXF1, that FOXF1 is an antifibrotic factor and loss of FOXF1 in myofibroblasts promotes pulmonary fibrosis. Interestingly, FOXF1 directly binds to the N-cadherin (Cdh2) and Cdh11 promoters in fibroblasts and differentially regulates their transcription. Deletion of FOXF1 increased myofibroblast invasion, proliferation, and collagen secretion and promoted cadherin switching from Cdh2 to Cdh11, leading to a fibrotic phenotype. Inhibition of Cdh11 expression reduced invasion of FOXF1-deficient myofibroblasts, confirming the role for Cdh11 in mediating invasion.

Given the expression of Cdh11 on macrophages and the importance of macrophages in fibrogenesis, it is possible that Cdh11 modulates macrophage behavior during fibrogenesis. Bronchoalveolar lavage cells and primary alveolar macrophages from Cdh-11 deficient mice produced less TGF-β1 compared with the wild type controls [46]. Furthermore, skin from Cdh11-deficient mice injected with bleomycin had significantly less TGF-β1 mRNA and bone-marrow-derived macrophages from Cdh11-deficient mice produced significantly less TGF-β1 than the wild type mice [45]. These data suggest that Cdh11 regulates TGF-β1 production by macrophages.

Recent studies by Lodyga *et al.* [52▪▪] have shed light on how Cdh11 may regulate macrophages during fibrogenesis. These studies confirmed the expression of Cdh11 on macrophages in the fibrotic lung. Cdh11 was expressed within the fibrotic foci of mouse and human lung tissues where it localized to contact points between macrophages and myofibroblasts. These data suggest that Cdh11 could enable the interaction between macrophages and fibroblasts, potentially providing TGF-β1 to enhance

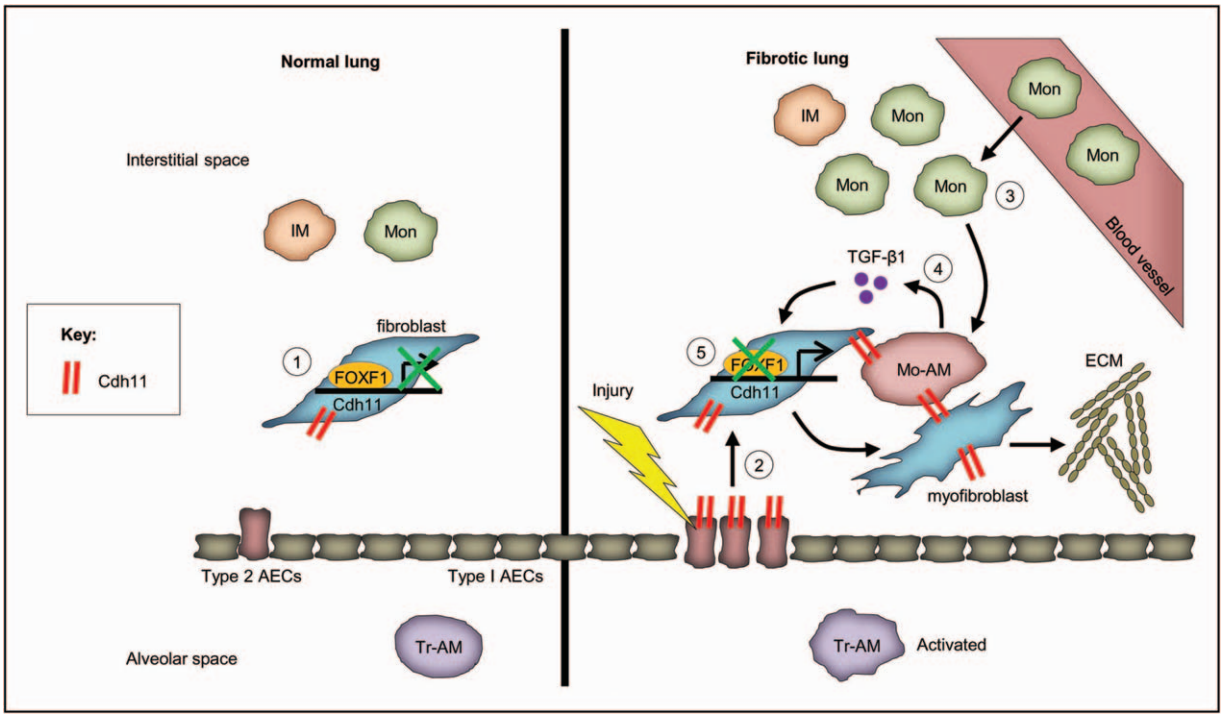


**FIGURE 1.** Working model for the role of cadherin-11 (Cdh11) in lung fibrosis. In a healthy normal lung, Cdh11 (marked in red) is expressed on resident fibroblasts (1). During fibrosis, injury to the epithelium induces the mesenchymal transformation of type 2 alveolar epithelial cells (AECs) into fibroblasts (2). Monocytes are recruited into the interstitial space and differentiate into monocyte-derived alveolar macrophages (3). Cdh11 expression is increased on fibroblasts, myofibroblasts, alveolar macrophages, and injured type 2 AECs. Cdh11 expression on alveolar macrophages and fibroblasts/myofibroblasts likely brings the two cell types in close contact so that transforming growth factor β1 produced by macrophages can lead to persistent activation of fibroblasts into myofibroblasts to secrete extracellular matrix (4). Loss of FOXF1 in the fibrotic lung may also induce expression of Cdh11 which may regulate fibroblast invasion, proliferation, and collagen secretion (5).

myofibroblast activation (Fig. 1). Consistent with this hypothesis, in-vitro studies demonstrated that Cdh11 expression on macrophages was more pronounced on bone marrow derived M2 macrophages compared with M1 macrophages. Furthermore, myofibroblasts formed stronger Cdh11-mediated cellular adhesion with M2 macrophages than M1 macrophages. This strong binding could allow profibrotic M2 macrophages to maintain themselves near myofibroblasts for persistent and close cell–cell communication during fibrogenesis. These data suggest that blocking the homotypic Cdh11 interactions may separate macrophages from myofibroblasts to prevent formation of a profibrotic niche between the two cell types.

## CONCLUSION

Macrophages play a central role in the pathogenesis of SSc and fibrosis. Cdh11 is expressed on macrophages and implicated in the development of fibrosis. Functional studies reveal an important role for Cdh11 in regulating macrophage behavior, fibroblast migration, and invasion. Furthermore, Cdh11 may regulate the adhesion between macrophages and myofibroblasts making them an effective therapeutic target on macrophages to treat fibrosis.

### Acknowledgements

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### Conflicts of interest

*S.A. has patents related to targeting of cadherin-11 in nondermal fibrosis and has received royalties from Adhereon Therapeutics and Roche related to this patent. S.T. has no conflicts of interest.*

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New insights into the mechanism by which Cdh11 regulates fibroblast migration and invasion in fibrosis is presented in this study.

REVIEW

# Calcinosis in scleroderma made crystal clear

*Vivien Hsu*[a], *John Varga*[b], *and Naomi Schlesinger*[a]

**Purpose of review**
Review the current state of knowledge and recent developments in the field of scleroderma-related calcinosis [systemic sclerosis (SSc)-calcinosis], focusing on emerging information related to pathophysiology.

**Recent findings**
Recent studies have begun to characterize that factors that regulate ectopic mineralization, and those that underlie the imbalance of promoters and inhibitors of this process in SSc.

**Summary**
Calcinosis cutis due to ectopic mineralization is a common and highly troublesome complication of SSc. Despite its significant prevalence and clinical impact, the pathogenesis is poorly understood and effective treatment is lacking. More research to better understand the pathophysiology is needed for the identification of novel management strategies for this severe complication of SSc.

**Keywords**
calcinosis, crystals, systemic sclerosis

## INTRODUCTION

Calcinosis (or dystrophic calcification) is the deposition of insoluble calcified material in the soft tissues, occurring in the presence of normal calcium and phosphate metabolism.

Calcinosis is commonly seen in patients with systemic sclerosis (SSc). It has been estimated to complicate the in approximately 40% of patients with limited cutaneous SSc [1]. Very little is known about its pathophysiology and there is no effective medical therapy.

### Crystal composition

Calcinosis in SSc patients (SSc-calcinosis) has previously been identified as calcium hydroxyapatite. Indeed, we have found calcium hydroxyapatite to be the major constituent of SSc-calcinosis deposits [2]. Radiograph diffraction analysis of draining materials from SSc patients with established calcinosis identified calcium hydroxyapatite as the only inorganic material, but the component between organic and inorganic was variable, usually less than 50% inorganic, regardless whether the draining material was in solid or liquid state [2]. The remainder organic components have not been adequately studied.

The pathophysiology of how or why these crystals form in patients with SSc is not well understood. It has been speculated that chronic hypoxia (characterized by digital ulcers, loss of digital tip or abnormal capillary drop-outs seen by nailfold capilloroscopy), repetitive trauma (based on common locations of these deposits such as the fingertips and extensor surfaces of extremities), localized structural damage and other poorly understood factors contribute to calcinosis [3–5].

## REGULATION OF TISSUE MINERALIZATION

Circulating inorganic pyrophosphate (PPi) plays a key role in preventing unwanted soft tissue calcification. PPi has long been known to be byproducts of many intracellular biosynthetic reactions, and it was first identified as the key endogenous inhibitor in biomineralization in the 1960s [6]. The direct effects of PPi on hydroxyapatite formation have been well established, and PPi acts as a potent inhibitor of mineralization by

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## KEY POINTS

- Calcinosis is a common and disabling complication of SSc that has poorly understood pathogenesis and no effective treatment.
- Defect in regulation of tissue mineralization, whether due to genetic or environmental influences, may be important in the pathophysiology of calcinosis.
- The management of calcinosis in scleroderma is an unmet need in almost half of patients with long-standing disease duration.

binding strongly to the surface of nascent or growing hydroxyapatite crystals, thereby blocking their ability to act as a nucleation site for mineralization and therefore preventing crystal growth (Fig. 1) [6–8]. Ectopic calcification is usually associated with a deficiency of one or more of these inhibitors. The first association of circulating PPi levels and vascular calcification was found in a cohort of patients with advanced kidney disease undergoing hemodialysis, peritoneal dialysis or no dialysis. Specifically, plasma PPi level was negatively associated with vascular calcification in patients with end-stage renal disease and stage 4 chronic kidney disease [9–11]. Plasma PPi levels have *NOT* been studied in the autoimmune inflammatory rheumatic diseases such as SSc.

Enzymes with nucleoside triphosphate pyrophosphohydrolase (NTPPPH) activity such as plasma ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) are crucial in the regulation of circulatory levels of PPi [12]. ENPP1 is an ectoenzyme and needs its substrate, ATP, to be present in the extracellular environment. Cells release ATP via different mechanisms. The most important ATP release pathway for PPi formation involves ATP-binding cassette (ABC) subfamily C member 6 (ABCC6), an efflux transporter predominantly found in hepatocytes [13,14]. ABCC6-mediated hepatic ATP release accounts for about 60–70% of all PPi found in the circulation [14]. Accumulation of PPi is prevented by its degradation by tissue nonspecific alkaline phosphatase (TNAP) [15]. TNAP deficiency is associated with severe hypophosphatasia due to significantly increased plasma PPi levels. The ectonucleotidase CD73 forms an additional step

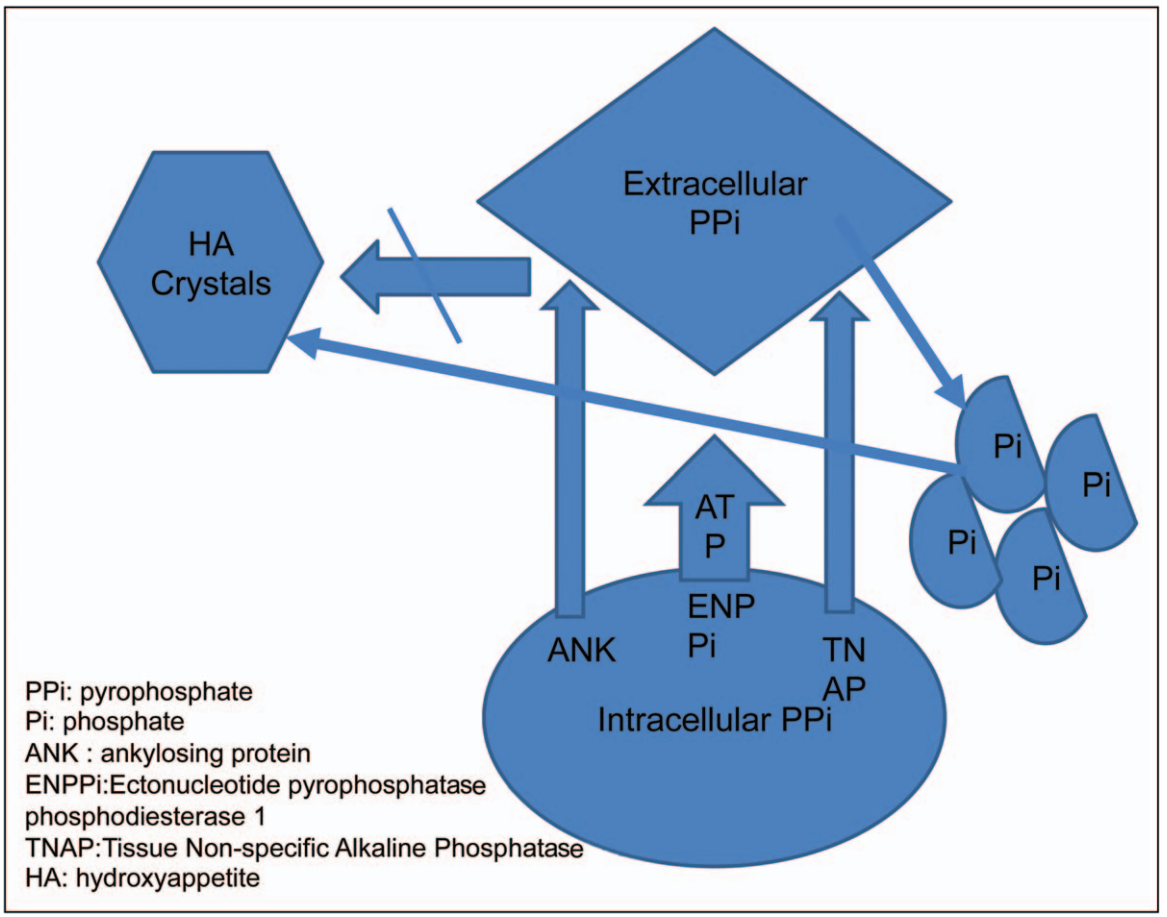


**FIGURE 1.** Extracellular pyrophosphate is hydrolyzed to phosphate, resulting in hydroxyapatite crystal deposition.

of regulation of PPi plasma levels by converting adenosine monophosphate into inorganic phosphate and adenosine, which serves as a TNAP inhibitor [19]. Deficiencies in ABCC6, ENPP1 and CD73 proteins lead to reduced plasma PPi levels, thereby promoting hydroxyapatite mineralization in peripheral tissues (Fig. 1). The PPi/inorganic phosphate balance is therefore critical for prevention of ectopic mineralization and maintenance of normal skeletal mineralization, and deserves further study in SSc patients with and without calcinosis.

Several years ago, Ferreira *et al.* [16] proposed that IL-1β contributed to pathologic mineralization by decreasing levels of NTPPPH activity in mesenchymal stem cells. Similar findings have been demonstrated in cartilage. Circulating levels of IL-1α have been found to be increased in SSc [17] and also elevated in patients with juvenile dermatomyositis with calcinosis [18].

Although the cause of SSc is unknown, significant advances have been achieved in the SSc genetics field [19]. There have been numerous genome-wide association studies completed in SSc and even in different ethnic backgrounds. Genetic susceptibility markers were found in the human leukocyte antigen (HLA) region and in non-HLA genes. In spite of the increasing number of SSc genetic susceptibility factors identified, very few of the studies stratified patients based on presence or absence of calcinosis. Specifically, the *HLA-DRB1∗04* allele was found to be associated with subcutaneous calcinosis in SSc patients in Korea [20]. Polymorphisms in the *MMP3* gene were associated with calcinosis in patients with SSc, suggesting the role of matrix metalloproteinases in the extracellular matrix protein deposition in SSc [21]. In addition, fetuin-A levels were found to be significantly lower in Italian SSc patients with calcinosis and in hemodialysis patients with arterial calcifications [22–25]. Fetuin-A, encoded by *AHSG*, is a major inhibitor of systemic calcification, and low serum levels have been associated with vascular and soft tissue calcification [26]. Any situation that lowers serum fetuin-A, including inflammatory conditions, could increase the risk of calcification, because fetuin-A is a negative acute-phase protein [27]. The relationship between fetuin-A levels and *AHSG* polymorphisms has been reported in SSc [25,26].

Another inhibitor of calciphyllaxis is carboxylated Matrix Gla protein (MGP), a vitamin K dependent factor in the soft tissues and circulation [28▪▪]. These are reduced in uremic patients with calciphyllaxis, resulting in lowered inhibitory effects on the promoters of calciphyllaxis, such as bone morphogenetic protein (BMP) 2 and BMP-4. Similarly, carboxylated MGP in susceptible SSc patients may be affected by lowered vitamin K levels [29].

## INFLAMMATION

Increased production of TNFα, IL-1, IL-6 and other proinflammatory cytokines has been reported in patients with SSc who have calcinosis [30]. Park *et al.* [31] reported elevated vascular endothelial growth factor (VEGF) levels in their small cohort of SSc patients with acro-osteolysis and osteoclastogenesis; 72% of their cohort also had calcinosis, suggesting hypoxia-induced imbalance between angiogenic factors (such as VEGF, platelet derived growth factors) and antiangiogenic factors (such as angiostatin, endostatin) may be common in the pathogenesis of tissue fibrosis and calcinosis [32–34]. In addition, chronic low nitric oxide state may promote fibroproliferative vascular and tissue damage [33,34].

## RISK FACTORS

Risk factors for calcinosis in patients with SSc include long disease duration, with a mean of 7–10 years [35–37]. It has been reported that calcinosis develops more commonly in limited cutaneous systemic sclerosis patients with anticentromere antibody [1]. However, examination of a large Canadian cohort found increased risk for future calcinosis in SSc patients with diffuse cutaneous disease and antipolymerase 3 antibody [3]. This study also found less calcinosis in patients who used calcium channel blockers. Another larger, international cohort of more than 5000 patients found other novel risk factors, including osteoporosis [36]; although dual energy X-ray absorptiometry scans were not available for many of the patients included in this largest retrospective study.

## CLINICAL MANIFESTATIONS

SSc-calcinosis can be asymptomatic or cause significant morbidity such as pain, intractable ulcers and recurrent infections. SSc calcinosis deposits can range in size from asymptomatic specs to large tumorous (>1 cm) deposits, often amorphous, causing significant pain, ulceration, joint contractures and disability. The lesions are most commonly found in the fingertips, upper extremities (along the forearms, the olecranon bursae at the elbows), shoulders and lower extremities (commonly on extensor surfaces of the knees) [36–38] where repetitive trauma may be a trigger. Draining materials from these deposits may be solid or semisolid (chalk-like) or liquid and can be associated with localized inflammation. In a European cohort, Bartoli *et al.* [37] classified calcinosis in their cohort of 52 patients with calcinosis, as visible versus palpable, then according to the shapes and consistency of the lesions on palpation or imaging, namely, mousse

(soft, cream-like material), net (diffuse thin network), plate (large uniform, flat) and stone (single or multiple solid deposits). Significantly, they reported nearly half of their cohort suffered from skin ulcerations; those patients with stone calcinosis had significantly more lung complications, and those with mousse calcinosis had more pulmonary arterial hypertension. Most of their cohort had limited cutaneous scleroderma with the anticentromere antibody. The authors did not clarify which shape or consistency was more prevalent in diffuse cutaneous versus limited scleroderma.

Acro-osteolysis of the fingertips is not uncommon in SSc with calcinosis. Johnstone *et al.* [4] reported more severe calcinosis and acro-osteolysis in patients with tissue damage due to severe digital ischemia and suggested that this may be a marker of more severe disease.

## IMAGING

Plain radiographs [38] are useful to confirm location of calcinosis and could be used to estimate the area of some of these deposits, whereas ultrasound [39] and computed tomography (CT) [40] are more helpful in showing the location of these deposits in the soft tissues and could be useful to quantitate the burden of calcinosis using volumetric measurements of many of these bulky and irregularly shaped deposits. Using standard CT with

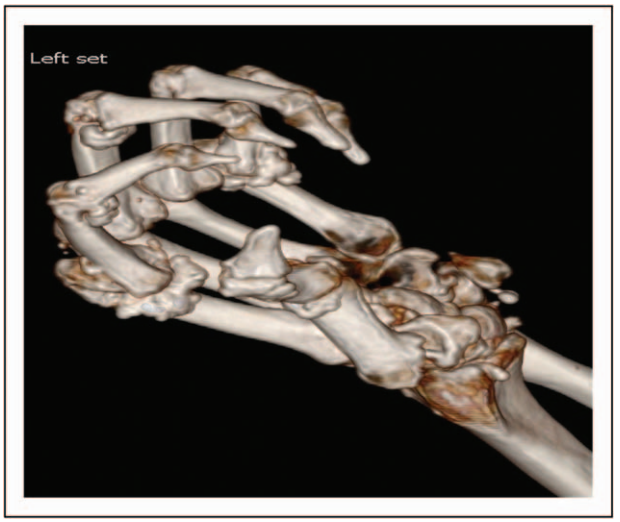


**FIGURE 2.** Calcinosis imaged by dual-energy computed tomography scan showing tumorous deposits attached to tendons and ligaments at the PIP, MCP and wrist joins. MCP, metacarpal phalangeal joint; PIP, proximal interphalangeal joint.

**Table 1.** Review of management of scleroderma-related calcinosis

| Medications | References [ref no.] | Efficacy – brief summary |
|---|---|---|
| Warfarin | Cukierman *et al.* [43] | 3 Patients – 2 regressed, 1 failed |
| | Lassoued *et al.* [44] | 6 Patients – failed |
| Bisphosphonates | Dolan *et al.* [45] | Diltiazem – 1 patient regressed |
| | Fujii *et al.* [46] | Risedronate – 1 patient regressed |
| | Murphy *et al.* [47] | Diltiazem – failed |
| Intravenous gammaglobulin | Schanz *et al.* [48] | 1 Patient – regressed |
| | Kalajian *et al.* [49] | 2 Patients – failed, dermatomyositis |
| Rituximab | de Paula *et al.* [50] | Regression |
| | Hurabielle *et al.* [51] | Failed |
| | Daoussis *et al.* [52] | Regression |
| | Narvaez *et al.* [53] | 8 Patients – 4 regressed, 4 failed |
| | Dubos *et al.* [54] | 2 Patients – failed |
| | Poormoghim *et al.* [55] | 1 Patient – failed |
| Sodium thiosulfate | Rosenbach [56] | Intravenous – failed |
| | Karthik *et al.* [57] | Topical – regressed |
| | Baumgartner-Nielsen and Olesen [58] | 5 Patients – regressed |
| Lithotripsy | Sultan-Bichat *et al.* [59] | More effective in smaller deposits |
| | Sparsa *et al.* [60] | 1 Patient – regressed |
| Carbon dioxide lase | Chamberlain and Walker [61] | 1 Patient – regressed |
| | Bottomley *et al.* [62] | 6 Patients – complete response in some, partial to no response in others |
| Colchicine | Fuchs *et al.* [63] | 2 Patients – regressed |
| Minocycline | Robertson *et al.* [64] | 9 Patients – regressed |

specialized software to permit dual-energy CT (DECT) techniques, DECT is an advanced imaging modality useful for assessing monosodium urate (MSU) crystal deposition in gout [41]. The DECT utilizes two energy beams, usually a combination of 80 and 140-kilovoltage peak beams, and differences in attenuation enable differentiation between calcium hydroxyapatite and MSU crystals. We found DECT imaging to be useful in the evaluation of SSc-calcinosis [42]. DECT enables visualization of calcium deposits by analysis of the chemical content of scanned materials (Fig. 2). Using DECT imaging, calcinosis was most commonly found in the subcutaneous fat pads of the fingertips and along tendon sheaths and muscle groups [42]. Standard CT and DECT imaging should be considered in the evaluation of any SSc patient with progressive hand deformities, especially in the presence of bulky calcinosis. However, there is still much to learn about optimal ways to locate and quantitate bulky calcinosis affecting the proximal extremities and trunk.

## MANAGEMENT

Therapies of SSc-calcinosis have been limited to case reports or series with anecdotal benefit, including warfarin, diltiazem, bisphosphonates, minocycline, intravenous immunoglobulin and colchicine (Table 1) [43–64] and randomized controlled trials are sorely lacking. The use of sodium thiosulfate, whether topical, intralesional or parenterally, have also shown mixed results [56–58] in SSc-calcinosis. MGP, a natural extracellular protein, is a vitamin K-dependent protein that inhibits dystrophic vascular calcification; thus, the use of warfarin for SSc-calcinosis is questioned as this can inhibit MGP carboxylation *in vitro* and promote calcinosis [65].

## CONCLUSION

The management of calcinosis in scleroderma is an unmet need, and is sorely needed for the almost half of SSc patients with long-standing disease duration. Its pathogenesis is poorly understood and there is no effective therapy. More research is needed to better understand its pathophysiology and to identify risk factors that may be amenable to early intervention, to avert this painful and potentially disabling complication.

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### Conflicts of interest

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# Biomarkers in systemic sclerosis

*Brian Skaug and Shervin Assassi*

**Purpose of review**
To discuss recent advances in identification of biomarkers in systemic sclerosis for disease severity, prognosis, and treatment response.

**Recent findings**
Recent reports describe novel circulating markers of disease severity, autoantibody associations with specific manifestations including cancer, and skin gene expression-based predictors of modified Rodnan skin score progression and treatment response. Moreover, there is converging evidence that C-reactive protein and pneumoproteins such as Krebs von den Lungen-6 and chemokine ligand 18 could serve as prognostic biomarkers in systemic sclerosis-associated interstitial lung disease.

**Summary**
Several novel biomarkers show promise in improving the assessment of systemic sclerosis (SSc) disease severity, prognosis, and treatment response. Their potential utility in prospective selection of patients for clinical trials and in individual patient management require additional research.

**Keywords**
biomarkers, scleroderma, systemic sclerosis

## INTRODUCTION

Heterogeneity is one of the hallmarks of systemic sclerosis (SSc, scleroderma). Reliable measures of disease activity as well as predictors of disease progression and treatment response are important for patient selection in clinical trials and to optimize individual patient outcomes. In this regard, clinical features such as diffuse vs. limited cutaneous involvement, progressive skin fibrosis, tendon friction rubs, and pulmonary function test trends are useful in estimating overall prognosis [1–5]. Specific SSc-associated autoantibodies, some of which were incorporated into the 2013 ACR/EULAR classification criteria for SSc [6], have also demonstrated prognostic value, particularly regarding organ involvement and malignancy (reviewed in [7,8]). The traditional biomarker C-reactive protein (CRP) may have a role in assessment of SSc disease activity and prediction of interstitial lung disease (ILD) progression (discussed more below), although the roles of CRP in clinical trial enrollment and patient management remain incompletely defined. Numerous other circulating factors (including proteins and microRNAs), as well as transcriptomic data from blood and skin biopsy specimens, have been characterized with reference to SSc disease manifestations, severity, prognosis, and treatment response in recent years (reviewed in [9–11]), and thus biomarker development in SSc is rapidly evolving. In this review we discuss advances in SSc biomarker identification and characterization from early-2018 to mid-2019. We focus particularly on biomarkers for monitoring disease severity (correlation with clinical evidence of fibrosis or end-organ damage), prognosis (predicting the course of a clinical manifestation over time), or response to treatment (predictive biomarkers).

## CIRCULATING BIOMARKERS ASSOCIATED WITH DISEASE SEVERITY OR SPECIFIC MANIFESTATIONS

Sonic hedgehog (SHH), previously shown to have a profibrotic effect in skin [12], was measured in serum samples from 154 SSc patients (80 limited, 74 diffuse) from eight European centers and 68 matched controls, then analyzed with reference to

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## KEY POINTS

- A variety of circulating proteins, including autoantibodies, may have utility in monitoring SSc disease severity or predicting prognosis with regard to specific manifestations such as skin fibrosis, interstitial lung disease, or cancer.
- Multiple reports indicate an ability of skin gene expression profiles to predict prognosis or treatment response.
- For many potential biomarkers, additional research is needed for validation and to define the roles of each biomarker in clinical trial design and in the management of SSc patients in clinical practice.

clinical disease features [13]. SHH levels were significantly elevated in SSc patients compared with controls, and associated positively with modified Rodnan skin score (mRSS), digital ulcers, and elevated pulmonary arterial pressure (estimated by echo).

The enhanced liver fibrosis (ELF) score, consisting of three circulating markers originally validated as a biomarker for chronic liver disease, was previously shown to be elevated in a majority of SSc patients compared with healthy controls and to correlate positively with mRSS and overall disease severity and negatively with diffusion capacity of the lungs for carbon monoxide (DLCO) [14]. In a recent validation study including 247 SSc patients from six European centers, the overall ELF score again correlated positively with mRSS, disease activity and severity, and negatively with forced vital capacity (FVC) and DLCO [15]. In a multivariate analysis, increased age, increased mRSS, and decreased DLCO were independently associated with ELF score. These studies suggest a potential role of SHH and ELF measurement in monitoring skin and lung disease severity. Since these studies were cross-sectional, the predictive significance of these markers is unknown.

Antibodies against U11/U12 RNP (anti-RNPC3 antibodies) were found to be associated with an increased risk of severe gastrointestinal dysfunction (defined as requiring total parenteral nutrition) in SSc in a case–control study. This finding was tested in an independent validation cohort, in which patients with anti-RNPC3 antibodies were significantly more likely to have moderate–severe gastrointestinal dysfunction [16].

A study of cancer risk in 2383 SSc patients in comparison with a representative sample of non-SSc patients in the general US population revealed no significant increased risk of cancer in SSc overall, but an increased risk among patients with anti-RNA Pol III antibody. In addition, a decreased risk of cancer was observed in patients with limited SSc and anticentromere antibody patients [17▪]. Adding additional depth to the understanding of specific autoantibodies and cancer risk, another recent report described the identification of antibodies against the large subunit of RNA Pol I (RPA194) and their association with decreased cancer risk [18▪]. Examining a subset of SSc patients with antibodies against RPC155, the large subunit of RNA Pol III, anti-RPA194 antibodies were found to be significantly more common in patients without cancer compared with anti-RPC155 antibody-positive, anti-RPA194 antibody-negative patients. Understanding of these associations between specific autoantibodies and cancer risk could lead to their future use as biomarkers to inform decisions about cancer screening in SSc patients.

A study of antineutrophil cytoplasmic antibodies (ANCA's) in a large, multicenter Australian cohort showed a relatively high prevalence of ANCA positivity in SSc patients and an association with ILD and increased mortality [19]. Screening ANCA testing is routinely performed on patients in this cohort, and the investigators found that 8.9% of 1303 SSc patients tested were ANCA positive. A total of 11.2% of ANCA-positive patients were positive for anti-myeloperoxidase antibodies, 13.8% for anti-PR3 antibodies. Only three patients had evidence of ANCA-associated vasculitis during the follow-up period, but ANCA-positive patients had a higher prevalence of ILD, synovitis, pulmonary embolism, and features of overlap with other connective tissue diseases. ANCA-positive patients had significantly higher mortality than ANCA-negative patients after adjustment for age of SSc onset and sex; cause-specific mortality was not determined. Further research, including validation in other cohorts and determination of cause-specific mortality, would be needed to define a potential role of ANCA screening as a prognostic biomarker.

A summary of circulating biomarkers [excluding chemokine ligand 18 (CCL-18) and Krebs von den Lungen-6 (KL-6) which are discussed later] is shown in Table 1.

## GENE EXPRESSION-BASED BIOMARKERS OF SKIN DISEASE PROGRESSION OR TREATMENT RESPONSE

The mRSS, a clinical estimate of overall skin fibrosis, is typically measured as part of the clinical assessment of SSc patients and has been a primary endpoint in clinical trials to treat diffuse cutaneous SSc (reviewed in [22]). However, prediction of mRSS

**Table 1.** Circulating biomarkers for severity monitoring, specific manifestations, or prognosis

| Reference | Biomarker | Population | Type of marker | Summary of findings |
|---|---|---|---|---|
| Beyer *et al.* [13] | Sonic hedgehog | European, multicenter | Severity monitoring | Correlation with mRSS, digital ulcers, PAP |
| Abignano *et al.* [15] | ELF score | European, multicenter | Severity monitoring | Correlation with mRSS (positive), lung function (negative) |
| McMahan *et al.* [16] | U11/U12 RNP (RNPC3) antibody | US Cohorts (Johns Hopkins, Pittsburgh) | Organ manifestation | Association with gastrointestinal dysmotility |
| Igusa *et al.* [17▪] | [a]SSc-associated autoantibodies | US Cohort (Johns Hopkins) | Cancer risk | RNA Pol III antibody: increased risk centromere antibody: decreased risk |
| Shah *et al.* [18▪] | RPA194 antibody | US Cohort (Johns Hopkins) | Cancer risk | RPA194 antibody: lower risk of cancer amongst patients with RPC155 antibody |
| Moxey *et al.* [19] | ANCA | Australian, multicenter | Prognostic | ANCA positivity associated with higher prevalence of ILD and increased mortality |
| Herrick *et al.* [20▪▪] | RNA Pol III antibody | European, multicenter | Prognostic | Earlier and higher peak in mRSS amongst patients with diffuse SSc |
| Ross *et al.* [21] | CRP | Australian, multicenter | Severity monitoring, prognostic | High CRP associated with high mRSS and decreased baseline FVC, and decline in longitudinal FVC |

ANCA, antineutrophil cytoplasmic antibody; CRP, C-reactive protein; ELF, enhanced liver fibrosis; FVC, forced vital capacity; ILD, interstitial lung disease; mRSS, modified Rodnan skin score; PAP, pulmonary arterial pressure; SSc, systemic sclerosis.
[a]Antitopoisomerase-I, anticentromere, and anti-RNA polymerase III.

progression remains quite challenging. Analyses of clinical trial results and observational cohorts have repeatedly demonstrated that a large percentage of diffuse SSc patients have stability or improvement in mRSS irrespective of targeted SSc treatment [20▪▪,23,24], highlighting a need for improved methods to predict progression. Two recent analyses of large, European multicenter observational cohorts each identified shorter disease duration and lower mRSS at baseline visit as predictors of subsequent mRSS increase [20▪▪,24]. The latter study also identified a unique mRSS progression profile associated with RNA polymerase III antibody, namely a higher and earlier peak in mRSS prior to improvement [20▪▪]. This suggests a potential prognostic role for this antibody in future mRSS-targeted clinical trials.

Skin gene expression profiling has shown potential in recent years for assessment disease activity, including changes over time (reviewed in [9,10]). In addition, associations have been observed between mRSS improvement during treatment and skin gene expression profiles (reviewed in [10]). Here we discuss recent reports on gene expression-based predictors of disease progression and treatment response (summarized in Table 2).

## Prognosis of skin disease based on skin gene expression

Analysis of skin gene expression from the placebo arm of the phase II study of Tocilizumab in diffuse SSc (faSScinate) [30] revealed multiple genes whose expression levels at baseline predicted change in mRSS at follow-up [25▪]. These observations were confirmed in a separate cohort of 20 diffuse SSc patients (some of whom were taking immunosuppressive therapy), although the correlation coefficients were generally smaller. Dividing the patients in the discovery cohort into three mRSS trajectory patterns (progressive, stable, or regressive), high expression of five genes (*CD14*, *IL13RA1*, *SERPINE1*, *OSMR*, and *CTGF*) was associated with a progressive, that is, worsening skin trajectory. The mRNA levels of these genes therefore showed potential as biomarkers to predict skin disease progression.

After applying a normalization method to reduce batch effects, differentially expressed genes in a compendium of eight previously generated,

**Table 2.** Skin gene expression-based prognostic or predictive biomarkers

| Reference | Biomarker | Population | Type of marker | Summary of findings |
|---|---|---|---|---|
| Stifano *et al.* [25▪] | Skin expression levels of CD14, IL13RA1, SERPINE1, OSMR, and CTGF | Placebo arm of clinical trial | Prognostic | High expression at baseline predicted worsening mRSS |
| Moon *et al.* [26▪] | 'Clusters' based on skin gene expression profiles | Placebo arm of clinical trial | Prognostic | Improved mRSS in patients in the inflammatory/immune cluster |
| Hinchcliff *et al.* [27] | 'Intrinsic subsets' of patients based on skin gene expression profiles | S. Cohort (Northwestern) | Predictive | mRSS improvement during MMF treatment more likely in inflammatory and mixed inflammatory/ fibroproliferative subsets |
| Higgs *et al.* [28▪] | Plasma cell gene expression signature in skin | Phase I trial of inebilizumab (anti-CD19) | Predictive | Increased plasma cell signature at baseline associated with greater mRSS improvement during anti-CD19 treatment |
| Martyanov *et al.* [29▪] | SASP gene expression signature in skin | SSc-ILD patients in single-arm trial of dasatinib | Predictive | High baseline SASP signature associated with greater mRSS improvement during treatment |

ILD, interstitial lung disease; MMF, mycophenolate mofetil; mRSS, modified Rodnan skin score; SASP, senescence-associated secretory phenotype; SSc, systemic sclerosis.

independent skin gene expression datasets (comprising 175 SSc patients and 61 healthy controls) were analyzed [26▪]. In one of these analyses, four subgroups of diffuse SSc patients were identified by nonnegative matrix factorization clustering, including a cluster enriched for inflammatory and immune cell signatures and another enriched for fibrosis signaling and a fibroblast signature. Examining skin gene expression and mRSS change from baseline to 24-week follow-up in the placebo arm of the faSScinate trial [25▪], patients in the inflammatory cluster had a significant improvement in mRSS from baseline to 24-week follow-up, suggesting that SSc skin with a prominent inflammatory gene expression profile has an improvement in skin score even without immunosuppressive treatment. Patients in the other clusters had variable mRSS progression. It should be noted that some of the patients' follow-up biopsies were in different gene expression-based clusters than their baseline biopsies, and that only four patients were analyzed for mRSS change in the inflammatory/immune cluster.

## Predicting treatment responses based on skin gene expression

As a follow-up to prior work showing that high baseline inflammatory gene expression in the skin was associated with mRSS improvement during mycophenolate mofetil (MMF) treatment [31], skin gene expression profiles of a cohort of patients taking MMF were analyzed with reference to mRSS progression over time [27]. Most patients whose mRSS improved over 12 months had inflammatory or mixed inflammatory/fibroproliferative gene expression profiles in baseline skin biopsies. The inflammatory gene expression signature was reduced in follow-up biopsies after 24 months of MMF therapy. Inflammatory gene expression rebounded in three patients who discontinued MMF treatment, but remained low in three patients who remained on MMF treatment. These results suggest that patients with increased inflammatory gene expression profiles in the skin are more likely to respond favorably to MMF, although the small sample sizes and lack of randomized treatment assignments were limitations.

In SSc patients in a phase I trial of an anti-CD19 antibody Inebilizumab (MEDI-551), analysis of skin biopsy microarray data indicated an elevated plasma cell signature in SSc skin compared with healthy controls. This signature correlated with baseline mRSS, and high plasma cell signature at baseline associated with greater improvement in mRSS during Inebilizumab treatment [28▪].

Skin gene expression related to senescence, termed senescence-associated secretory phenotype (SASP), was analyzed in 12 patients with SSc-associated ILD from a single-arm clinical trial of dasatinib [29▪]. A SASP gene signature was significantly higher at baseline in patients whose mRSS improved during dasatinib treatment, compared with those whose

mRSS did not improve. A greater decrease in SASP signature gene expression was also observed post-treatment in those with improving mRSS. These results suggest a role for baseline skin gene expression measurement in selection of patients for future trials of targeted immunosuppressive or antifibrotic therapies, although this approach would require prospective testing in larger patient samples for validation.

Building on prior work identifying distinct subsets of SSc patients based on skin gene expression, termed 'intrinsic subsets [10]', a recent report described a machine learning approach used to develop a classifier for these subsets that can be utilized for individual patient samples with the ultimate goal of using this method for risk stratification [32]. Empiric testing is needed to determine the ability of this classifier to prospectively identify patients likely to progress and to respond to therapies.

While different reports on the predictive significance of skin gene expression profiles exist, it is important that the methods and transcript lists used for generating the predictive signatures are published in sufficient detail to allow independent validation. Moreover, development of skin gene expression-based predictors is complicated by the spontaneous improvement in skin fibrosis observed in many patients, which complicates interpretation of treatment effect. For example, there seems to be conflicting data on whether the observed associations of inflammatory gene expression signatures with mRSS improvement reflect the natural history of disease [26▪] or treatment effect [27].

## PROGNOSTIC BIOMARKERS FOR INTERSTITIAL LUNG DISEASE

Lung involvement is the primary cause of disease-related death in SSc [33]. Lung tissue is not obtained during routine clinical care, and skin gene expression profiling shows only limited correlation with ILD severity [34] and is unlikely to be informative for predicting ILD course as the natural history of skin fibrosis and ILD is often divergent. Plasma/serum samples obtained during routine clinical care are therefore an attractive source of biomarker development in SSc-ILD. Herein, we review the recently published evidence for use of serum proteins as prognostic biomarkers in SSc-ILD.

### Pneumoproteins

Pneumoproteins are linked to lung parenchymal injury and may be more specific markers for monitoring and predicting ILD course than general fibrotic and inflammatory markers, which can be influenced by extra-pulmonary fibrotic processes such as cutaneous fibrosis or infections. Among pneumoproteins, two serum/plasma proteins have been shown to have prognostic significance for ILD course in several studies: KL-6 and CCL-18 (other name: pulmonary and activation-regulated cytokine [PARC]).

A previously published study in 50 Japanese untreated SSc-ILD patients indicated that high KL-6 levels were predictive of long-term development of end-stage lung disease, defined as % predicted FVC (FVC%) of less than 50%, requiring oxygen, or ILD-related death. A cutoff of 1273 U/ml was proposed to define KL-6 positivity [35]. A follow-up study in a multiethnic observational cohort of 82 early SSc-ILD patients, which also included patients treated with immunosuppressive agents, confirmed the predictive significance of KL-6 [36▪]. In this study, higher KL-6 was predictive of short-term decline in FVC% (12 months) after correction for baseline disease severity. Using the previously defined cut-off value of 1273 U/ml, SSc-ILD patients with positive KL-6 had on average 7% more decline in their annualized percentage change of FVC%. The predictive significance of KL-6 was independent of antitopoisomerase I positivity. In this study, CCL-18 was not predictive of change in FVC%.

In a European observational study of 234 SSc-ILD patients, predictive significance of KL-6 and CCL-18 was investigated [37▪]. This study also included patients that were treated with immunosuppressive agents, and a relatively small portion of patients (14.5%) had a change in FVC% more than 10% during the mean 3.2-year follow-up time. CCL-18 and KL-6 levels were dichotomized based on their correlation with baseline disease severity. Neither KL-6 nor CCL-18 were predictive of short-term (1 year) lung fibrosis worsening. However, CCL-18 was predictive of FVC decline more than 10% during the entire follow-up period, while positive KL-6 (cutoff value = 923 U/ml) did not show predictive significance.

Predictive significance of KL-6 and CCL-18 was also investigated in SSc-ILD patients ($n = 133$) enrolled in the Scleroderma Lung Study II (SLSII) [38▪]. Contrary to aforementioned observational studies, the investigated SSc-ILD patients were off immunosuppressive agents at the baseline visit and were subsequently treated with either cyclophosphamide or mycophenolate according to standardized treatment protocols. The course of FVC% during the first year of study (3–12 months) when patients in both treatment arms were on active treatment was the primary outcome. In both treatment arms (mycophenolate and cyclophosphamide), higher levels of KL-6 and CCL-18 predicted

**Table 3.** Studies showing predictive significance of circulating Krebs von den Lungen-6 or chemokine ligand 18

| KL-6 | | |
|---|---|---|
| **Reference** | **Population** | **Summary of findings** |
| Kuwana *et al.* [35] | Nippon University | Predictive of ESLD development |
| Salazar *et al.* [36▪] | GENISOS (US, Texas) | Predictive of worse FVC decline in 12 months |
| Sumida *et al.* [39] | Tokyo University | Predictive of lower DLCO |
| Volkmann *et al.* [38▪] | SLSII (US, multicenter clinical trial) | Predictive of worse FVC decline in 12 months |
| **CCL-18 (PARC)** | | |
| **Reference** | **Population** | **Summary of findings** |
| Tiev *et al.* [40] | French cohort | Predictive of ILD event |
| Elhaj *et al.* [41] | GENISOS (US, Texas) | Predictive of worse FVC in 12 months |
| Hoffmann-Vold *et al.* [42] | Norwegian cohort | Annual decline in FVC |
| Elhai *et al.* [37▪] | French/Norwegian cohort | Predictive of 10% decline in FVC |
| Volkmann *et al.* [38▪] | SLSII (US, multicenter clinical trial) | Predictive of worse FVC decline in 12 months |

CCL-18, chemokine ligand 18; DLCO, diffusion capacity of the lungs for carbon monoxide; ESLD, end-stage lung disease; FVC, forced vital capacity; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; PARC, pulmonary and activation-regulated cytokine; SLS, scleroderma lung study.

worse ILD progression based on the lower levels of serially obtained FVC% after adjustment for baseline disease severity.

Table 3 summarizes results of previously published studies on predictive significance of KL-6 and CCL-18. Higher KL-6 levels showed predictive significance for worse ILD course in untreated patients [35], in an observational cohort with mixed treatment regimens [36▪], as well in the SLSII cohort where all patients were treated with immunosuppressive agents [38▪], indicating that this serum protein is a prognostic rather than predictive biomarker, predicting worse ILD course regardless of treatment regimen. Of note, KL-6 did not predict ILD course in the above mentioned European study [37▪] where a validated, conventional ELISA was used, while in the other three studies [35,36▪,38▪] KL-6 was measured using latex-fixed anti-KL-6 monoclonal antibody with an automated analyzer. The differing assay accuracy might have influenced the discrepant results.

Clara cell secretory protein (CC16) is a marker of bronchial epithelial cell damage and another potential pneumoprotein biomarker in SSc-ILD. In a retrospective study of 106 SSc patients (half had ILD) [43], the predictive significance of baseline CC16 was investigated for a composite ILD event during the 4-year follow-up period. The outcome variable was defined as a 10% decrease in total lung capacity or FVC% from baseline, or death. The risk for this combined lung event was significantly higher in those patients with higher baseline CC16 levels.

## C-reactive protein

Previous data from observational cohorts have indicated that higher baseline CRP levels are predictive of reduced survival [44] and faster FVC% decline in SSc [45]. High CRP levels were recently utilized as an enrichment criterion for a placebo-controlled tocilizumab clinical trial [30]. In this trial, early diffuse patients enrolled in the placebo arm had a relatively large mean FVC% decline of 6.3% at 12 months despite the fact that ILD was not an inclusion criterion and skin involvement was the primary focus. In the Australian Scleroderma Cohort [21], the longitudinal correlates of raised CRP (defined as $\geq$5 mg/l) were investigated. Raised CRP was significantly associated with mRSS more than 20 and FVC% less than 80. Notably, a two-fold increase in CRP was associated with a 10% decrease in FVC between corresponding visits in the whole cohort and among those with ILD. In a retrospective Japanese study of 24 SSc-ILD patients, the predictive significance of CRP and KL-6 was investigated for treatment response after 6 monthly infusions of cyclophosphamide [39]. Unlike previously published studies in which FVC% was utilized for response assessment [46,47], good response in this study was defined as an increase of at least 6% in % predicted DLCO (DLCO%) while the remainder of patients were categorized as having poor response. Higher baseline CRP and KL-6 were significantly associated with poor response. These data cumulatively support the notion that CRP might serve as a prognostic biomarkers for worse ILD course. Future studies

conducted in large, well phenotyped SSc-ILD clinical trials could contribute importantly to establishing CRP as a clinically useful biomarker in routine clinical care and clinical trials.

As evident by the above review, cross-comparison and validation of biomarkers in SSc-ILD is hampered by the fact that differing outcome measures and biomarker cutoff values are used. Although FVC is the primary outcome variable in SSc-ILD clinical trials [46–48], a widely accepted and validated definition of FVC% improvement or worsening is currently not available. Therefore, progress in this field can be accelerated if prognostic/predictive biomarker studies first show the predictive significance of the investigated candidate biomarker as a continuous variable for the undichotomized FVC% outcome and then consider other secondary analyses.

## CONCLUSION

The results highlighted in this review suggest the possibility of more effective risk stratification and treatment selection for SSc patients based on circulating biomarkers and skin gene expression profiles. However, additional research is needed to determine the utility of these biomarkers in prospective patient selection or stratification in clinical trials and for management of individual SSc patients. It may also prove beneficial to test multifaceted prediction models incorporating clinical data with serologic (including specific autoantibodies) and transcriptomic/proteomic biomarker data. In addition, with improving knowledge of genetic and epigenetic contributions to SSc progression, gene expression and serum protein profiles of SSc patients, clinical trials of novel therapies, and longitudinal outcomes of SSc patients, it is likely that important biomarkers remain to be discovered.

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### Conflicts of interest

*B.S. has no competing interests to disclose. S.A. has received consultancy fees and grant support from Boehringer Ingelheim, and has received grant support from Momenta, Biogen, and Bayer HealthCare.*

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# Pulmonary involvement in antisynthetase syndrome

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**Purpose of review**
Lung involvement is a distinctive feature of antisynthetase syndrome (ASS) and it is considered a basic disease-classifying criterion. In this review, we go over clinical features, radiological patterns, prognostic factors, pathogenesis and treatment of lung involvement in ASS patients, focusing on the clinical differences linked to the different antibody specificities known so far.

**Recent findings**
The lung is the most common extramuscular organ involved in ASS and has the greatest impact on patient prognosis. The pulmonary disease-defining manifestation in ASS is interstitial lung disease (ILD), yet a proportion of patients also develop pulmonary arterial hypertension and, less frequently, obstructive bronchiolitis or acute respiratory failure according to drivers not yet fully understood but likely associated with the underlying autoantibody pattern. Clinical presentation of pulmonary involvement can range from milder forms to a rapidly progressive disease which may lead to chronic lung damage if misdiagnosed and not properly treated.

**Summary**
The knowledge of risk factors associated with progressive or refractory lung damage is important to identify and properly treat patients with the poorest prognosis. For those with a disease not responsive to conventional therapy the efficacy of other therapeutic option is under evaluation.

**Keywords**
antisynthetase antibodies, interstitial lung disease, prognosis, T cells, vascular disease

## INTRODUCTION

Antisynthetase syndrome (ASS) is a severe, autoimmune condition classified as a new entity among the immune inflammatory myopathies (IIM) [1▪▪] and defined by the presence of mutually exclusive autoantibodies directed against an aminoacyl-tRNA synthetase along with typical clinical manifestations. To date, eight antibodies have been identified. The most frequent are anti-Jo1 (histidyl), anti-EJ (glycyl), anti-PL7 (threonyl) and anti-PL12 (alanyl); anti-OJ (isoleucyl), anti-KS (asparaginyl), anti-Zo (phenylalanyl), anti-Ha (tyrosyl) are less frequently detected [2▪]. Anti-Jo1 account for 10–40% of cases, anti-PL7 for 10–15% and anti-PL12 for 5–10% of myositis patients [3–5]. Interstitial lung disease (ILD) is included either in the ASS classification criteria proposed by Connors *et al.* [6] in 2010 or by and Solomon *et al.* [7] in 2011 (Table 1). Recent findings suggest that the patient phenotype and ILD clinical and radiological features may vary according to the associated positive autoantibody. Nevertheless, other conditions must be considered when evaluating lung involvement in ASS patients including the presence of pulmonary arterial hypertension (PAH) [8] and pleuritis with pleural effusion [9,10].

## INTERSTITIAL LUNG DISEASE

ILD is the most common extramuscular manifestation with a prevalence ranging from 67 to 100%, higher than that reported in non-ASS IIM where it ranges from 20 to 75% [11]. It represents the most severe organ involvement, leading to an excess 5-year mortality up to 45% [12]. The strongest predictive factor of its development is the presence of antiaminoacyl-tRNA synthetase antibodies [13]. According to multiple series, patients with antisynthetase-related

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## KEY POINTS

- ILD, whose strongest predictive factor is the presence of antiaminoacyl tRNA synthetase antibodies, is the most common extramuscular manifestation in ASS and the major determinant patients' prognosis.
- Pulmonary arterial hypertension has a low prevalence in ASS compared with other connective tissue diseases but it independently affects prognosis and survival.
- Male sex, older age, the presence of fever, low CD3+CD4+ cell counts at diagnosis and black ethnicity are predictors of a more severe lung involvement at high-resolution computed tomography.
- Presence of nonanti-Jo1 antibodies or co-occurrence of anti-Jo1 and anti-Ro52 are associated with an earlier, more severe, isolated and progressive form of ILD with higher risk of misdiagnosis.
- Glucocorticoids and immunosuppressants are the cornerstone of the treatment and rituximab is emerging as an effective therapy in severe-progressive forms or refractory cases.

ILD show a restrictive pulmonary pattern and impaired gas exchange with a mean FVC (forced vital capacity) 65.5% or less and a mean DLCO (diffusing capacity of the lung for carbon monoxide) 55.4% or less of predicted at the time of the diagnosis [14,15▪▪,16▪]. Patients with anti-PL7, and to an even greater extent patients with anti-PL12 antibodies, were shown to have a more severe lung involvement, a higher degree of lung fibrosis and lower DLCO and FVC values than those with anti-Jo1 [16▪]. Nonanti-Jo1 antibodies are reported to be more prevalent in African-Americans than in whites [12], with an increased rate of anti-PL-12 among black patients that are also those with the worse reported prognosis [16▪]. It is not yet completely clear if the severity of lung involvement in these patients is due to nonanti-Jo1 antibodies or to interfering variables such as socioeconomic factors, or if black ethnicity *per se* is an independent determinant of a more severe ILD; however, at multivariate analysis both autoantibody specificity and black race independently predicted ILD severity, even after adjusting for confounding factors [16▪].

Beyond the autoantibody subtype, the main predictive factors for a progressive pulmonary disease were male sex, age at least 55, low DLCO at diagnosis, decreased FVC over time, co-occurrence of anti-Ro52 antibodies, muscle weakness and increase of the fibrosis score on high-resolution computed tomography (HRCT) [11,17–22]. A recent Japanese study found that the presence of fever and low CD3+CD4+ cell counts at the time of the diagnosis were positively associated with ILD deterioration and were also independent prognostic factors of lung involvement on HRCT [23▪▪].

Regarding radiologic features, Waseda *et al.* [24] described the computed tomography characteristics of lung involvement in a cohort of 64 patients with ASS. They observed a distribution of reticulation, consolidation and ground glass opacities predominantly in the lower, peripheral and/or peribronchovascular areas, displaying a pattern mainly suggestive of nonspecific interstitial pneumonia (NSIP), organizing pneumonia or NSIP with organizing pneumonia overlap (Table 2) with similar findings also confirmed in other series [7,19,25,26]. Significantly, a study carried out in 36 patients found that middle lobe traction bronchiectasis were significant predictors of long-term deterioration, conversely, their absence might lead to a good response and long-term stabilization [15▪▪]. During the follow-up consolidations and traction bronchiectasis, considered sings of permanent fibrosis, tended to resolve even though some patients experienced a disease progression toward fibrosis with increase occurrence of honeycombing [26]. In general, a HRCT finding compatible with NSIP associates with a better survival compared with an usual interstitial

**Table 1.** Proposed classification criteria for antisynthetase syndrome

| **Connors *et al.* [6]** | **Solomon *et al.* [7]** |
|---|---|
| **Serum positivity for an antiaminoacyl tRNA synthetase antibody** | |
| **Plus** | **Plus** |
| 1 or more of the following | 2 major criteria or 1 major and 2 minor criteria |
| Raynaud's phenomenon | Major |
| Arthritis | Interstitial lung disease |
| Interstitial lung disease | Polymyositis or dermatomyositis (Bohan and Peter's criteria) |
| Fever not attributable to other causes | Minor |
| Mechanic's hands | Arthritis |
| | Raynaud's phenomenon |
| | Mechanic's hands |

**Table 2.** Patterns of pulmonary involvement in patients with antisynthetase syndrome divided for antibody specificity

| HRCT/ BIO | Antibody specificity | No. of patients in the study | No. with HRCT/BIO | NSIP (%) | OP (%) | NSIP/ OP (%) | UIP (%) | Other patterns (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| **Anti-Jo1** | | | | | | | | | |
| HRCT | | 75 | – | (62.8) | (19.6) | – | (17.6) | – | Marie *et al.* [4] |
| HRCT | | 103 | 85 | 44 (51.76) | 19 (22.4) | 3 (3.5) | 11 (12.9) | 8 (9.41)[a] | Zamora *et al.* [11] |
| BIO | | 103 | 21 | 9 (30) | 6 (20.0) | – | 6 (20.0) | – | Zamora *et al.* [11] |
| BIO | | 28 | 8 | 4 (50.00) | 4 (50.0) | – | – | – | Johnson *et al.* [12] |
| HRCT | | 48 | 34 | 28 (82.35) | 5 (14.7) | – | 1 (2.9) | – | Stanciu *et al.* [19] |
| HRCT | | 66 | 66 | 39 (59.09) | 11 (16.7) | – | 16 (24.2) | – | Marie *et al.* [20] |
| HRCT | | 64 | 64 | 35 (54.68) | 26 (40.6) | – | 1 (1.6) | 2 (3.12)[a] | Waseda *et al.* [24] |
| HRCT | | 9 | 9 | 6 (66.67) | 2 (22.2) | – | 1 (11.1) | | Maturu *et al.* [25] |
| HRCT | | 68 | 17 | 6 (35.29) | 5 (29.4) | 4 (23.5) | – | 2 (11.76)[a] | Debray *et al.* [26] |
| HRCT | | 44 | 44 | 19 (43.18) | 25 (56.8) | – | – | – | Mejía *et al.* [29▪] |
| HRCT | Ro52+ | 66 | 28 | (60.7) | (17.9) | – | (21.4) | – | Marie *et al.* [30] |
| HRCT | Ro52− | 66 | 38 | (65.8) | (15.8) | – | (18.4) | – | Marie *et al.* [30] |
| **Anti-PL7/PL12** | | | | | | | | | |
| HRCT | PL7/PL12 | 20 | 20 | (50.00) | (16.7) | – | (33.3) | – | Marie *et al.* [4] |
| BIO | PL7 | 4 | 3 | 1 (33.33) | 1 (33.33) | – | 1 (33.3) | – | Johnson *et al.* [12] |
| BIO | PL12 | 5 | 5 | 1 (20.00) | 1 (20.00) | – | 3 (60.0) | – | Johnson *et al.* [12] |
| HRCT | PL12 | 68 | 13 | 9 (69.23) | – | 4 (30.77) | – | – | Debray *et al.* [26] |
| HRCT | PL7 | 12 | 12 | 9 (75.00) | 2 (16.67) | – | – | 1 (8.33)[b] | Hervier *et al.* [31] |
| HRCT | PL7 | 15 | 14 | (42.9) | (14.2) | – | (42.9) | – | Marie *et al.* [32] |
| HRCT | PL7 | 8 | 8 | 3 (37.50) | – | – | 5 (62.50) | – | Yousem *et al.* [33] |
| BIO | PL7 | 8 | 8 | 1 (12.5) | 2 (25.0) | – | 4 (50.0) | 1 (12.5)[c] | Yousem *et al.* [33] |
| **Other ASS antibodies** | | | | | | | | | |
| BIO | EJ, OJ | 4 | 2 | – | – | – | 2 (100.0) | – | Johnson *et al.* [12] |
| HRCT | KS | 5 | 5 | 1 (20.0) | – | – | 4 (80.0) | – | Schneider *et al.* [34] |
| BIO | KS | 5 | 5 | – | 1 (20.0) | – | 4 (80.0) | | Schneider *et al.* [34] |
| HRCT | EJ | 4 | 4 | – | – | – | 2 (50.0) | 2 (50.00)[d] | Schneider *et al.* [35] |
| HRCT | EJ | 3 | 3 | 1 (33.3) | 1 (33.3) | 1 (33.3) | – | – | Giannini *et al.* [36] |

ASS, antisynthetase syndrome; BIO, biopsy; HRCT, high-resolution computed tomography; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; UIP, usual interstitial pneumonia.
[a]Undetermined.
[b]Obliterative bronchiolitis.
[c]Lymphoid interstitial pneumonia.
[d]Diffuse alveolar disease.

pneumonia (UIP) pattern [23▪▪] and, in turn, UIP pattern in ASS-associated ILD has a better prognosis compared with the same found in patients with idiopathic pulmonary fibrosis [11,27▪▪] although it still represents a risk factor for lung disease progression [18]. The good correlation between lung function variables (DLCO and FVC) and extent of ILD on HRCT suggests that lung capacity and radiological abnormalities are closely correlated [28].

## PULMONARY ARTERIAL HYPERTENSION

PAH is defined as resting mean pulmonary artery pressure at least 25 mmHg with a pulmonary capillary wedge pressure 15 mmHg or less at right heart catheterization in absence of left heart and thromboembolic disease. Although rarely reported in ASS patients, PAH is characterized by a 3-year survival rate of 58%. Its diagnosis takes up a mean time of 7 years after the ASS diagnosis, when the great majority of patients had already developed severe dyspnea [8]. Prevalence of precapillary PAH assessed by RHC in IIM ranges from 7.9 [8] to 9% [37]. The retained mechanism was initially thought to be ILD-associated but the severe values of pulmonary hypertension found were not fully explained by the interstitial lung involvement, thus a vascular contribution due to vessels remodeling was also hypothesized and supported by the finding that precapillary pulmonary hypertension is frequently associated with Raynaud's phenomenon, capillaroscopic abnormalities and dramatic increase in pulmonary vascular resistance [8]. Despite further investigations to better clarify PAH pathogenesis and prevalence in patients with ASS are needed, these results highlight the importance of an echocardiographic screening in all cases with suspected PAH and suggest an independent contribution of PAH on ASS prognosis and survival.

## OTHER CLINICAL FEATURES OF LUNG INVOLVEMENT

Pleural effusion is not reported to be a common finding in ASS patients but Katz *et al.* [9] described a prevalence of 44% in a cohort of 93 patients, suggesting that it might be more frequent than previously thought; its underestimation may be due to a subclinical course [5]. In most cases it is bilateral and is significantly less frequent in anti-Jo1 patients than in those with other ASS-related autoantibodies [9].

Anecdotal cases and small case series of acute respiratory failure as initial manifestation are reported in the literature [38–41] and few case reports of bronchiolitis obliterans with organizing pneumonia (BOOP) are also present [42,43]. Data on BOOP are more abundant in patients with IIM in general, in which an association with a better prognosis than UIP pattern is also reported [44,45].

## PATHOGENESIS

The lung is thought to be the starting site of the syndrome as it is continuously exposed to environmental immunogenic stimuli (pollutants, infectious agents or cigarette smoke) [46,47]. These inflammatory triggers may cause cellular distress or death, eventually leading to enhanced release of microparticles and aberrant self-antigen exposure. It follows the break of self-tolerance [48] with consequent release of aminoacyl-tRNA synthetases which are themselves chemoattractive for lymphocytes and activated monocytes [49]. Dendritic cells, once attracted to the inflammatory site, present the antigen to T cells promoting their proliferation via a major histocompatibility complex-II dependent process. The contribution of T cells to ILD is strengthened by the retrieval of large amounts of CD3+ lymphocytes in the pulmonary infiltrate of ILD-bearing IIM patients which displayed a restricted T-cell receptor repertoire in their variable region, thus suggesting an antigen-driven oligoclonal lung infiltration [50].

An important role in the pathogenesis is also suggested for natural killer cells found in massive infiltrates inside the lungs of anti-Jo1-positive patients in histological studies [51]. They are thought to contribute to protein cleavage and generation of self-antigens by producing granzyme B.

Within this articulated framework, complexes containing anti-Jo1 or anti-Ro52/anti-Ro60 antibodies stimulate the secretion of IFN-α by plasmacytoid dendritic cells [52,53]. Eloranta *et al.* [52] showed that sera from patients with ILD had significantly higher IFN-α inducing capability compared with sera from patients without ILD. This association was not observed for other clinical manifestations suggesting a role for IFN-α in the maintenance of lung inflammation (Fig. 1).

Once the immune response organizes in the lungs, it then spreads to all organs and preferentially to the muscles, according to pathways still poorly understood; it is possible that a second hit such as an infection or a mechanic trauma could act as a propagating factor by amplifying the expression of aminoacyl-tRNA synthetase in the affected tissues [46] enhancing the cross-reactivity in those sites.

## AUTOANTIBODY-RELATED PHENOTYPES

### Anti-Jo1-positive patients

Anti-Jo1 antibodies are associated with a typical phenotype characterized by muscle weakness and lung involvement, with ILD occurring in 70–90% of the cases [20]. ILD time of onset is variable as shown in a study conducted on a large Spanish cohort [54] where 80 out of 145 anti-Jo1-positive patients (55.2%) presented ILD at the time of the diagnosis, out of whom 33 (22.8%) had also associated myositis, whereas 47 (32.4%) had only lung involvement. Considering a mean follow-up period of 70.3 months, ILD involved 119 patients (80%) and respiratory failure was responsible for nearly a quarter of deaths [54]. Even though in others cohorts the prevalence of amyopathic ASS was reported to be lower [19,55], the still high percentage of patients without myositis symptoms at onset underscores the importance of considering ASS in the differential diagnosis of patients presenting with idiopathic ILD and with interstitial pneumonia with autoimmune features [56▪▪] to refer them to a multidisciplinary evaluation involving rheumatologists, pneumologists and radiologists [14,57].

In Anti-Jo1 patients NSIP is the most common radiological pattern found on HRCT, followed by organizing pneumonia and UIP; the most frequent elementary lesions seen are ground glass opacities, interlobular septal thickening, reticulation and consolidations, whereas honeycombing is quite rare [11,19,20,24,37].

### Anti-PL7/PL12-positive patients

Anti-PL7 and anti-PL12-positive patients have a high prevalence of lung disease, being present in 60–90% of cases [31–33,58]. They are markedly linked to a milder and rapidly resolutive myositis but to an early and severe ILD [4,32,59]. The time of onset of lung involvement in relation to muscular symptoms is variable and it could proceed (20%), concur (70%) or follow (10%) myositis [59]. In a

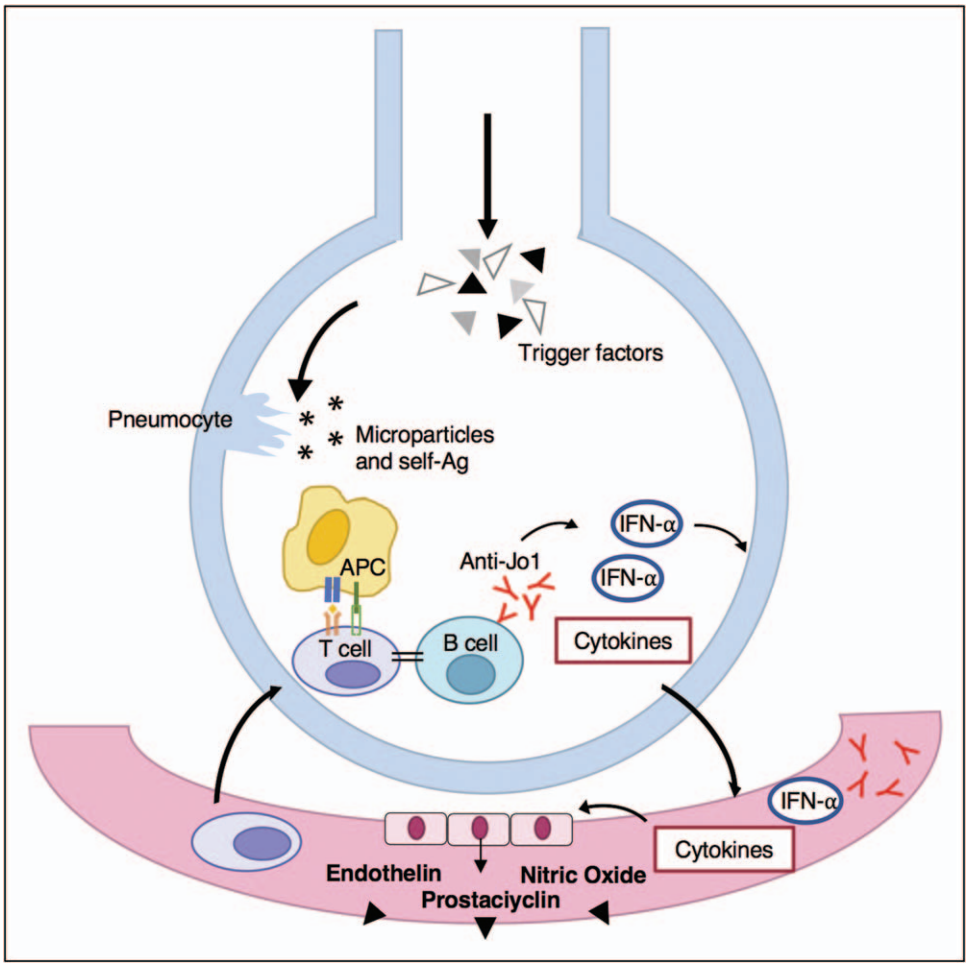


**FIGURE 1.** Pathogenetic mechanisms of pulmonary involvement in antisynthetase syndrome. Pollutants, infectious agents or cigarette smoke are irritative stimuli for alveolar cells. The resulting damage causes the exposure and the release of self-antigens which in turn lead to the break of self-tolerance. Hence the inflammatory and autoimmune response, mainly based on a major histocompatibility complex-II dependent process, give rise to the production of inflammatory cytokines. They sustain the local immune response and also act on endothelial cells of the alveolar capillary promoting vasoconstriction. Production of antibodies by B cells and the formation of immune complexes sustains the release of IFN-α that has a role in the development of interstitial lung disease. APC, antigen presenting cell; IFN-α, interferon-alpha.

comparative study by Marie *et al.* anti-PL7/PL12 patients more often experienced an acute symptomatic onset or a progressive lung injury in comparison with anti-Jo1 positive. Even though no substantial differences have been shown between the two groups in pulmonary function test (PFT) findings at onset and radiological patterns at HRCT, a higher frequency of UIP and a higher median score of fibrosis were reported during follow-up in anti-PL7/PL12 patients [4]. In particular anti-PL7 antibodies seem to be associated with a higher frequency of UIP with honeycombing and this might explain why these patients show a more deteriorated lung function, higher morbidity, resistance to corticosteroids and a worst overall prognosis [32]. Anti-PL12 patients are instead those with the highest prevalence of pleural effusion [9].

## Patients with other antisynthetase syndrome antibodies

Only few case reports or small case series describing lung involvement in patients with other ASS-associated antibodies are available. Nevertheless, patients with anti-EJ, anti-KS or anti-OJ are reported to develop ILD in nearly 100% of cases [34,60,61]. There are no significant differences in the pattern of lung involvement compared with patients with the other ASS-related antibodies but in anti-EJ-positive patients a UIP pattern [35,62,63] and an acute onset with diffuse alveolar damage are more often described [64]. Anti-KS positivity has been associated with a higher prevalence of NSIP or UIP as well [35,65]. Reports agree in confirming that nonanti-Jo1 patients tend to have a worse prognosis as they more often develop atypical features characterized

by aggressive pulmonary involvement, sometimes as an isolated manifestation, mild myopathy and absence of other ASS typical organ involvement with higher rates of lung fibrosis due to a delayed diagnosis [3,17,29▪,64,66].

### Anti-Ro/SSA association

Ro52 and Ro60 are part of a ribonucleoprotein complex known as SSA/Ro; however, only antibodies against Ro52 subunit are considered markers of IIM [67] and are encountered in up to 56% of anti-Jo1-positive patients, but never associated with other ASS-related antibodies [30]. This association however does not seem to be a cross-reaction between the two antibodies [68]. La Corte *et al.* [69] reported that patients with coexisting anti-Ro52 antibodies have more severe lung symptoms and greater ILD than those without. Marie *et al.* [30] have further observed that anti-Jo1-positive patients with anti-Ro52 antibodies less commonly exhibited an asymptomatic form of ILD and that ILD complications were responsible of 66.7% of overall causes of death. These findings are important to underline the value of a prompt searching of anti-Ro52 antibodies in patients with ASS to recognize those who may need a tighter follow-up and more aggressive therapeutic strategies.

## TREATMENT

### Interstitial lung disease

The response to therapy in patients with ILD depends on the radiological pattern of lung involvement, with organizing pneumonia and NSIP being the best responding [20]. For the treatment of the mildest and chronic forms glucocorticoids alone are reported to be efficacious in more than 80% of cases [70▪] but for more severe or steroid-resistant manifestations treatment is based on the association of glucocorticoids and immunosuppressants such as methotrexate, mycophenolate mofetil, cyclosporine A, tacrolimus and azathioprine [57,70▪,71], despite no specific guidelines for the management of ASS-ILD are available to date [70▪]. Among patients treated with glucocorticoids alone or in association with immunosuppressants, disease progression over time remains relevant in 32–35% of cases [26,41]. The use of methotrexate has previously been a real concern due to its possible pulmonary toxicity, yet the fear of its actual threatening potential has downsized over the years. Among calcineurin inhibitors cyclosporine A is the most commonly used but tacrolimus seems to have a comparable efficacy and safety profile. Their effectiveness in stabilizing or reducing pulmonary disease burden confirms a role of T-cell activation in the pathogenesis of ILD [70▪,72–75]. Cyclophosphamide is used as third-line therapy, in more severe or refractory cases. Growing evidence is emerging on the efficacy of rituximab (RTX) in the treatment of severe-progressive or refractory ILDs [76,77] as in patients with co-occurrence of anti-Jo1 and anti-Ro52 antibodies. Treatment with RTX leads to an improvement in both PFTs and ILD extent, especially if administration occurs in patients with a disease duration less than 12 months or in case of acute onset or exacerbation [78]. RTX treatment provided evidence of improvement/resolution of ground-glass opacities and stability/improvement of fibrosis allowing also corticosteroid sparing [79,80]. Data on intravenous immunoglobulins are limited but hint to a beneficial effect. They can be used as a rescue therapy even in patients with a severe contraindication to immunosuppression [81,82].

### Pulmonary arterial hypertension

Therapeutic approaches for PAH in ASS patients are similar to that of idiopathic form even though the survival rates seem to be better. Treatment is based on the use of supplemental oxygen to maintain a pulse oximetry saturation more than 90% at rest or under exercise and on drugs acting on the prostanoid, endothelin or nitric oxide pathways [83]. As these medications act on different molecular mechanisms, association therapies are relatively common in clinical practice to obtain a synergic effect [84]. In patients with progressive forms surgical and palliative interventions such as atrial septostomy or lung transplantation strategies are required [85].

## CONCLUSION

ASS is a rare autoimmune inflammatory condition characterized by a worse prognosis compared with polymyositis and dermatomyositis especially due to the severity of lung involvement that is the main determinant of the patient survival. Multidisciplinary evaluation is fundamental as the disease can have a variable clinical presentation. More data on prognostic factors are emerging and they could help in the identification of patients who require an aggressive therapeutic approach or a tighter follow-up. Recent studies have underlined the relationship between clinical manifestations and autoantibody specificity but larger cohorts are needed to validate this association. Glucocorticoids and immunosuppressants, alone or combined, are variably used to treat mild-to-severe cases and in refractory forms RTX is showing efficacy by improving PFT and ILD extent.

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## Conflicts of interest

*There are no conflicts of interest.*

## REFERENCES AND RECOMMENDED READING

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- ■ of special interest
- ■■ of outstanding interest

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In 2017, a multidisciplinary task force updated the European League Against Rheumatism/American College of Rheumatology classification criteria for immune inflammatory myopathies (IIM). The experts delineated the major subgroups of IIM and also introduced the definitions of 'possible', 'probable' and 'definite' diagnosis based on the calculation of a probability score.

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REVIEW

OPEN

# Genetics of idiopathic inflammatory myopathies: insights into disease pathogenesis

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**Purpose of review**
To review the advances that have been made in our understanding of the genetics of idiopathic inflammatory myopathies (IIM) in the past 2 years, with a particular focus on dermatomyositis and polymyositis.

**Recent findings**
Fine-mapping studies in the major histocompatibility complex region in Caucasian and Korean populations have identified novel human leukocyte antigen (HLA) variants that are associated with autoantibody subgroups in IIM. Differences in HLA associations have been identified between Caucasian adult-onset and juvenile-onset patients with anti-TIF1 autoantibodies, suggesting distinct aetiologies in these patients. For some autoantibodies, the strongest associations identified are specific amino acid positions within HLA molecules, providing mechanistic insights into disease pathogenesis.
A meta-analysis combining data from four seropositive rheumatic diseases identified 22 novel non-HLA associations in IIM, of which seven were previously reported at suggestive significance in IIM. A genome-wide association study conducted in the Japanese population identified a significant association with *WDFY4* in patients with clinically amyopathic dermatomyositis.

**Summary**
Considerable progress has been made in understanding the genetics of IIM, including differences in clinical and autoantibody subgroups. As research continues, there should be a focus to increase statistical strength and precision by conducting meta-analyses and trans-ethnic studies.

**Keywords**
genetics, genome-wide association study, human leukocyte antigen, idiopathic inflammatory myopathies, myositis

## INTRODUCTION

The idiopathic inflammatory myopathies (IIM), collectively known as myositis, are a group of rare autoimmune diseases. They are characterized primarily by skeletal muscle weakness and muscle inflammation, and commonly present with extramuscular manifestations such as skin rash, interstitial lung disease, polyarthritis and cancer. IIM are heterogeneous, and are clinically subclassified as dermatomyositis, inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM), polymyositis and anti-synthetase syndrome (ASS) [1,2]. IIM are complex diseases thought to be initiated by immune activation following specific environmental events in genetically predisposed individuals. Significant progress has been recently made in studying the genetics of IIM, and we are beginning to understand more about the genetic architecture of these rare diseases. The present article reviews the advances that have been made in the past 2 years in our understanding of the genetics of IIM, in particular dermatomyositis and polymyositis.

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## KEY POINTS

- A large HLA imputation study in the Caucasian population has identified strong associations with classical HLA alleles and amino acid positions with myositis autoantibodies.
- Differences in HLA associations have been identified between Caucasian adult-onset and juvenile-onset patients with anti-TIF1 autoantibodies, suggesting distinct causes in these patients.
- HLA association studies in non-European populations suggest unique immunogenetic backgrounds.
- A meta-analysis combining data from four seropositive rheumatic diseases has identified novel non-HLA associations in IIM.

## MAJOR HISTOCOMPATIBILITY COMPLEX GENETICS

In IIM, as in many other autoimmune and immune-mediated diseases, the strongest genetic risk for disease susceptibility lies within the major histocompatibility complex (MHC), and is thought to localize to specific human leukocyte antigen (*HLA*) alleles [3]. The 8.1 ancestral haplotype (AH), a common haplotype of extensive linkage disequilibrium in Caucasian populations, is a strong risk factor for IIM and many other autoimmune diseases. Identifying which genes in the MHC region are causal is an ongoing subject of debate.

Distinct *HLA* associations have been described in IIM in different populations, clinical subgroups and with specific clinical features, however the strongest *HLA* associations are found when stratifying by autoantibody status [3]. Using data from a large recent genetic association study in IIM, we correlated autoantibody type with imputed *HLA* genotype in patients to identify novel risk variants in the MHC region that confer susceptibility to IIM autoantibodies [4▪▪]. SNP2HLA was used to impute HLA gene and amino acid variants using a large imputation reference panel [5]. Associations with alleles of the 8.1 AH were observed for patients with anti-Jo-1, anti-PM/Scl and anti-cN1A autoantibodies. Anti-HMGCR and anti-Mi-2 were the only autoantibodies tested that were associated with *HLA* alleles not forming part of the 8.1 AH. For patients with anti-Jo-1 autoantibodies, there was an additional independent effect of the class I allele *HLA-B*08:01*. This allele also forms part of the 8.1 AH suggesting that multiple independent risk factors within this haplotype confer risk of autoimmunity. Indeed, other genes on the 8.1 AH may predispose individuals to immune-mediated diseases, such as NF-κB and tumour necrosis factor-α polymorphisms [6,7], and copy number variants of complement genes [8]. In addition, a recent study in anticitrullinated protein antibody (ACPA) positive rheumatoid arthritis (RA) has identified a risk within *HLA-DOA*, a nonclassical *HLA* gene, suggesting *HLA* alleles not imputed by SNP2HLA may contribute to disease risk [9].

There is also evidence of genetic differences between adults and juveniles with the same autoantibody, as has been reported previously with anti-HMGCR autoantibodies [9,10]. Of particular interest in IIM are the different haplotype associations for adult-onset and juvenile-onset patients with anti-TIF1 autoantibodies [4▪▪], where there is a strong association with cancer in adult-onset disease but not juvenile-onset disease [11,12]. After stratifying by age, an association between anti-TIF1 autoantibodies and the 8.1 AH was present in juvenile-onset patients but not adult-onset patients. This suggests that there may be distinct causes that differentiate adult-onset and juvenile-onset disease. For example, genetic modifications including somatic mutations and loss of heterozygosity in the *TRIM33* gene encoding TIF1γ have recently been reported in tumours in adults with cancer-associated myositis [13▪]. In juvenile-onset patients there may be environmental triggers such as infection [14,15].

To refine the associations within the *HLA* region, SNP2HLA imputes amino acid locations within HLA proteins, which may give mechanistic insights in to disease pathogenesis. For some IIM autoantibodies, amino acid locations were more strongly associated than classical alleles alone [4▪▪]. For example, amino acid position 74 of HLA-DRB1, which faces inwards of the peptide binding groove in HLA DR molecules, was the most strongly associated amino acid position in patients with anti-Jo-1, anti-PM/Scl and anti-cN1A autoantibodies. This location has been implicated in other autoimmune diseases in Caucasian individuals [16–18]. Identification of shared amino acid signatures across different ethnicities may give insight in to whether these positions are functionally important for susceptibility to certain autoantibody profiles [19].

Many of these risk associations have been validated in a large phenome-wide association study (PheWAS), which examined relationships between classical *HLA* variants and amino acid positions, and a range of human disease phenotypes taken from electronic health records in European ancestry individuals [20]. An online catalogue reported associations of *HLA-DRB1*03:01* and *HLA-B*08:01* in a range of autoimmune diseases, including strong associations with polymyositis and dermatomyositis.

Another study investigating *HLA*–autoantibody associations has been conducted in the Korean population. *HLA-DRB1* and *HLA-DPB1* alleles were typed in 179 IIM patients with dermatomyositis ($n = 129$) or polymyositis ($n = 50$) [21▪]. Correlations between individual *HLA* alleles and anti-MDA5, anti-aminoacyl-tRNA synthetase (anti-ARS), anti-TIF1, anti-SRP and anti-Mi-2 autoantibodies were investigated. Strikingly, the observed associations suggest a unique immunogenetic background of Korean patients with myositis compared to the Caucasian population. Differences can also be seen in the frequency of autoantibodies. The most common autoantibody in this study was anti-MDA5 in 26.8% of patients tested ($n = 48$), and a strong association was reported with *HLA-DRB1*12:02* ($P_{corr} = 0.001$; OR = 5.46; 95% CI, 2.67–11.20). In Rothwell *et al.* [4▪▪], anti-MDA5 autoantibodies were detected in 1.7% of patients tested ($n = 35$); however, no *HLA* allele was associated at a study wide significance level of $P < 2.9 \times 10^{-5}$. Notably, *HLA-DRB1*12:02* is very rare in Caucasian populations (http://www.allelefrequencies.net) [22]. There was no strong association with *HLA-DRB1*03:01* on the 8.1 AH in Korean IIM patients, likely because of the rarity of this haplotype in Asian populations [23,24]. An association was identified with anti-ARS autoantibodies and *HLA-DRB1*08:03* ($P_{corr} = 0.02$; OR = 4.15; 95% CI, 1.89–9.09). The only antibody with the same association in the Korean population and Caucasians is anti-Mi-2 (*HLA-DRB1*07:01*, $P_{corr} = 0.0003$; OR = 10.23; 95% CI, 3.81–27.51). Although classical *HLA* associations differ across ethnicities, there may be features of risk alleles or haplotypes that are shared across populations. *HLA* fine-mapping analysis results reflect the linkage disequilibrium structure of examined populations, therefore including different ethnic populations could contribute to identification of additional independent association signals by breaking down population specific haplotype blocks. Further work investigating ethnic heterogeneity within the MHC in IIM therefore may be informative.

*HLA* associations have been described in other Asian populations, including the Japanese [25] and Vietnamese [26]. There have also been multiple candidate gene studies in the Han Chinese population, reporting novel associations with *HLA-DRB1* and *HLA-DPB1* alleles and clinical features of disease [23,27], and with anti-MDA5 autoantibodies [27,28]. Because of the smaller studies in the Han Chinese population, genetic associations with clinical subgroup or autoantibody status are often conflicting, or underpowered. Conducting a meta-analysis or systematic review of these studies to identify genuine associations in this population could be revealing.

## GENOME-WIDE ASSOCIATIONS

The largest genetic association study to date in IIM was conducted using the Immunochip including 2566 patients with polymyositis, dermatomyositis, juvenile dermatomyositis (JDM) and IBM [29]. The *HLA* region and *PTPN22* gene were both associated at genome-wide significance, and there were a number of loci that reached the study-wide significance threshold of $P < 2.25 \times 10^{-5}$. A recent meta-analysis combined GWAS data from four systemic seropositive rheumatic diseases; systemic sclerosis, systemic lupus erythematosus, RA, and IIM [30▪▪]. The authors report 26 shared genome-wide significant loci, five of which have never been associated with these diseases before. IIM contributed to 22 of the observed associations, and interestingly seven of these associations were the same as those found in the Immunochip study reaching study-wide significance (*PTPN22, NAB1, STAT4, DGKQ, FAM167A-BLK, YDJC*). *TYK2* has also been reported before in IIM in a separate candidate gene study [31]. Validating what we already know about the genetic architecture of autoimmune disease, many of the associations identified are significantly enriched in regulatory regions in relevant immune cells, and many are expression quantitative trait loci (eQTL) for genes involved in the immune system [32]. This analysis strengthens our confidence in associations previously reported as suggestive in IIM. Many of the reported associations are enriched in drug targets either being tested or currently used in these diseases, showing how genetic data can be used to identify potential novel therapies in IIM or for drug repositioning.

Until recently, GWAS conducted in IIM have been in the Caucasian population [29,33,34]. The first GWAS in the Asian population was recently conducted in a Japanese cohort comprising 236 patients with polymyositis and 340 patients with dermatomyositis, of which 33 patients had clinically amyopathic dermatomyositis (CADM) [35▪▪]. Interestingly, there was no association with the MHC region in contrast to the strong genetic risk seen in Caucasian studies. No genome-wide significant associations ($P < 5 \times 10^{-8}$) were found with the total IIM cohort, or the dermatomyositis and polymyositis subgroups. A significant association with a SNP intronic of *WDFY4* was reported in 33 patients with CADM (rs7919656; $P = 1.5 \times 10^{-8}$; OR = 3.87; 95% CI, 2.23–6.55). This variant is associated with higher expression of a truncated *WDFY4* isoform and increased expression of NF-κB associated genes. Functional analysis in IIM suggests that WDFY4

 

interacts with pattern recognition receptors, with isoforms of *WDFY4* differentially augmenting NF-κB signalling. Notably, the lead association in the Japanese CADM subgroup was not significant in a separate analysis of 21 European CADM cases, however independent variants in *WDFY4* were seen at nominal significance (rs2889697, $P = 0.0058$).

## GENE–ENVIRONMENT ASSOCIATIONS

IIM are thought to be initiated by immune activation following specific environmental events in genetically predisposed individuals. As yet, there are few validated environmental risk factors identified for IIM. As extensive genetic data are being generated on more myositis patients and broader clinical data collected, we are able to replicate some of the gene–environment interactions that have previously been suggested in IIM.

Smoking is thought to be a risk factor in IIM for the development of anti-Jo-1 autoantibodies [36]. A recent study by Schiffenbauer *et al.* [37] explored this association, showing that smoking was associated with an increased risk of developing polymyositis, anti-Jo-1 and anti-synthetase autoantibodies, with the greatest risk attributable to those who smoked and carried the *HLA-DRB1*03:01* allele. The risk of only one of these two risk-factors was intermediate. Interestingly, an inverse association was found with anti-TIF1, where smokers with *HLA-DRB1*03:01* were less likely to have these autoantibodies.

A further environmental risk factor for IIM appears to be ultraviolet (UV) radiation, as suggested by data showing that the likelihood of developing dermatomyositis over polymyositis significantly increases towards the equator [38], attributable to higher UV exposure [39]. In particular, one study has shown that UV radiation is associated with the relative proportion of individuals with anti-Mi-2 autoantibodies, where patients' characteristic skin changes are more prominent on the sun-exposed parts of the body [40]. A recent study investigated whether there may be genetic risk factors that could explain the latitudinal gradient of dermatomyositis prevalence, in addition to UV exposure [41]. The authors analysed the association of latitude with classical *HLA* alleles and SNPs associated with IIM and dermatomyositis autoantibodies in healthy control subjects. The authors confirm an increase in prevalence of dermatomyositis towards the equator, and report a novel finding that the frequency of anti-TIF1 autoantibodies is negatively correlated with latitude. In addition, *HLA* alleles significantly associated with anti-Mi-2 and anti-TIF1-γ autoantibodies also were strongly negatively associated with latitude, suggesting that genetic background, in addition to UV exposure, may contribute to the distribution of dermatomyositis.

## GENE EXPRESSION STUDIES IN IDIOPATHIC INFLAMMATORY MYOPATHIES

As the cost of RNA sequencing drops, we will begin to understand in finer detail genes that are differentially expressed in subgroups of IIM, and between different target tissues. A recent study investigated differential gene expression profiles in T cell subsets that differ between polymyositis and dermatomyositis [42]. Inflammatory cells infiltrating muscle fibres is a hallmark feature of IIM. In polymyositis, there is a predominance of CD8+ T cells, whereas in dermatomyositis CD4+ T cells predominate, along with plasmacytoid dendritic cells and B cells. RNA-sequencing was conducted on CD4+ T cells (polymyositis = 8 and dermatomyositis = 7) and CD8+ T cells (polymyositis = 4 and dermatomyositis = 5) isolated from peripheral blood mononuclear cells. Although overall gene expression was similar between polymyositis and dermatomyositis in T cells, differential gene expression analysis revealed 176 genes expressed in CD8+ T cells, that differ between patients with polymyositis compared to dermatomyositis. Many of these genes are involved in lymphocyte migration and T-cell differentiation. In contrast, in the CD4+ analysis, only two genes were significantly differentially expressed; *ANKRD55* and *S100B*. Interestingly, *ANKRD55* is a strong genetic risk factor for a number of autoimmune diseases [43–46].

A study by Pinal-Fernandez *et al.* [47] used RNA-sequencing to quantify the expression of IIM autoantigens in muscle and regenerating muscle, and to investigate whether autoantigen expression correlates with the corresponding autoantibody. RNA from 106 muscle biopsies from patients with IIM autoantibodies (anti-HMGCR, anti-Jo-1, anti-NXP2, anti-TIF1γ, anti-Mi-2, anti-SRP, and anti-MDA5) and 20 healthy controls were sequenced. All IIM autoantigens studied were expressed in muscle biopsies, and the levels positively correlated with markers of muscle regeneration. Autoantigens were also expressed in regenerating mouse muscles and in cultured human myoblasts. Notably, the expression of IIM autoantigens was not associated with the presence of the corresponding autoantibody. It is still unknown why one autoantigen is preferentially targeted by the immune system in patients with IIM.

Many studies in IIM report a strong interferon (IFN) signature in which there is upregulation of type I IFN inducible transcripts and activation of the type I IFN pathway [48]. This is in keeping with

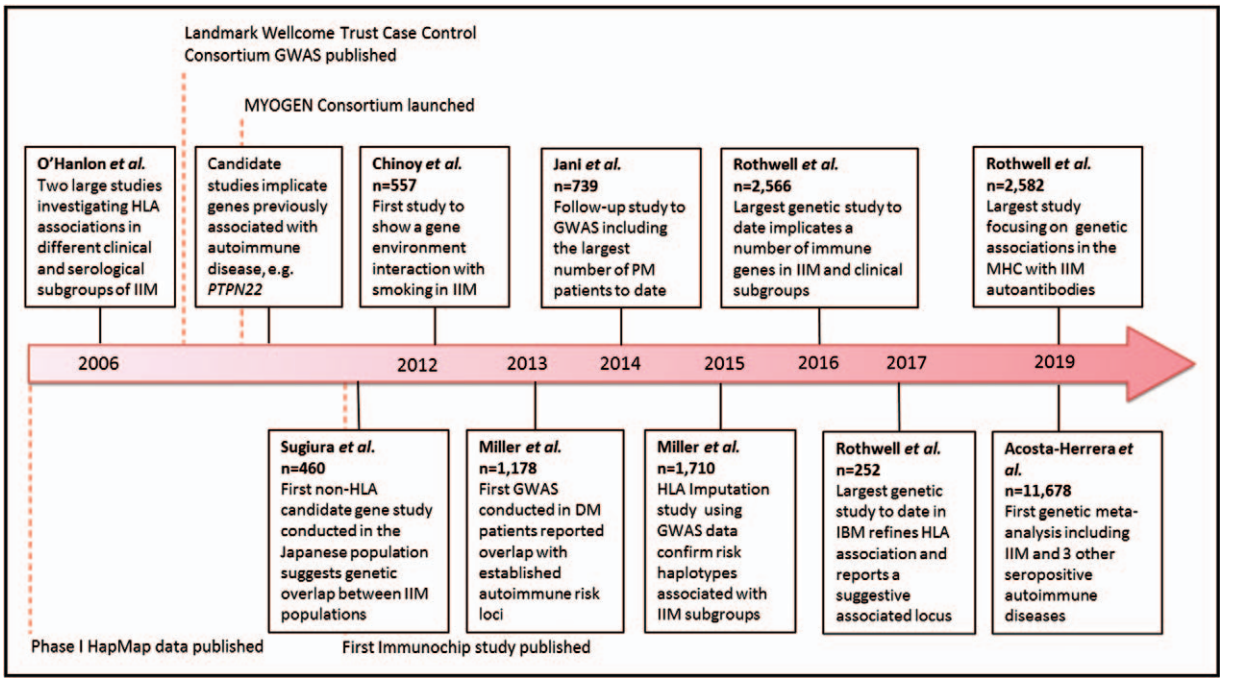


**FIGURE 1.** Timeline of key genetic studies published in IIM. Landmark studies that have been published in IIM genetics research, and the progress that has been made since the last IIM genetics review in 2016 [52].

the function of genes that have been reported in the GWAS meta-analysis discussed above, such as *NAB1*, *TYK2*, *PTPN11*, *IRF5*, and *IRF8* [30▪▪]. Moreover, recent studies have stratified patients by clinical subgroup and found differences in IFN signature in muscle biopsies [49,50]. Dermatomyositis is commonly associated with a type I-IFN signature, but distinct associations with IBM and ASS and type-II IFN, and the lack of an IFN signature in IMNM, suggest differences in pathogenesis and potential for targeted therapies in IIM.

## CONCLUSION

Considerable progress has been made in our understanding of the genetics of IIM, as summarized in Fig. 1. The strongest genetic risk lies within the MHC region, and we are beginning to understand in greater detail how heterogeneity within this region contributes to disease susceptibility in different clinical and autoantibody subgroups of IIM. As research continues in different populations, there should be a focus to increase statistical strength and precision by combining the results in trans-ethnic meta-analyses. As we have seen from genome-wide association studies, leveraging power from other related diseases, either by meta-analysis or novel statistical techniques [51], will continue to reveal more about the genetic architecture of IIM.

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### Conflicts of interest

*There are no conflicts of interest.*

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- ▪ of special interest
- ▪▪ of outstanding interest

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This is the largest study to date reporting novel risk variants in the MHC region that confer susceptibility to IIM autoantibodies. Associations were seen with many myositis autoantibodies and classical HLA alleles, although for some autoantibodies the strongest association was with amino acid positions within HLA molecules. Differing associations between adult-onset and juvenile-onset patients with anti-TIF1 autoantibodies suggests that there may be distinct aetiologies that differentiate these patients.

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# Using the tools of proteomics to understand the pathogenesis of idiopathic inflammatory myopathies

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**Purpose of review**
One of the most important advances in medical research over the past 20 years has been the emergence of technologies to assess complex biological processes on a global scale. Although a great deal of attention has been given to genome-scale genetics and genomics technologies, the utility of studying the proteome in a comprehensive way is sometimes under-appreciated. In this review, we discuss recent advances in proteomics as applied to dermatomyositis/polymyositis as well as findings from other inflammatory diseases that may enlighten our understanding of dermatomyositis/polymyositis.

**Recent findings**
Proteomic approaches have been used to investigate basic mechanisms contributing to lung and skin disease in dermatomyositis/polymyositis as well as to the muscle disease itself. In addition, proteomic approaches have been used to identify autoantibodies targeting the endothelium in juvenile dermatomyositis. Studies from other inflammatory diseases have shown the promise of using proteomics to characterize the composition of immune complexes and the protein cargoes of exosomes.

**Summary**
There are many relevant scientific and clinical questions in dermatomyositis/polymyositis that can be addressed using proteomics approaches. Careful attention to both methodology and analytic approaches are required to obtain useful and reproducible data.

**Keywords**
dermatomyositis, juvenile dermatomyositis, polymyositis, proteomics

## INTRODUCTION

The past 15 years have seen the emergence of multiple technologies to survey biological processes on a global scale. These include tools to analyze whole genomes [1,2], transcriptomes [3,4], epigenomes [5,6], and metabolic products [7,8] in ways that were previously unimaginable. These technologies, thus provide the opportunity to detect and model complex biological processes and human diseases in ways that take us beyond the small-scale, one-pathway-at-a-time analyses that previously characterized the biological sciences and sometimes resembled the efforts of mythical blind men and the elephant [9]. In recent years, the cost efficiency and scalability of tandem mass spectrometry (MS) has also allowed the analysis of proteins, whether from cells or in body fluids, on a similar global scale [10▪▪,11▪]. In this review, we describe how these approaches have been adapted to the study of inflammatory myopathies, such as polymyositis, dermatomyositis, and juvenile dermatomyositis (JDM). We also discuss new opportunities to use address relevant clinical and biological questions using proteomic approaches.

## PROTEOMIC METHODS

Proteomic methods have been used as a tool to identify and quantify specific proteins, to compare protein expression between groups (for example, individuals with a disease and healthy people), to detect and quantify posttranslational modification of proteins and the sites of those modifications, to assess binding specificities (e.g. binding specificities between proteins and drugs), and to analyze protein complexes [12–17,18▪]. Proteomic approaches may be applied to identify candidate markers associated with different disease phenotypes, to identify drug targets for developing new therapies, and to identify

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## KEY POINTS

- Proteomic methods carry considerable potential for advancing our understanding of the pathobiology of dermatomyositis/polymyositis and provide a promising method to develop clinically useful biomarkers.
- Biological samples, including muscle, skin biopsies, blood, BALF, and immune complexes, have been analyzed by proteomics in dermatomyositis/polymyositis.
- As with global-scale genetic/genomic methods, the utility and reproducibility of proteomic data is dependent on careful attention to both sample preparation and analytic methods.
- Further development of the established database on proteomic analyses would allow a better understanding of the pathobiology of dermatomyositis/polymyositis.

disease biomarkers to assist in diagnosis, the assessment of disease activity, and to clarify prognosis [16,17,18▪,19,20]. In addition, information from proteomic analyses has the potential to elucidate disease pathophysiology and the pharmacological action of therapeutic drugs [21,22]. As proteomic methods permit the use of a wide range of biological samples, such as blood [15,17,23], urine [8,16,24], spinal fluid [25,26], tissue [18▪,27], cultured cells [28,29] (Fig. 1), and exosomes [30,31▪], proteomic methods are becoming an increasingly attractive tool for many fields of biology and medicine.

A challenge to any proteomic analysis is the broad range proteins and biological samples that might be analyzed. Although this broad range is part of what makes this approach attractive, it also presents challenges in establishing standard techniques for proteomic methods. As with genomics and other global scale biological analyses, the quality of the information provided by proteomics approaches is strongly dependent on analytical techniques, analysis methods, sample types, sample conditions (whether freshly obtained, frozen, or embedded in paraffin prior to analysis), and methods for sample preparation. Understanding the limits of proteomic analyses (the actual handling and analysis of the sample) and the statistical methods used to analyze

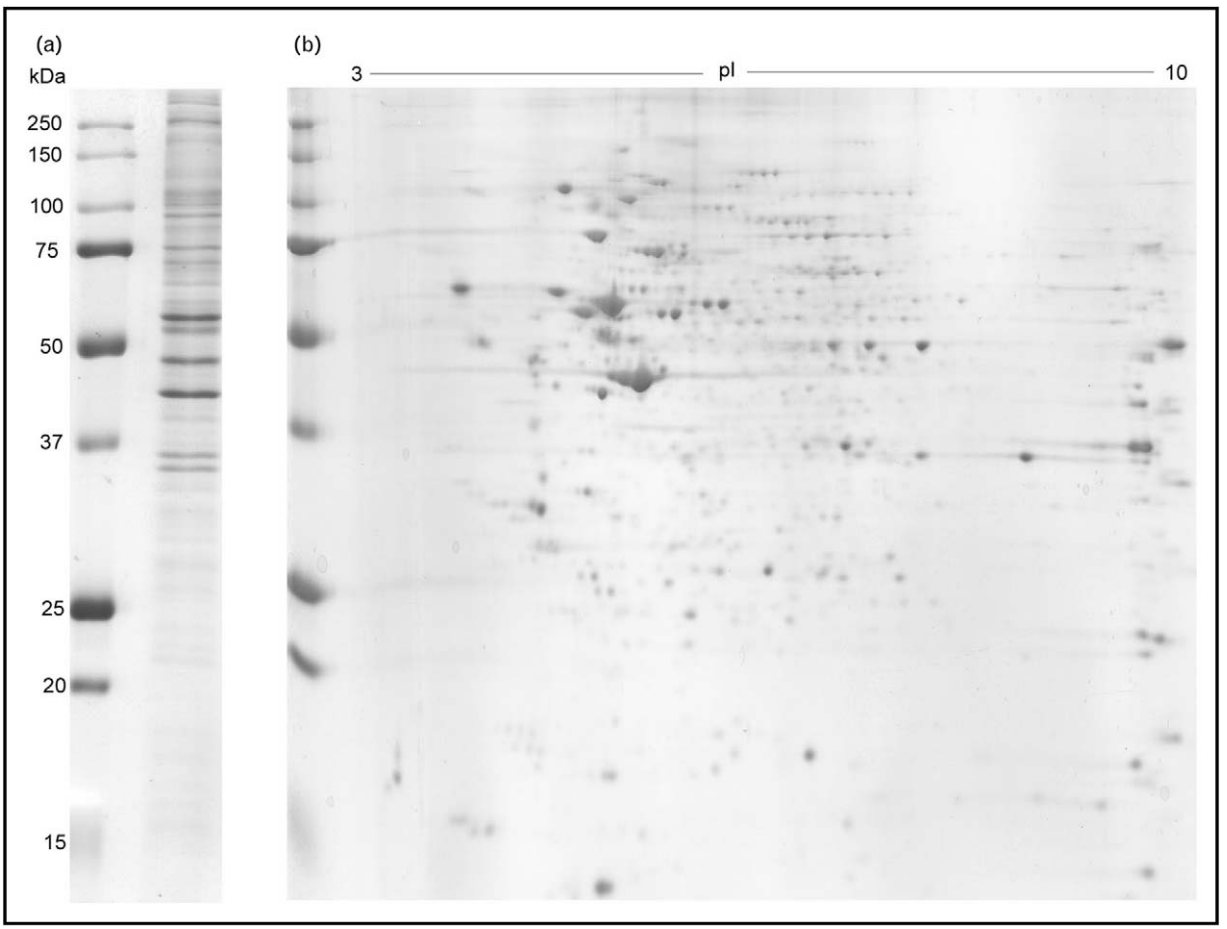


**FIGURE 1.** SDS-PAGE and two-dimensional gel electrophoresis using human aortic endothelial cell extracts. Proteins extracted from HAEC were separated by SDS-PAGE (a) and 2DE (b), respectively. Gels were then stained with Coomassie brilliant blue R-250 (CBB). 2DE, two-dimensional gel electrophoresis; HAEC, human aortic endothelial cells.

the raw data are important in both evaluating the reliability of the experimental results and ensuring reproducibility.

## PROTEOMIC METHODS IN DERMATOMYOSITIS/POLYMYOSITIS

In dermatomyositis/polymyositis, muscle and skin biopsies [32,33], blood [34], bronchoalveolar lavage fluid (BALF) [35] and circulating immune complexes [36] have been analyzed using proteomics techniques. The use of labeling techniques, such as isobaric tags for relative and absolute quantification (iTRAQ) provides a method to quantify protein abundance as well as identify proteins in several different samples simultaneously [32,33]. The primary methods of proteomics include protein separation followed by analysis (protein identification or quantification) using MS. The extracted proteins are usually separated by two-dimensional gel electrophoresis (2DE) [34,35] or by chromatography [32,33,36] prior to analysis by MS. High separation performance is achieved by 2DE, which allows rapid detection of multiple proteins. However, 2DE methods lack the ability to analyze low abundance, insoluble, or high molecular weight proteins (>200 kDa) [37]; it is also insensitive for the detection of proteins outside of an isoelectric point (pI) range of 3–10. High-coverage data and high-throughput analysis may be achieved using high-performance liquid chromatography (HPLC) compared with that of 2DE. HPLC methods generate large numbers of peptide fragments for MS compared with 2DE methods. In either case, as all proteins in compared samples are targets for both HPLC and MS analyses, high capacities for information processing are required to properly interpret the complex data from MS whether HPLC or 2DE are used as the first step in separation, but robust and accurate informatics is especially important when HPLC separation is the first step. It is important to note, as well, that 3D protein structures are lost during MS analyses because of the use of proteases in sample preparation. MS analysis combined with chemical cross-linking of proteins, that is, crosslinking-MS, may overcome some of these limitations in settings where proteomics is used for investigations into structural biology [38]. Understanding the advantages and limitations of the different separation and analysis approaches will guide investigators in selecting samples to study and the combination of techniques that are most appropriate.

## PROTEOMIC RESEARCH IN DERMATOMYOSITIS/POLYMYOSITIS

In dermatomyositis/polymyositis, interstitial lung disease (ILD) is a severe clinical complication. The frequency of ILD in these patients is 20–86%, and 80% of deaths from dermatomyositis/polymyositis have been attributed to ILD [39,40]. Analysis of protein expression in BALF may provide important clues to understanding lung disorders. Passadore *et al.* investigated protein expression profiles in BALF from patients with three different subsets of inflammatory myopathy (dermatomyositis/polymyositis, antisynthetase syndrome, and overlap syndrome) to determine whether there were differences in protein expression that correlated with clinical phenotype. The protein expression of BALF was analyzed using immunoblotting and proteomic analyses using 2DE coupled with mass spectrometry (2DE–MS). The mean number of spots on Coomassie-stained 2DE gels was $258 \pm 48$ in dermatomyositis/polymyositis, and 9 of these spots were identified only in patients with polymyositis/dermatomyositis. These 9 spots contained 11 different proteins, including cytoskeleton proteins, tissue architecture proteins, and proteins related to oxidative stress [35]. The authors concluded that these proteins might serve as useful biomarkers for diagnosis or to monitor disease activity, but acknowledged that larger studies would be necessary. To compare the protein expression profiles in BALF from dermatomyositis/polymyositis with those from controls and /or control diseases (other than different forms of inflammatory myopathy) may be useful in better understanding the differences in the different myopathic phenotypes and to provide a firmer grasp of the underlying biology of these diseases.

Skin lesions are a characteristic of dermatomyositis, including mucin deposition and lymphocytic infiltration, findings that are also seen in systemic lupus erythematosus (SLE) [41,42]. Furthermore, increased expression of interferon-related genes is detected in the skin from patients with both conditions [43,44]. Nakamura *et al.* investigated the participation of mucin deposition and interferon signature in development of skin lesions in patients with dermatomyositis using proteomic methods. To assess protein expression changes in skin samples from dermatomyositis, samples were compared with those from healthy controls by quantitative proteomic analysis. Twenty-six up-regulated proteins and 34 down-regulated proteins were detected in dermatomyositis skin. Out of 60 proteins identified, proteasome subunit beta type 9 (PSMB9) and versican V1 were upregulated in the epidermis of dermatomyositis and SLE skin, and type I collagen was downregulated in the dermis of dermatomyositis and SLE skin. Upregulated expression of PSMB9 was specific to dermatomyositis and SLE in some skin diseases. Overexpression of PSMB9 induced by interferon led to expression of transforming growth

factor (TGF)-β2/β3 in epidermal keratinocytes, which induces increased expression of versican in dermal fibroblasts. Interestingly, the expression of TGF-β2 was increased only in epidermis of dermatomyositis patients. The pathway via ΔDiHS-diS1 and TGF-β2 have the potential to be involved in protein expression changes in dermatomyositis skin [32]. A larger number of patients will likely need to be studied to fully understand the exact roles that PSMB9 plays in dermatomyositis skin lesions.

Previous reports have suggested that mitochondrial dysfunction and/or changes are involved in a broad range of muscle diseases [45,46]. Sunitha *et al.* investigated whether protein changes in mitochondria, specifically, the state of mitochondria and the effect of protein oxidation, are observed in muscle biopsy samples from patients with muscle diseases, including polymyositis. Using quantitative proteomic analysis, mitochondrial extracts from muscle samples were compared between muscle diseases and healthy controls. These authors detected 36 up-regulated proteins and 95 down-regulated proteins in samples from patients with polymyositis. Interestingly, the authors identified three proteins that were down-regulated only in polymyositis but not in other muscle diseases. The down-regulated proteins were mainly characterized as electron transport chain complex subunits, assembly factors, or Krebs cycle enzymes. In this study, the up-regulated proteins were not analyzed. More tryptophan oxidation was also observed in mitochondrial proteins from polymyositis compared with controls. The authors concluded that alterations of muscle mitochondria, including changes in mitochondrial proteins and protein oxidation, play an important role in mitochondrial damage in human muscle diseases, including polymyositis [33].

Prominent features of vascular and perivascular inflammation are observed in muscle biopsies of children with JDM and contribute to the observed clinical manifestations. Anti-endothelial cell antibodies (AECA) are detected in a variety of infectious, and inflammatory diseases, such as vasculitis [47–49]. However, the target antigens for AECA are generally not known, and thus clinical and pathological significance of AECA remains uncertain [50]. Karasawa *et al.* investigated the presence of AECA in JDM using extracted proteins from human aortic endothelial cells (HAEC) and plasma from patients with clinically active disease, to identify target antigens for AECA using proteomic methods. Using immunoblotting and proteomic analyses using 2DE–MS, 22 candidate target autoantigens were identified, including heat shock cognate 71 kDa protein (HSC70), prelamin-A/C, and heat shock protein 90-beta. Four-fifths of the autoantigens detected were proteins associated with antigen processing and protein trafficking, a provocative finding given the known role of endothelial cells in antigen processing and presentation. Using ELISA assays, these authors confirmed the presence of antibodies to HSC70 in JDM plasma. Furthermore, HSC70 antibodies showed a strong correlation with untreated disease. This study shows that AECA are detected in plasma from patients with JDM and the presence of autoantibodies to HSC70, a target of AECA, may have the potential to be an useful biomarker in JDM [34]. The limitations of this study are that 2DE gel patterns of proteomes do not detect all expressed proteins in the cells, and thus do not provide a comprehensive analysis of all potential autoantigens. Furthermore, reproducibility of ELISA data should be evaluated in large number of plasma samples.

## OTHER POTENTIAL USES OF PROTEOMICS IN DERMATOMYOSITIS/POLYMYOSITIS

Circulating immune complexes have long been recognized as a feature of rheumatic diseases, including dermatomyositis [51]. There is a considerable literature reporting efforts at characterizing the composition and biological behavior of immune complexes in rheumatic diseases [52–54]. However, available methods in these early studies were largely limited to gradient density centrifugation (to characterize immune complex size), and immunodiffusion and western blotting (e.g. for complement proteins and immunoglobulins) to characterize immune complex content. The relatively high sensitivity of MS provides the opportunity to revisit some of these earlier studies with an aim toward identifying potential autoantigens. For example, Low *et al.* [55] have described a proteomic analysis of immune complexes from sera of children with juvenile idiopathic arthritis (JIA) analyzed by 2DE and MS. These authors identified high levels of GAPDH, α-1AT, and a precursor to serotransferrin in JIA immune complexes, although whether the presence of these protein antigens within immune complexes were a cause or a consequence of the chronic inflammatory state could not be ascertained. More recently, Aibara *et al.* [56] analyzed immune complexes immobilized on protein A from the cerebrospinal fluid (CSF) of patients with inflammatory disorders of the central nervous system, including neuropsychiatric systemic lupus erythematosus (NPSLE), multiple sclerosis, neuromyelitis optica, Alzheimer's disease, and Hashimoto's encephalopathy. Using nanoliquid chromatography combined with tandem MS, they identified

176 antigens within immune complexes from the CSF samples. Although there was considerable overlap in immune complex-associated proteins identified in the different diseases, a protein called suprabasin (SBSN) was identified only in patients with NPSLE, suggesting that this protein may serve as a diagnostic biomarker for NPSLE. Finally, Ohyama *et al.* [36] have demonstrated the feasibility of identifying antigens within immune complexes in a broad range of rheumatic/autoimmune diseases, including dermatomyositis. These studies suggest that isolation and characterization of immune complexes from patients with inflammatory muscle diseases, such as dermatomyositis/polymyositis may be equally fruitful.

More recently, there has been increasing interest in characterizing the protein contents of extracellular vesicles, such as exosomes in a broad range of infectious and rheumatic diseases [57,58] in efforts to better understand the pathobiology of these conditions. Exosomes and other microvesicles play an important role in intercellular communication and, while there remain significant challenges to obtaining accurate and reproducible proteomic data from exosomes purified either from body fluids or cell cultures [59], this is likely to be a fruitful area of investigation that will complement parallel efforts to examine the RNA cargoes in exosomes and other microparticles. Specifically, proteomic analysis of exosomes in polymyositis/dermatomyositis can be expected to provide useful insight into the mechanisms through which the immune system identifies and injures the affected target tissues.

## CONCLUSION

Proteomic methods are used to compare protein expression between groups, to identify and quantify specific proteins, and to detect and quantify post-translational modification of proteins in dermatomyositis/polymyositis study. Proteomic methods are an important tool for detection and/or identification of key proteins involved in diagnosis, monitoring of disease activity, therapeutic effect, and disease pathophysiology in dermatomyositis/polymyositis. Using this approach, however, there are no target proteins developed for clinical application in dermatomyositis/polymyositis. Like genomic analyses, the development of standardized methods and further development of the established database on proteomic analyses will be required to solve this problem.

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### Conflicts of interest

*There are no conflicts of interest.*

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# Diagnosis and treatment of myasthenia gravis

*Renato Mantegazza and Paola Cavalcante*

**Purpose of review**
This article provides an update on the most recent advances in diagnostic procedures and therapeutic approaches for myasthenia gravis, spanning from autoantibody and neuroelectrophysiological tests as diagnostic tools, to innovative and promising treatments based on biological drugs.

**Recent findings**
Novel studies performed by cell-based assays (CBAs) indicate an improvement in the chance of identifying serum autoantibodies in myasthenic patients. Clinical trials on the use of biological drugs were recently concluded, providing important data on safety and efficacy of eculizumab, efgartigimod and amifampridine phosphate: the first, a complement blocker, showed long-term safety and efficacy in acetylcholine receptor (AChR)-positive myasthenic patients with refractory generalized disease; the second, the neonatal Fc receptor blocker, was well tolerated and clinically effective in both AChR-specific and muscle-specific kinase receptor (MuSK)-positive patients; the third, a blocker of presynaptic potassium channels, was found to be well tolerated and effective in MuSK-positive patients.

**Summary**
CBAs can lead to a significant reduction of seronegative patients, improving myasthenia gravis diagnostic process. New biological drugs offer innovative approaches to treat myasthenic patients with generalized disease, promising to change the paradigm of treatment and to significantly enhance therapeutic success within a precision medicine framework.

**Keywords**
autoantibodies, biologic agents, immunosuppressive drugs, myasthenia gravis, precision medicine

## INTRODUCTION

Myasthenia gravis is an autoimmune disease causing neuromuscular junction (NMJ) impairment, characterized by weakness and easy fatigability on exertion involving different skeletal muscle districts [1]. The autoimmune attack is mediated by autoantibodies targeting key functional and structural NMJ proteins: the acetylcholine receptor (AChR), the muscle-specific kinase receptor (MuSK) or the low-density lipoprotein receptor-related protein 4 (LRP4) [1]. In AChR-MG, morphological and functional changes (i.e. follicular hyperplasia and thymoma) of the thymus are pathologically relevant, and a wealth of data indicates that this organ is a main site of anti-AChR autosensitization, ultimately leading to autoantibody production and chronic autoimmunity [2]. Myasthenia gravis diagnosis is clinical, instrumental and pharmacological; the latter particularly used in seronegative patients [3▪]. Recently, recommendations for myasthenia gravis treatment have been published [4,5▪]. However, treatment improvement of myasthenic patients is still a medical need as a substantial proportion of them are refractory or intolerant [6].

The current article provides an update on the diagnostic and therapeutic strategies for myasthenia gravis in relationship with the clinical heterogeneity of the disease, highlighting how the introduction of novel biological agents and the development of precision medicine approaches, based on predictive biomarkers, could significantly improve therapeutic success in a cost/effective manner.

## MYASTHENIA GRAVIS: CLINICAL PRESENTATION AND CLASSIFICATION

Clinical examination and disease course are crucial in myasthenia gravis diagnosis: muscle weakness

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## KEY POINTS

- Cell-based assays, based on autoantigen-transfected cells, represent the future development of diagnostic assays for myasthenia gravis because of their high sensitivity and ability to reflect antibody binding as in pathophysiological context.
- Biological drugs (such as eculizumab or efgartigimod) promise to improve and modify the therapeutic approaches for myasthenia gravis, significantly increasing the quality of life of patients with refractory disease.
- The identification of biomarkers able to predict drug efficacy in individual patients, or specific patients' subgroups, could promote the rapid development of precision medicine approaches for efficient myasthenia gravis treatment.

upon repetitive exercise and fluctuating over time is suggestive of the disease. Ocular, skeletal, bulbar and respiratory muscles are variably involved. Ocular symptoms are frequently observed at onset and may convert into a generalized form, usually within the first 2–5 years [7], as described in a recent retrospective population-based study [8▪]. When myasthenia gravis becomes bulbar and respiratory, the disease can be life-threatening [1].

Clinical manifestations may vary because of severity and muscle group involvement, and patients can be stratified into disease subgroups (Fig. 1) [9,10], according to autoantibodies, age at onset and thymic histology [1,11,12]. Myasthenia gravis stratification may be relevant to prognosis and treatment response, thus becoming functional to a personalized approach. MuSK-MG, predominantly ocular and bulbar, has a high risk of a severe clinical course with a lower chance of achieving complete stable remission than AChR-MG; however, improvement of treatment has induced a reduction of respiratory crisis and a better clinical outcome [13]. MuSK-MG is usually not associated with thymic abnormalities and, after thymectomy, a favorable clinical outcome was not observed, as recently reported by a retrospective multicenter study [14▪]. On the other hand, a thymic hyperplasia has been detected in 23% of patients in whom

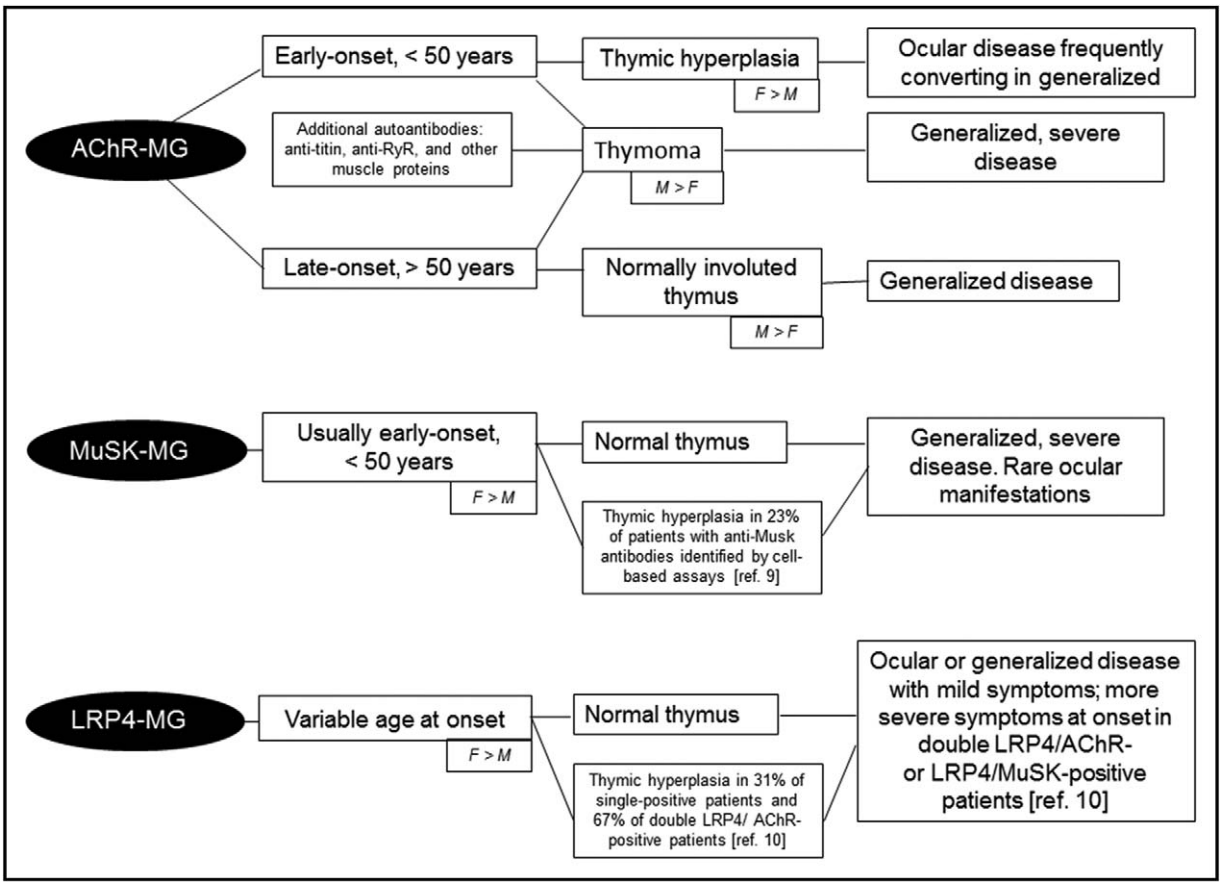


**FIGURE 1.** Clinical features of myasthenia gravis subgroups classified based on autoantibody status. AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor-related protein 4; MG, myasthenia gravis; MuSK, muscle-specific tyrosine-kinase; RyR, ryanodine receptor.

anti-MuSK antibodies were identified only by a cell-based assay (CBA) [9]. LRP4-MG shows clinical features and response to treatment similar to those of AChR-MG, thymoma is usually absent, and thymic hyperplasia was found in 31% of single LRP4-positive and 67% of double LRP4/AChR-positive patients [10]. A recent report showed an absence of hyperplasia in the thymus of four LRP4-MG patients, and one of them went into clinical remission after thymectomy alone, and another one improved after thymectomy in combination with immunosuppressive therapy [15▪]. A clinical examination of Chinese LRP4-MG patients reported that symptoms were mild and responses to acetylcholinesterase inhibitors and prednisone were mostly successful [16▪].

Thymoma-associated myasthenia gravis is usually more severe than non-thymomatous disease [1,17]. Such a finding was recently observed in a retrospective study performed in 230 Italian myasthenia gravis patients in which thymoma patients reached higher clinical severity and higher antibody titers than patients without thymoma [18▪▪]. In the same study, novel HLA associations were detected: DQB1∗05:01 was correlated with thymoma, and DQB1∗05:02/DRB1∗16 haplotype with late-onset (>60 years) non-thymoma AChR-MG, underlying distinct susceptibility to the disease [18▪▪].

## THE DIAGNOSTIC PROCESS

Serum autoantibody determination is the most specific diagnostic tool for the disease. Electromyography (EMG) and clinical response to cholinesterase inhibitors are important for diagnosis confirmation, particularly for seronegative patients, who need differential diagnosis to distinguish myasthenia gravis from other neuromuscular transmission disorders (Fig. 2).

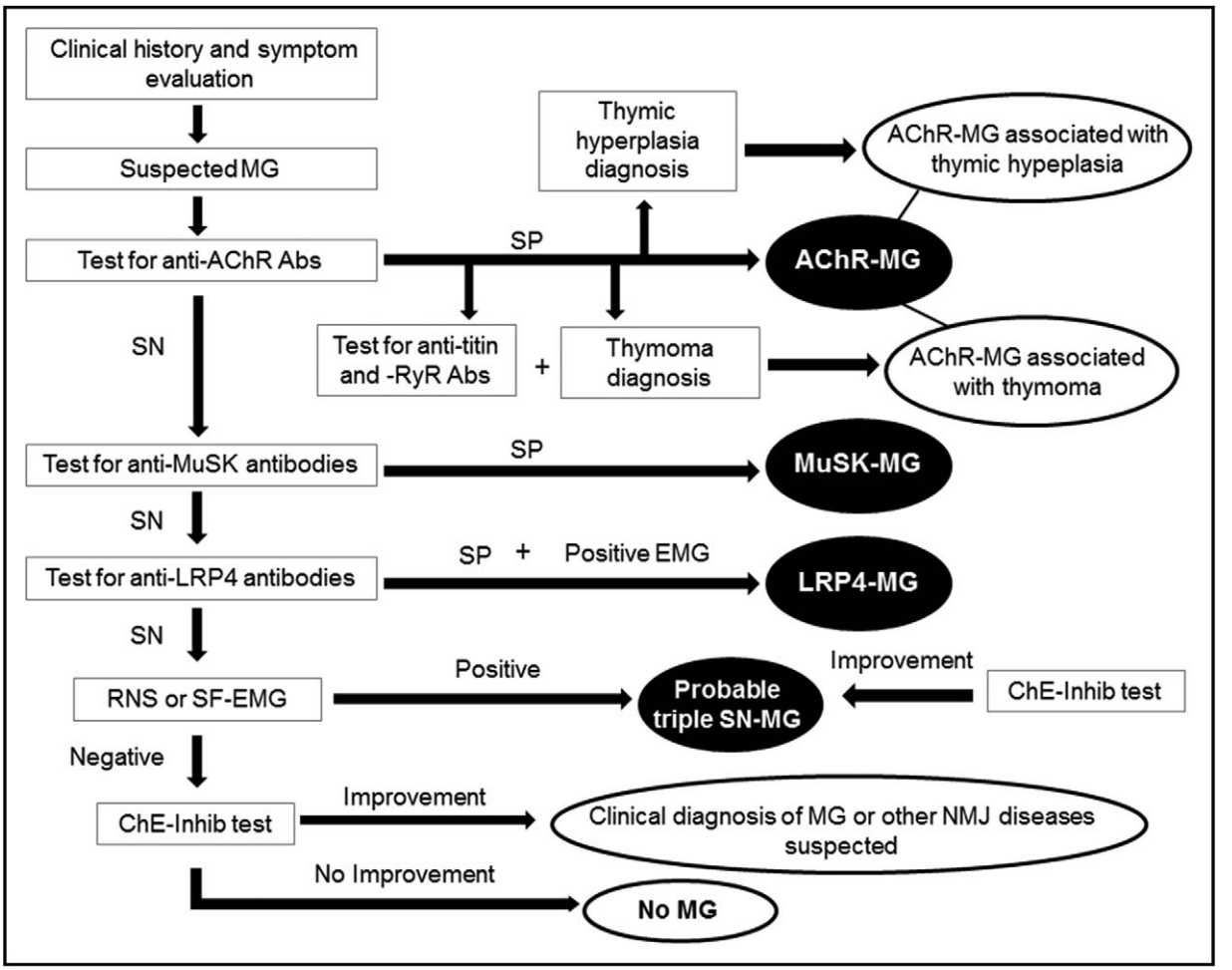


**FIGURE 2.** Diagnostic algorithm for myasthenia gravis. Abs, antibodies; AChR, acetylcholine receptor; ChE-Inhib, cholinesterase inhibitor; LRP4, low-density lipoprotein receptor-related protein 4; MG, myasthenia gravis; MuSK, muscle-specific tyrosine-kinase; NMJ, neuromuscular junction; RyR, ryanodine receptor; RNS, repetitive nerve stimulation; SF-EMG, single-fiber electromyography; SP, seropositive; SN, seronegative.

### Autoantibody testing as specific diagnostic tool

Determination of anti-AChR antibodies, observed in ~80% of patients [1], is the first assay to be performed; if negative anti-MuSK antibodies, present in 5–8% of patients [1], should be searched (Fig. 2). Anti-LRP4 antibodies, the more recent diagnostic marker for the disease [10], should be tested in double AChR/MuSK-negative patients. They are rarely found in anti-AChR or anti-MuSK antibody-positive patients, possibly representing a subgroup of patients more severely affected at onset [10]. Antibodies to LRP4 were occasionally found in amyotrophic lateral sclerosis in patients with neuromuscular transmission defects and a weak response to cholinesterase inhibitors [19].

The most sensible diagnostic assay for anti-AChR and anti-MuSK antibodies is radioimmunoassay (RIA) [20,21]. The introduction of CBAs has significantly increasing the chance to identify autoantibodies to low affinity clustered AChR, MuSK and LRP4, thus improving myasthenia gravis diagnosis [22,9,10], although commercial kits are not available. A rapid (less than 1 h) and simple immunostick immunosorbent ELISA has been recently developed for qualitative detection of anti-AChR and anti-MuSK antibodies in serum or whole blood, with 99.1% of specificity and 91.1% of sensitivity for antibodies to AChR [23▪]. Detection of antibodies to titin by ELISA and to ryanodine receptor (RyR) by western blot is suggestive of thymoma but also present in late-onset myasthenia gravis [1]. Recently, by using a combination of CBA and flow cytometry (cytometric CBAs), antibodies to titin, RyR and voltage-gated Kv1 were found in thymoma, in late-onset myasthenia gravis patients, and in myasthenia gravis patients with concomitant myositis and/or myocarditis; the latter patients had a severe generalized form, and anti-striational antibodies suggested an association of myasthenia gravis with myositis and/or myocarditis [24▪]. In another study, detection of neuronal autoantibody in thymomatous patients using immunohistology and CBA suggested that these antibodies could serve as biomarkers for neuromyotonia or tumour recurrence [25▪].

### Neuroelectrophysiological diagnostic tests

Neuroelectrophysiological tests are confirmatory of myasthenia gravis and are particularly important in seronegative patients for providing evidence of a neuromuscular transmission defect. These tests include repetitive nerve stimulation (RNS) and single-fibre EMG (SF-EMG) [3▪]. In myasthenic patients, a positive RNS at low frequency (2–5 Hz) is decremental at the fourth/fifth response; such a positivity seems to be less evident in patients recently diagnosed (<4 weeks) [26], likewise RNS is frequently abnormal in patients with myasthenia gravis crisis [27▪]. Optimal stimulation parameters for an accurate ocular myasthenia gravis diagnosis, using repetitive ocular vestibular-evoked myogenic potentials, were recently determined: a robust decrement in the inferior oblique muscles was observed at repetition rates between 20 and 50 Hz, with an optimum at 30 Hz [28▪].

SF-EMG, measuring the neuromuscular jitter during voluntary muscle contraction, is the most sensitive test for myasthenia gravis diagnosis; indeed, examination of limb and facial muscles produces positive results in greater than 90% of patients [29,30]. A recent serial stimulated jitter analysis of the orbicularis oculi muscle in juvenile myasthenia gravis patients showed a significant correlation between electrophysiological data and the Myasthenia Gravis Foundation of America (MGFA) score, suggesting that stimulated jitter values are sensitive biomarkers in this disease subgroup [31▪].

## THERAPEUTIC TREATMENT

Current treatments for myasthenia gravis include symptomatic therapy with cholinesterase inhibitors, immunosuppression, thymectomy in selected patients and plasmapheresis or immunoglobulins for acute exacerbations [3▪,4]. New biological drugs are promising for refractory disease (approximately 10% of patients), and also to reduce/eliminate chronic immunosuppression and the associated side effects [6]. Myasthenia gravis is clinically heterogeneous and exhibits variable treatment response, hence its treatment should be, as much as possible, personalized and possibly falling into the precision medicine. Therapeutic algorithms for myasthenia gravis, including possible new flow-charts, are shown in Fig. 3.

### Current therapies

Most myasthenic patients are chronically treated with immunosuppressive drugs, and prednisone remains essential though with a deleterious side-effect burden [32]. In a recent retrospective study, intravenous methylprednisolone (IVMP) therapy (1000 mg/day administered one to three times within 6 months) was found to provide faster improvements in ocular myasthenic patients compared with conventional oral prednisolone (5–10 mg/day), indicating that IVMP may be a well tolerated and efficient therapeutic option for ocular disease [33▪].

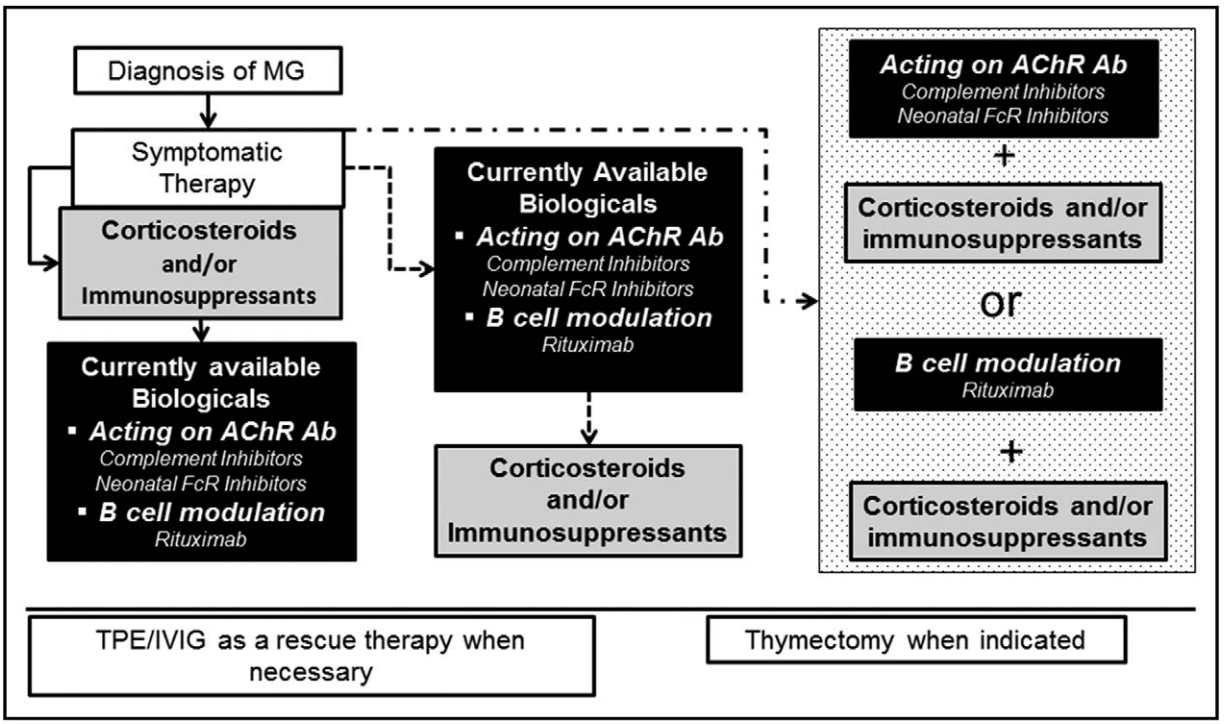


**FIGURE 3.** Therapeutic algorithm for myasthenia gravis. The dashed lines represent future flow-chart of treatment not yet applied. Ab, antibodies; AChR, acetylcholine receptor; IVIG, intravenous immunoglobulin; MG, myasthenia gravis; TPE, therapeutic plasma exchange.

Plasma exchange or intravenous immunoglobulin are recommended for severely affected patients. A recent study showed 96% complete response rate to plasma exchange irrespective of antibody status [34▪].

Using a multivariate model, an increased dose of corticosteroid therapy, mycophenolate mofetil, and plasma exchange, was associated with infections (e.g. pneumonia, sepsis and opportunistic infections) in a 10-year retrospective study performed including myasthenic patients, thus highlighting the risk of infections with the current immunosuppression [35▪].

The beneficial effect of thymectomy in non-thymomatous myasthenia gravis was demonstrated by the MGTX clinical trial [36] and its 2-year extension [37▪▪]. Such a benefit was also recently shown in non-thymomatous elderly (≥50 years) patients with generalized myasthenia gravis [38▪].

## Myasthenia gravis in the era of 'biologicals'

Therapies based on biologic drugs, or 'biologicals', targeting molecules involved in the specific immunopathological mechanisms, represent a novel care strategy for myasthenia gravis patients aimed at more specific and effective interventions. The most recent randomized clinical trials (RCT) of biologicals in myasthenia patients are listed in Table 1.

Retrospective studies and meta-analyses showed that rituximab (RTX), a B-cell-depleting monoclonal antibody (mAb) could be beneficial in myasthenia gravis, especially in MuSK-MG [45,46]. Hence, a phase 2 BeatMG (NCT02110706) RCT was performed but did not reach the primary end-point: it was decided not to proceed to a phase 3 study. A recent uncontrolled prospective study showed the beneficial effects of RTX at 12 months on muscle function in 55% of patients with severe, refractory generalized AChR-MG [47▪]. Furthermore, a low-dose RTX treatment over a 6-month period was able to reduce B cells and to increase regulatory T cells' percentage, thus improving symptoms in refractory generalized myasthenia gravis [48▪].

Efficacy and safety of belimumab (BEL), a mAb against the B-cell activating factor (BAFF), was investigated in a phase 2 RCT (NCT01480596; BEL115123) in generalized myasthenia gravis patients, who remained symptomatic despite standard of care [39▪]: in this study the primary end-point, that is, a mean change from baseline for Quantitative Myasthenia Gravis (QMG) score at week 24, as well as the secondary end-points, were not reached, questioning BEL as possible treatment for myasthenia gravis; however, the small sample

**Table 1.** List of recently completed clinical trials of biological drugs in myasthenia gravis

| Drug | Target | Function | Clinical trial | Phase | Patients | Intervention/treatment | Results and references |
|---|---|---|---|---|---|---|---|
| Rituximab (RTX) | CD20 | B-cell depletion | BeatMG, NCT02110706 | Phase II | AChR-positive with generalized MG | Matched placebo: vehicle control infusion Treatment: two cycles of RTX [one infusion of 375 mg/m$^2$ intravenously (IV)] per week for 4 consecutive weeks, separated by 6 months | No related published article Results available at: https://clinicaltrials.gov/ct2/show/results/NCT02110706 |
| Belimumab (BEL) | B-cell-activating factor (BAFF) | B-cell survival inhibition | BEL115123, NCT01480596 | Phase II | AChR-positive and MuSK-positive with generalized MG and current standard of care therapy | Matched placebo: IV infusion Treatment: 10 mg/kg IV infusion | Class I evidence that belimumab did not significantly improve QMG score at week 24 compared with placebo (Hewett *et al.* [39▪]) Results available at: https://clinicaltrials.gov/ct2/show/NCT01480596 |
| CFZ533 | CD40L | B-cell activation inhibition | CCFZ533X2204, NCT02565576 | Phase II | AChR-positive and MuSK-positive with generalized MG | Matched placebo Treatment: CFZ533 | No related published article Study description at: https://clinicaltrials.gov/ct2/show/results/NCT02565576 |
| Eculizumab (ECU) | Terminal complement protein C5 | Prevention of C5b-induced MAC formation and reduced destruct of the NMJ | REGAIN Study, ECU-MG-301, NCT01997229 | Phase III | AChR-positive with refractory generalized MG | Matched placebo: buffer components (induction and maintenance scheme as the drug) Treatment: induction phase, 900 mg weekly for 4 doses (every 7 days ± 2 days) followed by 1200 mg 1 week later for the fifth dose (week 4); maintenance phase, 1200 mg every 2 weeks (14 days ± 2 days) from the fifth dose onwards (week 6 through week 26) | Safety profile; improvements in activities of daily living, muscle strength, functional ability, and quality of life, but no significant changes in the MG-ADL score between the drug and placebo (Howard *et al.* [40▪▪]); Results available at https://clinicaltrials.gov/ct2/show/NCT01997229 |

**Table 1** (*Continued*)

| Drug | Target | Function | Clinical trial | Phase | Patients | Intervention/treatment | Results and references |
|---|---|---|---|---|---|---|---|
| Eculizumab (ECU) | Terminal complement protein C5 | Prevention of C5b-induced MAC formation and reduced destruct of the NMJ | ECU-MG-302: Open-label, Extension Trial of ECU-MG-301, NCT02301624 | Phase III | Patients who completed the ECU-MG-301 study | Treatment: 1200 mg every 2 weeks for 22.7 months [median] | Evidence that improvements in ECU-MG-301 were maintained through 3 years (Muppidi *et al.* [41▪]) Improvements in perceived fatigue correlated with improvements in MG-ADL, QMG, and MG-QOL15 scores (Andersen *et al.* [42▪]) Study description at: https://clinicaltrials.gov/ct2/show/NCT02301624 |
| Amifampridine phosphate (AP) | Kv1.5 | Depolarization of the presynaptic membrane at the NMJ | MSK-001 | Phase IIb | MuSK-positive with generalized MG | Matched placebo: tablets matching AP, three to four times a day Treatment: 30–100 mg/day divided into doses taken three to four times a day; max single dose: 25 mg | Safety and efficacy of 30–60 mg daily dose; significant differences between drug and placebo both in the primary (QMG, MG-ADL) and secondary endpoints (MGC, MG-QOL15) (Bonanno *et al.* [43▪]) |
| Efgartigimod (ARGX-113) | Neonatal Fc receptor of IgGs | Reduction of levels of pathogenic IgG antibodies | ARGX-113-1602, NCT02965573 | Phase II | AChR-positive and MuSK-positive with generalized MG and stable standard of care therapy | Matched placebo: four doses over a 3-week period of 10 mg/kg IV Treatment: Four doses over a 3-week period of 10 mg/kg IV efgartigimod | Class I evidence of safety and efficacy: rapid decrease in total IgG and anti-AChR autoantibody levels; rapid and long-lasting disease improvement assessed by QMG, MG-ADL, MGC, and MG-QOL15 in 75% of patients [Howard *et al.* [44▪▪]) Study description at: https://clinicaltrials.gov/ct2/show/results/NCT02965573 |

AChR, acetylcholine receptor; Kv1.5, non-specific voltage-dependent potassium channel; IgG, immunoglobulin G; MAC, complement membrane attack complex; MG-ADL, Myasthenia Gravis-specific Activities of Daily Living; MGC, Myasthenia Gravis Composite score; MG-QOL15, Myasthenia Gravis Quality of Life scale-15; MuSK, muscle-specific kinase; NMJ, neuromuscular junction; QMG, Quantitative Myasthenia Gravis score.

size and the mild clinical severity of patients recruited in the study may have jeopardized the final results.

The results of a RCT (NCT02565576) to evaluate safety, tolerability, pharmacokinetics and efficacy of CFZ533, an anti-CD40 mAb inhibiting B-cell activation, in myasthenic patients have not yet been published.

Eculizumab, a mAb preventing the formation of C5b-induced MAC at the NMJ, was used in a phase 3 RCT in refractory generalized AChR-MG without thymoma (REGAIN); its use was well tolerated and clinically relevant: whereas the primary end-point (a statistically significant change of at least points in the myasthenia gravis Activities of Daily Living (MG-ADL) score) was not reached, all the other end-points were reached [40▪▪]. Subsequently, both Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved Eculizumab for treatment of myasthenia gravis. Recently, Muppidi *et al.* [41▪] reported the results of the open-label extension study of REGAIN showing a long-term safety and a sustained efficacy of eculizumab in refractory generalized myasthenia gravis patients. Furthermore, Eculizumab treatment was associated with improvements in fatigue, strongly correlated with Quality of Life in Neurological Disorders (Neuro-QOL) Fatigue scores, and myasthenia gravis-specific outcome measures (MG-ADL, QMG, and MG-QOL15) [42▪].

Recently, the first phase 2, cross-over RCT using amifampridine phosphate, a blocker of presynaptic potassium channels, in MuSK-MG patients (MuSK-001) was concluded [43▪]. Despite the low number of patients, amifampridine phosphate was well tolerated and effective as both the primary and secondary endpoints were reached. A large multicenter phase 3 trial (NCT03579966) to confirm the efficacy of amifampridine phosphate in MuSK-MG is presently recruiting.

The results of a phase 2 exploratory, multicenter RCT in patients with generalized AChR-MG using efgartigimod (NCT02965573), a functional blocker of the neonatal Fc receptor targeting IgGs, have been recently published [44▪▪]. Efgartigimod was well tolerated and clinical efficacy was concomitant to a rapid decrease in total IgG and anti-AChR autoantibodies; 75% of patients had a rapid and long-lasting disease improvement using four different scales, suggesting that reducing pathogenic autoantibodies would offer an innovative approach to treat generalized myasthenia gravis [44▪▪]. A recent assessment of efgartigimod in a passive transfer mouse model for MuSK-MG revealed reduction of IgG4 titers (about eight-fold), improvement of muscle strength and reduced myasthenic CMAP decrement in treated mice, thus suggesting that this drug could offer a good candidate therapy also for patients with anti-MuSK antibodies [49▪].

## The new frontier of personalized medicine

Variation in drug response and side effects highlight the importance to develop novel therapeutic strategies for myasthenia gravis, to improve clinical decisions, and hence, therapeutic success via a more targeted choice in individual patients. Thus, the identification of molecular factors able to modulate and predict patient-specific treatment response represents a crucial medical need. Among genetic factors, our previous studies identified an association between response to azathioprine and two haplotypes, the TPMT∗3E haplotype in the thiopurine S-methyltransferase and a haplotype in the ATP-binding cassette sub-family C member 6 transporter [50,51]. Moreover, non-responsiveness to glucocorticoid therapy in myasthenic patients was recently associated with genetic variants in the secreted phosphoprotein 1 (*SPP1*) gene, encoding osteopontin [52].

Serological levels of free immunoglobulin light chains (FLCs), indicative of B-cell activity, represent a useful predictor of RTX therapeutic efficacy in autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus [53,54]. Recently, Basile *et al.* [55▪] demonstrated a significant increase in free κ chains in both AChR-MG and MuSK-MG patients, and a significant reduction of both free κ and λ chains in a MuSK-MG patient after 2 months of RTX treatment, suggesting a potential role of FLC as biomarkers of RTX therapy response in myasthenia gravis patients.

Due to their well known function in modulating both immune response and drug metabolism [56,57], microRNAs (miRNAs) are promising 'pharmacoepigenetics' markers for autoimmune conditions. Dysregulated miRNA expression has been described in serum, peripheral blood cells and thymus of myasthenic patients [58,59▪▪,60▪], suggesting a significant contribution of these molecules to the disease pathogenesis, as well as their potential role as predictive biomarkers to improve stratification of patients within a personalized medicine framework. Among miRNAs, circulating miR-21-5p, miR-150-5p and miR-30e-5p were recently found to correlate with clinical improvement after initiation of immunosuppression in late-onset myasthenia gravis patients [61▪], and mir-30e-5p was described as predictor of generalization in patients with ocular disease [62▪▪], supporting a potential value of these molecules as disease biomarkers potentially associated with treatment response.

Personalized medicine is a big challenge in autoimmune conditions. Further investigations aimed at revealing serological, pharmacogenomics and pharmaco-miR biomarkers, able to predict patient-specific drug efficacy, promise to open new perspectives towards the development of novel and more efficient personalized therapeutic approaches.

## CONCLUSION

In the last few years, we have observed the onset of new categories of drugs, that is the complement inhibitors and the neonatal FcR blockers, which will progressively bring us into the reality of a precision medicine in myasthenia gravis. Results from the recently concluded clinical trials of biologicals, including eculizumab and efgartigimod, are expected to produce a significant change in the paradigms of treatment, illustrated in Fig. 3, with a strong impact on myasthenic patients' management. The identification of biomarkers able to predict the efficacy of these drugs in individual patients will lead to the development of personalized medicine, that could significantly increase therapeutic success and the cost/effectiveness ratio for the disease treatment.

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### Conflicts of interest

*R.M. has received compensation for participating on advisory boards in relation to MG clinical trial design, Congress participations and research support in the last 5 years from: Alexion Pharmaceuticals, ARGENX Pharma, Biomarin. P.C. has no conflicts of interest.*

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# Interferon-signature in idiopathic inflammatory myopathies

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**Purpose of review**
The present review describes the interferon (IFN)-signature currently emerging as a tool for the diagnosis of idiopathic inflammatory myopathies (IIMs), and aims at presenting the interests and limitations of this recent tool for the clinics and the research.

**Recent findings**
Recent in-vivo and in-vitro transcriptomic studies have evidenced the involvement of IFNs in the pathogenesis of IIMs. A correlation between the IFN-signature and the clinical severity of IIMs has been established. Moreover, studies pointed out differences in the IFN-signature regarding the IIM subgroup (dermatomyositis, polymyositis, inclusion body myositis, anti-synthetase syndrome, immuno-mediated necrotizing myopathies), raising the hypothesis of several pathogenic processes in IIMs.

**Summary**
IIM pathogenesis remains partially understood. IFN-signature represents one of the main recent advances in the field. IFN-signature was identified thanks to transcriptomic analyses of tissues or cells from IIM patients (muscle, skin, blood cells, muscle cells) and should allow to establish new diagnosis and better monitoring of IIM patients. It also provides a tool for investigation of IIM pathogenesis. Nevertheless, IFN-signature still requires accurate definition in order to standardize its use, notably in the clinical practice.

**Keywords**
idiopathic inflammatory myopathies, interferon score, interferon-signature

## INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) are classified as dermatomyositis, polymyositis, inclusion body myositis (IBM), anti-synthetase syndrome (ASS) and immunomediated necrotizing myopathies (IMNM) [1–4,5▪▪]. IIMs are characterized by muscle impairment and are associated with specific autoantibodies. Current medical care includes immunosuppressive drugs. Despite important efforts made during the last decades, the understanding of IIM etiopathogenesis remains fragmented, combining the involvement of adaptative and innate immunity dysfunction, of genetic background, and of environmental factors, such as virus infection or cancer [6]. Generally, elucidating specific molecular pathways involved in a disorder has important outcomes for defining disease subgroups, monitoring the disease activity and choosing therapies. In that context, interferons (IFNs) were identified as important actors in IIMs. In 1995, the analysis of cytokine expression demonstrated the up-regulation of IFN-γ in IIM muscle [7]. This finding led to identify an overexpression of the IFNγ-induced signal transducer and activator of transcription 1 (STAT1) in the altered perifascicular area of dermatomyositis muscle [8]. This was the beginning of considering transcriptomic changes in IIM pathogenesis. IFNs are widely expressed cytokines that exhibit antiviral, antiproliferative and immunomodulatory properties. IFN family is divided into three classes, which share overlapping signaling pathways (Fig. 1) [9–11]. The present review aims at summarizing the work recently published on the IFN-signature that was found in IIMs, and to discuss the establishment of an IFN-score as a tool for diagnosis and monitoring of IIM patients.

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## KEY POINTS

- ISG differential expression constitutes an IFN-signature in IIMs.
- IFN-signature (and IFN-score) correlates with the severity of the disease.
- IFN-signature (IFN-I and IFN-II) differs among IIM subgroups.
- IFN-signature needs to be standardized for a general use in clinics and for investigation of IIM pathogenesis.

## TRANSCRIPTOMIC EVIDENCE OF INTERFERON INVOLVEMENT IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Transcriptomic analysis was first conducted *in vivo* using IIM muscle, was expanded to skin, circulating leukocytes, then *in vitro* to myogenic precursor cells (MPCs) and to endothelial cells isolated from patients (Table 1). The most upregulated interferon-stimulated genes (ISGs) are varying depending on the cell type, albeit common ISGs are always upregulated, such as *OAS1*, *IFIT1*, *MxA*. These studies highlight that IFNs appear to be key effectors of IIM pathogenesis [12]. Interestingly, the different subtypes of IIMs exhibit distinct gene expression signatures [13–15,16▪▪]. Consecutively, a focus on the analysis of IFN-induced transcripts led to the establishment of a so-called IFN-signature, which represents a further advance in the understanding of the disease, as well as a useful tool for diagnosis and monitoring IIM patients.

### Transcriptomic analysis of skeletal muscle

The upregulation of ISG transcripts correlated with the over expression of the corresponding proteins in

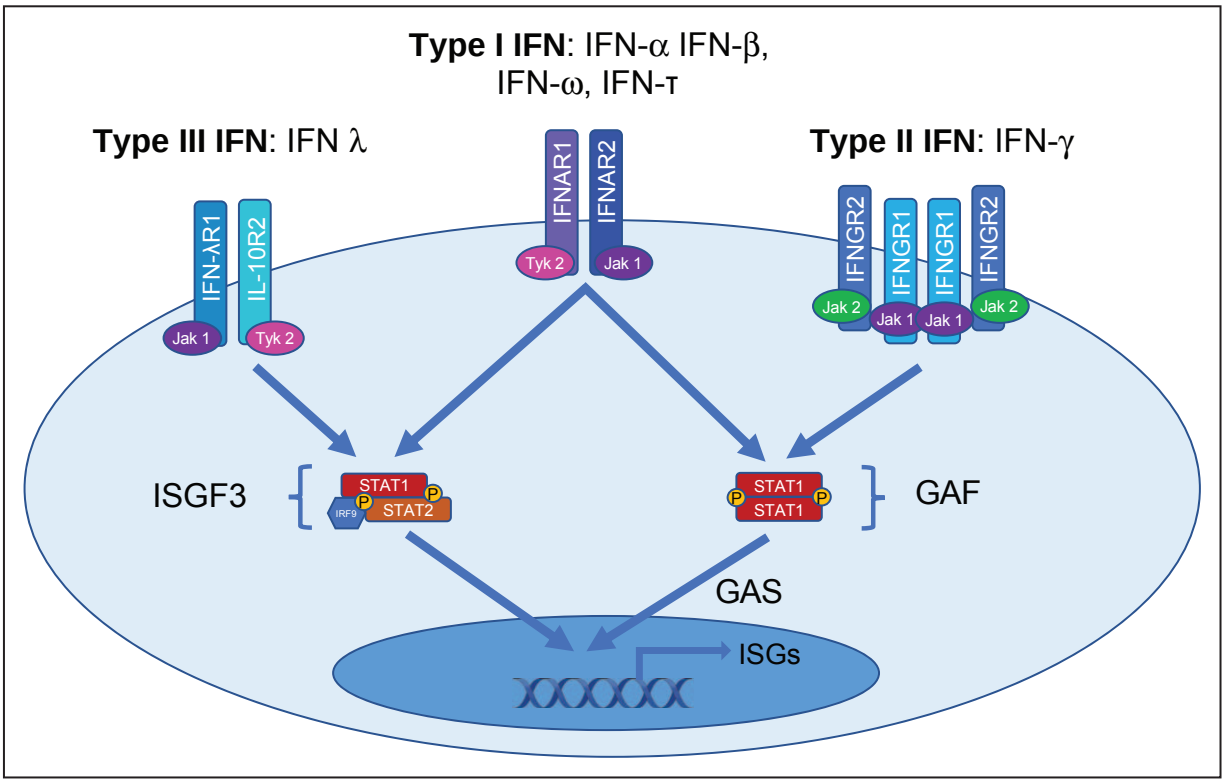


**FIGURE 1.** Interferon signaling pathways. Type I IFN-Is include IFN-α, IFN-β, IFN-ω, and IFN-τ, the two latter remaining poorly characterized because of their overlapping functions with IFN-α and IFN-β, their limited tissue expression, and species-to-species differences [9]. Type I IFNs bind to a specific heterodimeric IFN Alpha Receptor (IFNAR) that consists of IFNAR1 and IFNAR2. IFN binding triggers the recruitment of Janus Activated Kinases (JAK) TYK2 and JAK1, leading to tyrosine phosphorylation of STAT1 and STAT2 that bind to IRF9 to form the ISGF3 complex, that translocates to the nucleus to stimulate the transcription of IFN-inducible genes or IFN-stimulated genes (ISGs) via binding to specific DNA binding sites (ISRE). IFN-II, which is IFN-γ, binds to the IFNGR1/IFNGR2 receptor that recruits JAK 1 and JAK2 to phosphorylate STAT1 leading to the formation of STAT1 homodimers complexes [gamma-activated factors (GAFs)], which translocate to the nucleus and targets gamma activated site (GAS) elements in the promoters of ISGs, leading to their expression [10]. IFN-III, which is IFN λ (also known as interleukin 28 and 29), is structurally related to IFNs-I and to the IL-10 family. IFN-III binding to its receptor, composed of IL-28R-binding and IL-10R2 chains, causes the activation of JAK1 and TYK2, leading to the formation of the ISFG3 transcription factor [11]. IFN, interferon.

**Table 1.** Studies reporting upregulation of interferon-stimulated gene transcripts in idiopathic inflammatory myopathies

| Genes induced by | IFN-α/b | | | | IFN-γ | |
|---|---|---|---|---|---|---|
| Tissue | Skeletal muscle | PBMCs | Skin | MPCs/ECs | Skeletal muscle | Skin |
| DM | Greenberg *et al.* [13]; Greenberg *et al.* [14]; Zhou *et al.* [18]; Salajegheh *et al.* [19]; Allenbach *et al.* [29]; Rigolet *et al.* [16▪▪] | Walsh *et al.* [15]; Baechler *et al.* [23]; Liao *et al.* [28]; Greenberg *et al.* [33]; Reed *et al.* [30]; Zhu *et al.* [24]; Huard *et al.* [31]; Zhang *et al.* [25▪] | Wong *et al.* [12] | | Rigolet *et al.* [16▪▪] | Wong *et al.* [12] |
| JDM | Tezak *et al.* [17]; Salajegheh *et al.* [19] | O'Connor *et al.* [21]; Baechler *et al.* [23]; Reed *et al.* [30]; Huard *et al.* [31] | | Gitiaux *et al.* 2018 [20▪] | | |
| IBM | Greenberg *et al.* [14]; Salajegheh *et al.* [19]; Rigolet *et al.* [16▪▪] | Walsh *et al.* [15] | | | Greenberg *et al.* [13]; Raju *et al.* [26,27]; Rigolet *et al.* [16▪▪] | |
| PM | Zhou *et al.* [18]; Salajegheh *et al.* [19]; Greenberg *et al.* [14] | Walsh *et al.* [15] | | | Greenberg *et al.* [13] | |
| IMNM | Rigolet *et al.* [16▪▪]; Greenberg *et al.* [14] | | | | Rigolet *et al.* [16▪▪] | |
| ASS | Rigolet *et al.* [16▪▪] | | | | Rigolet *et al.* [16▪▪] | |

ASS, anti-synthetase syndrome; DM, dermatomyositis; ECs, endothelial cells; IBM, inclusion body myositis; IIMs, idiopathic inflammatory myopathies; IMNM, immunomediated necrotizing myopathies; JDM, juvenile dermatomyositis; MPCs, myogenic precursor cells; PBMCs, peripheral blood mononuclear cells; PM, polymyositis.

altered myofibers and in capillaries of dermatomyositis muscle assessed by immunohistochemistry [14]. These results led to the hypothesis of a production of IFN-I-inducible transcripts and proteins by the myofibers, and suggested that the alteration of myofibers may be directly linked to the IFN-I downstream activation. Such an upregulation was confirmed in a transcriptomic study on juvenile dermatomyositis (JDM) muscles [17]. Using gene expression microarrays, Greenberg *et al.* [13] reported a molecular signature that distinguished IIMs from nemaline myopathies, Duchenne muscular dystrophy, congenital myopathy, and normal muscle. Focusing on dermatomyositis, they reported that 84% of the top 25 upregulated genes were ISGs. These results were confirmed on six polymyositis and four dermatomyositis muscles [18]. In a cohort of various IIM subgroups, it was found that ISG transcripts were more abundant in dermatomyositis than in other IIM muscles [14], leading to define the specific status of acquired interferonopathy for dermatomyositis. In a large IIM cohort, the expression of the ISG15-conjugation pathway (*ISG15*, *HERC5*, *USP18*) was found upregulated specifically in dermatomyositis muscles [19]. Recently, a study established an IFN-γ score to classify IIMs, defining specific IFN-γ score for IBM and ASS subgroups [16▪▪]. *In vitro*, transcriptomic analysis of MPCs freshly isolated from JDM muscle showed that 18 of the 30 most upregulated genes were ISGs [20▪]. Similarly, endothelial cells isolated from the same JDM patients showed ISGs as 10 amongst the 30 most upregulated genes [20▪].

## Transcriptomic analysis of circulating leukocytes

Activation of the IFN-I pathway has also been identified in circulating leukocytes or peripheral blood mononuclear cells (PBMCs) from IIM patients. It was first assessed by the upregulation of *MxA* mRNA expression in PBMCs of JDM patients [21]. By comparing the IFN-signature observed in systemic lupus erythematosus (SLE), Baechler *et al.* [22] showed that blood cells of dermatomyositis patients present an IFN-signature. The cluster of ISGs upregulated in dermatomyositis patients was composed of 93 genes, amongst which 43 were also found in the SLE IFN-signature. Interestingly, a similar clustering was observed in both adult dermatomyositis and JDM PBMCs [23]. It is to mention that whereas most of the patients exhibited an upregulation of ISGs, 2 out of 12 did not present that molecular signature [23]. As observed in skeletal muscle, differential upregulation of ISGs in PBMCs was shown to distinguish IIM subgroups [24]. In a recent study, Zhang *et al.* [25▪], showed an up-regulation of 4 ISGs (*IRF7*, *STAT1*, *ISG15* and *Mx1*) in PBMCs isolated from anti-

MDA5 antibody associated dermatomyositis, as compared with ASS and seronegative dermatomyositis patients opening the question of the heterogeneity of IFN-signature within IIM subgroups, here dermatomyositis.

### Transcriptomic analysis of skin

Whole genome analysis using oligonucleotide arrays identified 946 genes differentially regulated in dermatomyositis versus normal skin [12]. Two third of those genes were up-regulated, including an important cluster of ISGs. The authors proposed a 'DM signature', which was confirmed using another microarray platform [12]. Moreover, comparative analysis of multiple inflammatory skin diseases indicates that the IFN-signature appears to be specific of dermatomyositis [12].

## INTERFERON-SIGNATURE IN IDIOPATHIC INFLAMMATORY MYOPATHIES

IFN appears a key feature in the pathogenesis of IIMs, especially in dermatomyositis and JDM. IFN-signature was established by multiple techniques from various tissues. Two main approaches were used to establish the IFN-signature in IIMs. One is to identify the gene ontology of differentially expressed genes after transcriptomic analysis of the tissue samples. The second strategy was to analyze the expression of predetermined ISGs by RTqPCR on tissue samples from IIM patients, which confirmation was sometimes made on normal cells treated *in vitro* by recombinant IFNs [12–15,16▪▪,17–19,20▪,21,23,24,25▪,26–31]. The definition of ISGs represents a key element for the analysis, and was used to establish a so-called IFN-score in eight IIM studies (Table 2).

### Interferon score

IFN-score (Table 2) was initially established in SLE, using PBMCs, and was based on the expression levels of genes included in the IFN cluster [22]. Thereafter, eight studies proposed to score the IFN-signature in IIMs [12,16▪▪,23,29–33] and two in genetic interferonopathies [34,35] using various strategies. Most of the studies established a median fold change on ISG expression in IIM versus healthy control samples on a number of ISGs ranging from 6 to 21. The cut-off fold change to establish the score ranged from 2.466 to 4 [12,16▪▪,29,31–33]. Two studies summed the normalized expression of ISGs in IIMs versus healthy controls [23,30]. Despite a high heterogeneity in the number of genes that were analyzed (3–43), in the tissue origin (PBMC, skin, skeletal muscle) and in the patient subgroup (dermatomyositis, IIM, IMNM, ASS, IBM), all studies have shown that the IFN-score discriminated IIM patients from healthy controls and in some cases, the severity of the disease. Therefore, the IFN-score seems a highly promising tool to investigate IIMs, although it requires standardization for a broader use.

### Type I interferon versus type II interferon expression and activity

Type I and II IFNs both induce the expression of a largely overlapping group of molecules whose relationship is highly dependent on the responding cell type and on IFN concentration on the target cell. However, a study on skin from dermatomyositis patients showed that over the 10 IFNs, IFN-β and IFN-γ both strongly correlated with the IFN-signature, whereas IFN-α did not, although it was the highest expressed in the samples [12]. This suggests an uncoupling between the expression of IFN subtypes and their biological activity [12]. Interestingly, outside the field of IIMs, an in-vitro study using human submandibular gland epithelial cell line showed that the majority of the genes that were highly up-regulated by IFN-α were also highly upregulated by IFN-γ, making the IFN-signature a broader marker of IFN activity [36]. Nevertheless, the authors identified two genes upregulated only by IFN-γ: *GBP1* and *GBP2* [36].

### Interferon-signature: bias and limitations

The variation in the range of fold changes of ISG expression, and more generally, the heterogeneity of magnitude of IFN-signature between studies remain subjects of caution. Although patients and tissue intervariability may explain that heterogeneity, the novel IFN-signature still suffers from a lack of technical standardization.

It is widely acknowledged that heterogeneity exists among IIM patients, regarding clinical and pathological phenotypes, clinical course and response to therapy. Variability may also be because of the timing of sample collection, as sample characteristics likely evolve with the course of the disease. Another limitation is the variability of the IFN-signature when patients receive a treatment. Indeed, it was shown *in vivo* [30] and *in vitro* [37] on SLE PBMCs that high doses of glucocorticoids, a standard treatment of disease flares, shut down the IFN-signature.

Moreover, important variations in the IFN-signature were observed between the tissues isolated from a same patient [15]. Variations may correlate

**Table 2.** Studies reporting interferon score in idiopathic inflammatory myopathies

| Reference | Baechler *et al.* [23] | Greenberg *et al.* [33] | Reed *et al.* [30] | Rice *et al.* [34] | Wong *et al.* [12] | Rice *et al.* [35] | Allenbach *et al.* [29] | Huard *et al.* [31] | Rice *et al.* [32] | Rigolet *et al.* [16▪▪] |
|---|---|---|---|---|---|---|---|---|---|---|
| Disease | SLE and IIM (DM) | IIM (DM and PM) | IIM (DM) | Genetic interferonopathies | IIM (DM), other inflammatory skin diseases | Genetic interfero-nopathies | IIM (DM) | IIM (DM) | Genetic interfero-nopathies, IIM (DM), SLE, sJIA | IIM (DM, IMNM, IBM, ASS) |
| Tissue | PBMC | PBMC | PBMC | PBMC | Skin | PBMC | Muscle | PBMC | PBMC | Muscle |
| Technique | Microarray +gene ontology | RTqPCR | RTqPCR | RTqPCR | Oligonucl. arrays + QRT-PCR | RTqPCR | RTqPCR | RTqPCR | qPCR | RTqPCR |
| IFN-score calculation | Sum of normalized expression values versus healthy controls | FC versus healthy controls: weak (FC <4), moderate (≦ 4 FC <10), high (FC >10) | Sum of normalized expression versus healthy controls adjusted to a 100-point scale | Median FC versus healthy controls | Median expression value (versus healthy controls) | Median FC versus healthy controls Positive score 2.466 | Mean RQ of the six genes | Mean FC: low less than 3, high greater than 3 | Positive score 2.466 | Median FC versus healthy controls |
| Number of ISGs | 43 | 13 | 3 | 15 | 21 | 6 | 6 | 10 | 6 | 6 |
| IFN-I ISGs | | | | | | | | | | |
| IFI27 | | x | | x | | X | | x | x | |
| IFI44L | X | x | | x | x | X | | x | x | |
| IFIT1 | X | x | x | x | x | X | | x | x | |
| ISG15 | | x | | x | x | X | | x | x | |
| RSAD2 | X | | | x | | X | | x | x | |
| SIGLEC1 | | | | x | | X | | | x | |
| Ly6E | X | | | x | | | | | | |
| MX1 | X | x | | x | | | | | | |
| USP18 | X | | | x | | | | | | |
| OAS1 | X | x | | x | | | x | | | |
| IFI44 | X | x | | x | | | | x | | |
| IFI6 | | x | | x | x | | | x | | |
| IFIT3 | X | x | | x | x | | | | | |
| IRF7 | X | | x | x | | | | | | |
| STAT1 | X | | | x | | | x | | | |
| ETV7 | X | | | | | | | | | |
| AW474434 | X | | | | | | | | | |
| FAM46A | X | | | | | | | | | |
| SAMD9 | X | | | | | | | | | |
| SNF7DC2 | X | | | | | | | | | |
| GBP1 | X | | | | | | | | | |
| SP100 | X | | | | | | | | | |
| TNFSF10 | X | | | | | | | | | |
| EIF2AK2 | X | | | | | | | | | |
| NM_00595 | X | | | | | | | | | |
| SN | X | | | | | | | | | |
| IL1RN | X | | | | | | | | | |
| MX2 | X | | | | x | | | | | |
| IFRG28 | X | | | | | | | | | |
| ZC3HDC1 | X | | | | | | | | | |

**Table 2** (*Continued*)

| Reference | Baechler *et al.* [23] | Greenberg *et al.* [33] | Reed *et al.* [30] | Rice *et al.* [34] | Wong *et al.* [12] | Rice *et al.* [35] | Allenbach *et al.* [29] | Huard *et al.* [31] | Rice *et al.* [32] | Rigolet *et al.* [16▪▪] |
|---|---|---|---|---|---|---|---|---|---|---|
| OASL | X | | | | x | | | | | |
| HERC6 | | | | | x | | | | | |
| HERC5 | X | x | | | x | | | x | | |
| OAS2 | X | | | | | | | | | |
| OAS3 | X | x | | | | | x | | | |
| HSXIAPAF | X | | | | | | | | | |
| G1P2 | X | | x | | | | | | | |
| G1P3 | X | | | | | | | | | |
| IFIT2 | X | | | | | | | | | |
| TRIM22 | X | | | | | | | | | |
| IFIT5 | X | | | | | | | | | |
| PLSCR1 | X | | | | | | | | | |
| IFI16 | X | | | | | | | | | |
| ZBP1 | X | | | | | | | | | |
| LAMP3 | X | | | | x | | | | | |
| TRIM5 | X | | | | | | | | | |
| PHF11 | X | | | | | | | | | |
| ZCCHC2 | X | | | | | | | | | |
| MDA5 | | | | | | | x | | | |
| RIG-1 | | | | | | | x | | | |
| CXCL10 | | | | | x | | | x | | |
| CMPK2 | | | | | | | | x | | |
| BATF2 | | | | | x | | | | | |
| IFIT2 | | | | | x | | | | | |
| IFIH1 | | | | | x | | | | | |
| SAMD9 | | | | | x | | | | | |
| IDO1 | | | | | x | | | | | |
| PARP14 | | | | | x | | | | | |
| ISG20 | | | | | x | | | | | |
| BST2 | | | | | x | | | | | |
| EPSTI1 | | x | | | x | | | | | |
| IFN-II ISGs | | | | | | | | | | |
| IFNg | | | | | | | x | | | |
| gBP2 | | | | | | | | | | x |
| Hla-DOB | | | | | | | | | | x |
| Hla-DPB | | | | | | | | | | x |
| ciita | | | | | | | | | | x |
| Hla-DrB | | | | | | | | | | x |
| Hla-DMB | | | | | | | | | | x |

ASS, anti-synthetase syndrome; DM, dermatomyositis; ECs, endothelial cells; FC, fold change; IBM, inclusion body myositis; IIMs, idiopathic inflammatory myopathies; IMNM, immunomediated necrotizing myopathies; JDM, juvenile dermatomyositis; MPCs, myogenic precursor cells; PBMCs, peripheral blood mononuclear cells; PM, polymyositis.

with the clinical phenotypes that differentially affect skin or muscle in each dermatomyositis patient [12]. Using a large cohort, Wong *et al.* [12] observed that PBMCs from dermatomyositis and SLE patients showed a weaker induction of ISGs than the skin samples from the same patients.

On top of patient and tissue variability, technical procedures should be carefully driven as sample

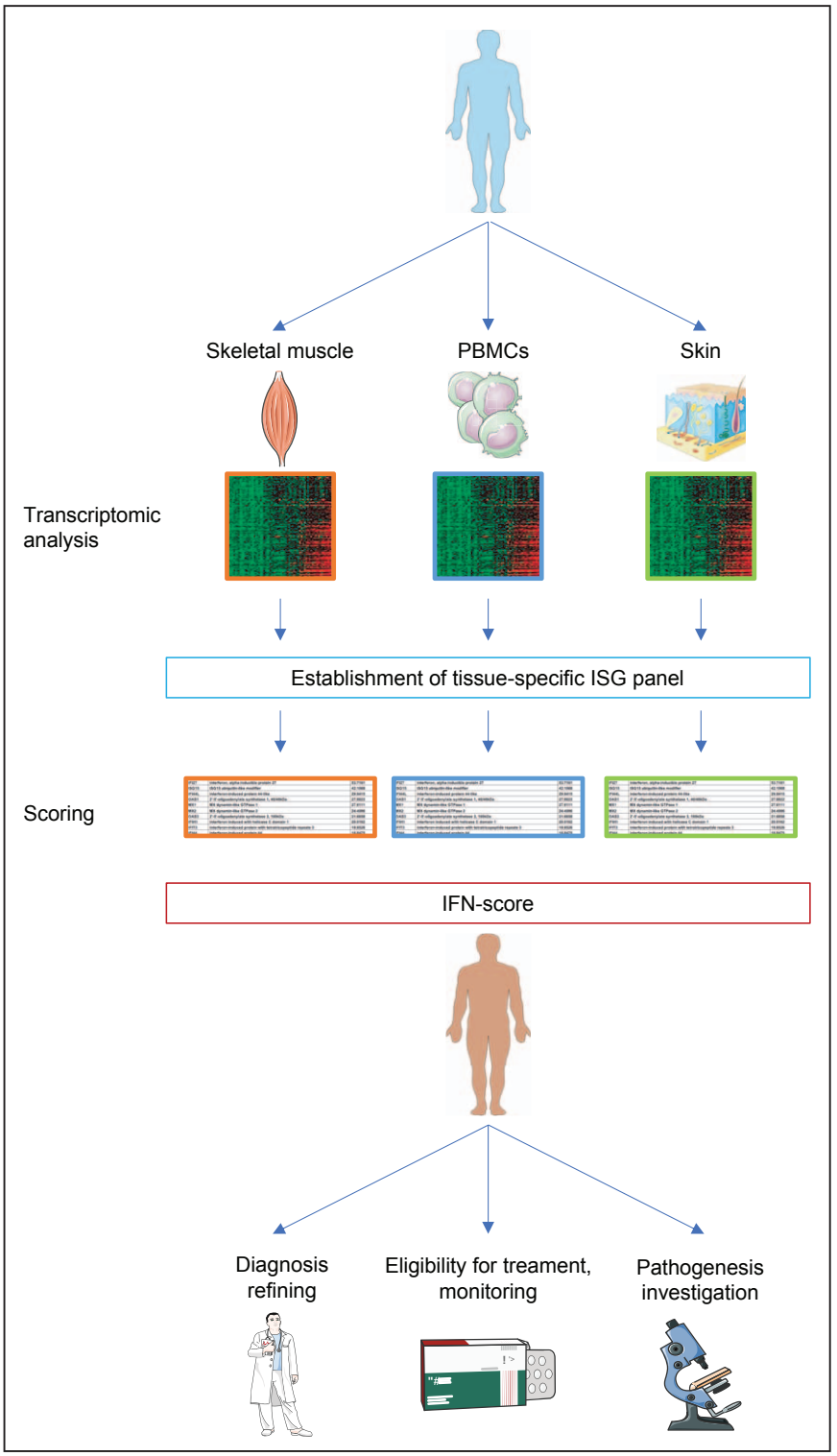


**FIGURE 2.** Establishment and standardization of tissue-specific IFN-score. IFN-I-signature should be standardized to establish tissue-specific IFN-score that will help to better diagnose the patients, monitor the evolution of the disease and select responding patients for future therapeutics. IFN, interferon.

handling may induce changes in gene expression (as shown in PBMCs, [22]) and is currently not standardized. For instance, blood sample handling was described to range from 2 to 72 h before gene expression analysis was performed [32,34,35]. Finally, the variety of probes that have been used so far also appears as so many causes of variability.

## APPLICATION TO THE CLINICS

A plethora of studies showed that the IFN-signature correlates with the disease severity [21,23,30,31,33]. Moreover, the IFN-score established from dermatomyositis-derived PBMCs correlated with the disease activity score based on muscle strength testing, muscle enzyme elevation, skin disorder and patient report of functional assessment [23]. Similarly, transcriptomic analysis of skin from dermatomyositis patients showed that patients with inactive-skin dermatomyositis clustered with the healthy group and segregated from the active-skin dermatomyositis patients [12]. According to the studies described above, it appears that IFN-signature varies depending on the IIM subgroup, with an IFN-I-signature for dermatomyositis and JDM, and an IFN-II-signature for ASS and IBM [14,16▪▪,19,29–31]. As IFN-signature appears to be an easy test, specifically when made on blood sample (i.e. PBMCs), it is being developed at the hospital for refining the diagnosis of IIM patients and will help to identify whose patients are eligible for anti-IFN therapies. Moreover, the establishment of a standardized IFN-signature will provide a tool for the evaluation of IIM treatments, including anti-IFN therapies [38▪▪], as it was done for SLE patients [39].

As described above, IFN expression does not correlate with IFN-signature and IFN activity. On a practical point of view, transcriptomic IFN-signature appears to be a more sensitive readout than the evaluation of the serum cytokine levels in IIMs [33,40]. Indeed, although studies reported a positive correlation between the severity of the disease and the level of IFN-β in dermatomyositis serum, and of IFN-γ, and IFN-α in JDM serum [41–43], others reported no correlation [40] or discrepancies between IFN-α and IFN-β correlation with dermatomyositis severity [31]. Moreover, the detection of IFN proteins by ELISA is hardly feasible in hospital routine, as the protein quantity ranges in atomollar concentrations, and thus requires specific technology for its detection, such as single-molecule assay (Simoa) [44], which remains currently in the research field [20▪].

## CONCLUSION

A dozen of publications from different laboratories have shown the interest and the robustness of using the IFN-signature in the analysis of IIMs. However, to date, there is no consensus on the list of genes to be included in the IFN-signature and the way to calculate an IFN-score, preventing the diffusion of this promising technique for a wide use. It is likely that standardization of the IFN-signature and/or IFN-score requires: the definition of a list of ISGs to be analyzed, the establishment of procedures in the handling of samples to limit variability among laboratories/hospitals, the definition of the techniques for the quantification of ISG transcripts, that will likely move towards RNAseq in the future (for which standardization will be also required). Moreover, discrepancies of the IFN-signature between tissues from the same patient suggest different signaling pathways, in accordance with the respective clinical outcomes (e.g. different evolution of the disease in skin and in skeletal muscle in dermatomyositis). This argues for the establishment of IFN-signatures that are tissue-specific/cell-specific (Fig. 2). Therefore, the IFN-signature requires an international consensus for its standardization in order to generalize its practice.

Standardized tissue-specific IFN-signature will allow to refine IIM subgroups, as well as to define responding patients who may be eligible for specific therapies (e.g. JAK/Stat inhibitors [38▪▪]). On the other hand, transcriptomic analysis may also link different disease pathogenesis. For example, the IFN-signature in dermatomyositis, SLE and HSV-2 (herpex simplex virus-2) skin samples is remarkably similar, suggesting that skin alteration of these disorders has a common pathophysiology [12].

The IFN-signature, through the analysis of ISG transcripts, appears to be a precious tool for the understanding of IIM pathogenesis, as well as for refining the clinical status, monitoring the evolution and evaluate therapeutic intervention of IIM patients.

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### Conflicts of interest

*There are no conflicts of interest.*

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# The role of cancer-associated autoantibodies as biomarkers in paraneoplastic myositis syndrome

*Xin Lu, Qinglin Peng, and Guochun Wang*

**Purpose of review**
The aim of this study is to provide a comprehensive overview of the current insight about the clinical utility of cancer-associated autoantibodies (CAAs) as biomarkers in paraneoplastic myositis syndrome (PMS). In addition, the possible mechanisms of the relationship between malignancy and myositis onset are discussed.

**Recent findings**
It has become increasingly clear that a subgroup of the myositis-specific autoantibodies could be considered as CAAs because they are closely related to the PMS. Increased risk of cancer was found in patients with antitranscriptional intermediary factor 1-γ (TIF1-γ), antinuclear matrix protein-2 (NXP-2), anti3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) or antismall ubiquitin-like modifier 1-activating enzyme (SAE) antibodies. However, the diagnosing sensitivity and specificity of these CAAs for PMS are different among different cohort studies. Abnormally expressed or mutated autoantigen genes in tumor could possibly induce cross immunity against self-proteins and subsequently lead to the development of PMS.

**Summary**
Anti-TIF1-γ, anti-NXP-2, anti-HMGCR and anti-SAE antibodies may act as CAAs in PMS. It is necessary to closely screen and monitor for cancer in patients with CAAs. The recent studies of the relationship between CAAs and PMS provided important new insights into the disease mechanisms.

**Keywords**
cancer-associated autoantibodies, cancer-associated myositis, myositis-specific autoantibodies, paraneoplastic myositis syndrome

## INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of systemic autoimmune diseases (collectively referred to as myositis), which mainly include the subtypes of polymyositis, dermatomyositis, immune-mediated necrotizing myopathy (IMNM) and sporadic inclusion body myositis (sIBM). The increased risk of cancer in patients with myositis, especially in adult dermatomyositis, has been recognized for a long time [1,2]. Myositis with cancer is often referred to as cancer-associated myositis (CAM), which is typically defined as the development of a malignancy within 3 years of the diagnosis of myositis. Observations of a close temporal relationship between myositis onset and cancer diagnosis and reports of cancer therapy halting CAM progression suggest that CAM is a paraneoplastic myositis syndrome (PMS).

In recent years, some improvements have been made in searching biomarkers and in understanding the underlying mechanisms of the PMS. One of these has been a subgroup of myositis-specific antibodies (MSAs), which are linked to an increased risk of malignancy[3▪▪,4▪▪,5▪,6▪,7–8]. The most common MSAs reported to be associated with PMS are antitranscriptional intermediary factor 1-gamma (TIF1-γ), antinuclear matrix protein-2 (NXP-2), anti3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and antismall ubiquitin-like modifier 1 activating enzyme (SAE) autoantibodies. This subgroup of MSAs is also called-cancer associated autoantibodies (CAAs).

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## KEY POINTS

- Patients with PMS present with similar clinical features as myositis without malignancy.
- Anti-TIF1-γ, anti-NXP-2, anti-HMGCR and anti-SAE may be considered as CAAs and act as the biomarkers of PMS.
- It is necessary to closely screen and monitor for cancer in patients when they present with CAAs.
- Autoantigens recognized by CAAs are expressed in malignancies and trigger antitumor immune responses, which cross-react with target tissues leading to the autoimmune-mediated skin, muscle and other organs damage in PMS.

Herein, we review the current state of knowledge regarding the role of CAAs as biomarkers in PMS and discuss their potential pathogenic roles in PMS.

## CLINICAL FEATURES OF PARANEOPLASTIC MYOSITIS SYNDROME

PMS is one of the typical paraneoplastic syndromes, with the manifestations of skin and skeletal muscle system from the underlying malignancy. Patients with PMS tend to be older. Usually, PMS shows a more severe cutaneous (with skin ulcerations) and muscular involvement (severe muscle weakness and dysphagia), but a lower incidence of interstitial lung disease than patients with other forms of myositis [9,10]. The muscle pathological features of the PMS are similar to the typical myositis without malignancy. However, there was a study that showed that vacuolated fibers and dense C5b-9 deposits on capillaries were the characteristic histopathologic findings in PMS, more specifically in anti-TIF1-γ positive PMS [11]. PMS usually responds to glucocorticoid therapy, although the skin manifestations can be recalcitrant. Most of the patients with PMS will go into remission after removal of the malignancy. In some cases, however, the symptoms of myositis recur even without a relapse of cancer, supporting the hypothesis that an initially tumor-triggered, but later self-perpetuating, immune response occurs against skin and muscle antigens. In general, the survival rate of the patients with PMS is considerably lower than that of patients without malignancy [2].

## CANCER-ASSOCIATED AUTOANTIBODIES ACT AS BIOMARKERS OF PARANEOPLASTIC MYOSITIS SYNDROME

### Anti-TIF1-γ antibodies

Anti-TIF1-γ antibodies are one of the most common MSAs in dermatomyositis. The frequency of the anti-TIF1-γ antibodies ranged from 13 to 30% among different myositis populations [12,13,14▪,15] (Table 1).

The association between anti-TIF1-γ antibodies and cancer in patients with adult dermatomyositis has been confirmed by a number of studies [3▪▪,4▪▪,5▪,11,16,17▪,18]. The prevalence of cancer in anti-TIF1-γ-positive patients (i.e. anti-TIF1-γ-related PMS) varied between different cohort studies, ranging from 38 to 80% as previously reviewed by Mammen [19]. More recently, a meta-analysis of 18 cohort studies showed that the pooled prevalence of CAM (or PMS) in patients with anti-TIF1-γ antibodies was 40.7% [95% confidence interval (CI) 0.36–0.45] [4▪▪]. In our cohort, we found that the overall risk of cancer was 17-fold higher [standardized incidence ratio (SIR) = 17.28, 95% CI 11.94–24.14] in the anti-TIF1-γ-positive patients than the age-matched and sex-matched general Chinese population [3▪▪].

**Table 1.** Frequency of the cancer-associated autoantibodies in idiopathic inflammatory myopathies, frequency of the paraneoplastic myositis syndrome and the standardized incidence ratio of cancer in the patients with cancer-associated autoantibodies

| CAA | Percent frequency of the CAA in IIMs | Percent frequency of the PMS in the patients with CAAs | SIR of cancer in the patients with CAAs |
|---|---|---|---|
| Anti-TIF1-γ | 13% ~ 31% in DM | 38% ~ 80% | 17.28 (95% CI 11.94–24.14) (our cohort) [3▪▪] |
| Anti-NXP-2 | 1.6% ~ 30% in DM | 7% ~ 37.5% | 3.68 (95% CI 1.2–8.6) (United States) [6▪];<br>8.14 (95% CI 1.63–23.86) (our cohort) [3▪▪] |
| Anti-HMGCR | ~5% in IIMs<br>44.9% in IMNM | 17.3% ~ 36% | 2.79 (95% CI 1.02–6.07) (France) [8];<br>9.1 (95% CI 4.5–16.2) (Japan) [7];<br>3.0 (95% CI 0.30–16.83) (our cohort) [3▪▪] |
| Anti-SAE | 1.3% ~ 10% in DM | 14% ~ 57% | 12.92 (95% CI 3.23–32.94) (our cohort) [3▪▪] |

95% CI, 95% confidence interval; CAA, cancer-associated autoantibody; DM, dermatomyositis; IIMs, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotizing myopathy; PMS, paraneoplastic myositis syndrome; SIR, standardized incidence ratio.
Adapted from [3▪▪,6▪,7,8] with permission.

The sensitivity of anti-TIF1-γ antibodies for diagnosing cancer-associated myositis/PMS was reported to range from 22 to 100%, and specificity from 54 to 98% in different studies [4▪▪]. The pooled estimated sensitivity was 52% (95% CI 0.47–0.57), and the specificity was 92% (95% CI 0.90–0.93). The overall log diagnostic odds ratio (DOR) for cancer in the presence of anti-TIF1-γ antibodies was 9.37 (95% CI 5.37–16.34) [4▪▪].

Interestingly, Abe *et al.* recently showed that anti-TIF1-γ antibody titers did not decrease after treatment in CAM patients, but they did reduce in IIM patients without malignancy. If anti-TIF1-γ antibody titers increase after treatment in IIM patients, it raises the possibility of an underlying malignancy [20▪▪]. In addition, Aussy *et al.* showed that the presence of anti-IgG2 isotype of anti-TIF1-γ antibodies, not other isotypes, was significantly associated with the occurrence of cancer during follow-up in patents with dermatomyositis, with a 100% positive predictive value of cancer when the mean fluorescence intensity of anti-TIF1-γ IgG2 was higher than 385 MFI [21▪].

The types of cancer occurred in anti-TIF1-γ-positive patients were comparable with those in the general population whenever stratified by age and sex. Most of them were solid cancers. On the other hand, the association of the antibody with haematological malignancies varied in different studies. In our cohort, the most common cancer was lung cancer (26%), followed by breast cancer (18%), and gynecological cancer (18%), and no haematological malignancies were observed [3▪▪]. However, in the UKMyoNet cohort, lymphoma was reported to be the third most common malignancy in anti-TIF1-γ-related PMS (14%) [5▪]. It is not clear whether the difference between these two results is because of ethnical differences. However, a recent meta-analysis study did not show significant increased risk of haematological malignancies in the presence of anti-TIF1-γ antibodies [4▪▪]. Moreover, Ogawa-Momohara *et al.* noted that among the dermatomyositis patients with cancer, those with anti-TIF1-γ antibodies presented with a higher frequency of advanced cancer than those who were anti-TIF1-γ-negative [17▪]. A similar phenomenon was also reported by Aussy *et al.* [21▪].

A close temporal relationship between PMS onset and cancer diagnosis was observed in patients with anti-TIF1-γ antibodies. In our cohort, in most cases, PMS and cancer were diagnosed simultaneously in patients with anti-TIF1-γ antibodies. The median duration of dermatomyositis (or PMS) at cancer-diagnosis was +0.19 years [interquartile range (IQR) − 0.02 to +0.19 years, the plus sign signifies cancer developing after myositis onset and the minus sign signifies cancer developing before myositis] [3▪▪]. Similar results were also observed in the Japan cohort. Hida *et al.* [11] reported that 97% of the cancers were detected within 1 year of PMS diagnosis in anti-TIF1-γ-positive patients. More recently, Oldroyd *et al.* reported that the median time from dermatomyositis (or PMS) onset to subsequent malignancy diagnosis throughout the entire follow-up period was shorter for anti-TIF1-γ positive compared with anti-TIF1-γ negative cases [1.4 years (IQR 0.7–2.5) vs. 5.0 (2.5–10.4)]. All detected cancers in anti-TIF1-γ positive cases occurred within 2.5 years following dermatomyositis onset; no further cases of cancer were detected within the remaining follow-up period (up to 10 years). Cox proportional hazard modelling, adjusted for age, sex and other status, revealed that anti-TIF1-γ positivity was significantly associated with a shorter time between dermatomyositis and cancer onset [hazard ratio 3.2 (95% CI 1.8–5.5)] [5▪].

## Anti-NXP-2 antibodies

Anti-NXP-2 antibodies are one of the dermatomyositis-specific autoantibodies. The association between anti-NXP-2 antibodies and cancer in patients with adult dermatomyositis has been reported by a few studies as well. The frequency of PMS in patients who carried anti-NXP-2 antibodies varied from 7 to 37.5% (Table 1) [3▪▪,6▪,22,23]. In our cohort, 7% of the anti-NXP-2-positive dermatomyositis had cancer, and the temporal relationship between dermatomyositis onset and cancer diagnosis was also very strong with the median duration of dermatomyositis at cancer-diagnosis +0.5 years (IQR −0.08 to +0.75 years) [3▪▪]. A study of a Japanese dermatomyositis population by Ichimura *et al.* showed that 37.5% of the anti-NXP-2-positive dermatomyositis patients developed malignancy, mostly within 1 year around the diagnosis [22]. A study by Albayda *et al.* [6▪] revealed that patients with anti-NXP-2 antibodies had a 3.68-fold higher risk of cancer (95% CI 1.2–8.6) compared with the US general population of the same age and sex. We also compared our patients with their age-matched and sex-matched counterparts in the general Chinese population, and the overall cancer risk in anti-NXP-2 positive patients was about 8-fold higher (SIR = 8.14, 95% CI 1.63–23.86) [3▪▪].

The cancer types associated with anti-NXP-2 antibodies were similar to those observed in anti-TIF1-γ-positive population: most of them were solid cancers, and haematological malignancies were not common. Moreover, Ichimura *et al.* [22] reported that all of the malignancies were discovered at an advanced stage (stages IIIb–IV) in anti-NXP-2-positive patients, and that all of the patients were men

above the age of 50 years. Similar results were also showed by Fiorentino *et al.* [23] in another Japanese dermatomyositis population. However, whether this particular phenomenon exists in other ethnic cohorts still requires further studies.

### Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies

Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase is one of the IMNM-specific autoantibodies. The frequency of the anti-HMGCR antibodies was about 5% in IIM, and 44.9% (95% CI 33.8–56.6%) in the IMNM population, reported by a large international multicenter study [24]. There were studies that reported that the prevalence of cancers was increased in patients with anti-HMGCR antibodies [7,8,25,26]. Allenbach *et al.* [8] reported that malignancy occurred in 17.3% of anti-HMGCR-positive patients. The mean duration between the diagnosis of cancer and the myopathy was $4.2 \pm 4.9$ years in anti HMGCR-positive patients, but two-thirds of the malignancies occurred within 3 years of or before the diagnosis of anti-HMGCR myopathy. They further compared the patients with the general age-matched and sex-matched general population and found that anti-HMGCR positive patients have a significantly increased SIR (2.79, 95% CI 1.02–6.07) for developing cancer. A study by Kadoya *et al.* showed [7] that the prevalence of cancer detection within 3 years of myopathy diagnosis among the patients with anti-HMGCR antibodies was 36%. In the analysis of the risk of cancer, the SIR within 3 years of myopathy diagnosis was 9.1 (95% CI 4.5–16.2), and that within 1 year was 22.1 (95% CI 10.6–40.7) [7]. However, in our cohort, the frequency of PMS in anti-HMGCR-positive patients was 4.7%, and the SIR was 3.0 (95% CI 0.30–16.83), but it did not reach statistical significance [3▪▪]. The risk of cancer in the presence of anti-HMGCR antibodies needs to be further investigated in large cohort studies with different ethnic groups.

### Antismall ubiquitin-like modifier 1 activating enzyme antibodies

Anti-SAE is one of the dermatomyositis-specific autoantibodies, which occurs with a lower frequency ranging from about 1.3 to 10% [27–32]. Most of the studies on anti-SAE antibodies have reported a high frequency of cancers in anti-SAE-positive patients, ranging from 14% (1/7) to 57% (4/7) [29–32]. However, all of them were very small sample studies. In our cohort, we found that the risk of cancer was significantly increased in anti-SAE positive patients (SIR = 12.92, 95% CI 3.23–32.94). All of the cancers occurred in patients with anti-SAE antibodies were adenocarcinoma from cervical, pulmonary, esophageal or rectal origin. The median duration of myositis at cancer diagnosis in the anti-SAE group was +0.46 years (IQR −1.4 to +1.0 years).

In addition, there were cases that reported the myositis patients with other MSAs, such as anti-Jo-1 and anti-PL12 antibodies, complicated with cancer. However, larger sample studies did not confirm that the cancer risk would significantly increase in those antibody-positive population.

## PATHOGENESIS OF CANCER-ASSOCIATED AUTOANTIBODIES

There were few studies regarding whether CAAs contribute to the development of myositis or cancer in PMS patients. Arouche-Delaperche *et al.* [33▪▪] demonstrated that the in-vitro cultured muscle cells stimulated by anti-HMGCR antibodies showed significantly increased myotube and myofiber atrophy, obviously up-regulated transcription of *MAFbx* and *TRIM63*, and produced high levels of inflammatory cytokines including TNF, IL-6 and reactive oxygen species. In addition, the differentiation of myoblasts after stimulation by anti-HMGCR antibodies was impeded, and the generation of myotube was reduced [33▪▪]. Further in-vivo study illustrated that passive transfer of IgG from IMNM patients to mice provoked muscle deficiency, and that immunization with recombinant HMGCR to mice induced the production of anti-HMGCR antibodies and subsequent muscle strength deficiency [34▪]. These results suggested that the pathogenic role of anti-HMGCR autoantibodies as a significant contributor, but not just a biomarker, in myositis.

It is also important to clarify whether autoantigens of CAAs contribute to the development of myositis or malignancy in PMS. It is widely speculated that the abnormally expressed or mutated autoantigen genes in tumor could possibly induce cross immunity against self-proteins and subsequently lead to the development of PMS. Pinal-Fernandez *et al.* performed whole-exome sequencing analysis to examine the presence of somatic mutations and loss of heterozygosity (LOH) in the *TIF1* genes of anti-TIF1-γ-positive CAM patients [35▪▪]. Intriguingly, in seven anti-TIF1-γ-positive CAM patients, they found one somatic mutation and five cases of LOH in one or more of the four *TIF1*genes, whereas only one case of LOH in tumors from anti-TIF1-γ-negative patient was observed. Further in-silico analysis revealed that both the normal peptide and the mutated peptides had high binding affinity to HLA-A and HLA-B, leading to the

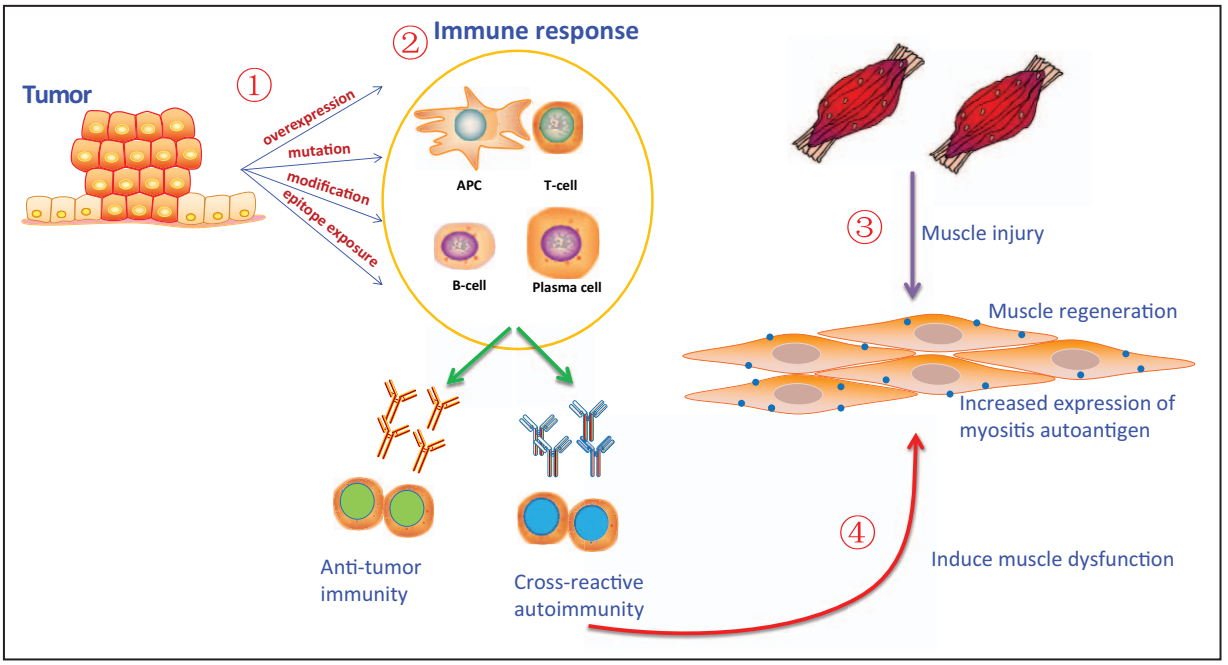


**FIGURE 1.** Hypothetical model of cancer-triggered cross-reactive autoimmunity. (1) Tumor antigens may be mutated, overexpressed, abnormally modified, or expose concealed antigens. (2) Alongside the induced antitumor immune response, the abnormally changed tumor antigens may result in cross-reactive autoimmunity, which is directed to autologous tissue. (3) Under certain muscle injury, regenerating muscle cells with overexpressed myositis autoantigens expanded. (4) The cross-reactive immune effector cells or molecules may result in ongoing muscle damage.

hypothesis that the elevated expression of TIF1-γ with strong binding affinity to HLA class I may generate an intense immune response, and consequently affect the tumor neoantigen availability. Interestingly, investigations on scleroderma, another significant paraneopalastic syndrome, has also demonstrated that genetic mutations of the POLR3A locus existed in patients with anti-RPC1 antibodies, but not in patients without anti-RPC1 antibodies [36]. Further analysis revealed that POLR3A mutations triggered cellular immunity and cross-reactive humoral immune responses [36], supporting the idea that tumor antigen could trigger autoimmune response by inducing cross-reactive immunity (Fig. 1).

However, the cross-reactive immune response hypothesis, although plausible, raises additional questions as to why apparently most of myositis patients do not develop malignancy in their whole lifetime. A possible explanation is that, according to the cancer immunoediting theory [37], once cancer cells survived the immune clearance, they can exist peacefully with the immune system in a 'Equilibration' state. As such, a second hit causing tissue injury, such as muscle damage and abnormal muscle regeneration is of importance to break this equilibration and results in autoimmune myositis.

## CONCLUSION

PMS is a special subtype of IIM. Early detection of cancers is essential to improve the prognosis of patients with PMS. Therefore, it is of great clinical significance to find specific and sensitive biomarkers in this population for diagnosing cancer early. In this review, we address that a subgroup of MSAs, which includes anti-TIF1-γ, anti-NXP-2, anti-HMGCR and anti-SAE may act as CAAs in PMS. It is necessary to closely screen and monitor for cancer within patients present with CAAs. However, our study revealed that the risk of cancers is also significantly increased in the dermatomyositis patients without MSA [3▪▪], suggesting that there may be other new CAAs, which have not been found, and that it is worth further investigating. In addition, the relationship between CAA and PMS also provides a crucial clue for us to understand the pathogenesis of IIM and PMS.

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## Conflicts of interest

*There are no conflicts of interest.*

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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- ■■ of outstanding interest


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This is a large longitudinal cohort study that estimated the cancer risk associated with different MSAs in patients with IIM by SIR. The study demonstrated that a subgroup of MSAs, that is anti-TIF1-Ι` gamma, anti-NXP-2 and anti-SAE1, are associated with an increased risk of malignancy, and that other MSAs might also be driven by malignancy in some cases.

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A systematic review reported that the pooled prevalence of cancer-associated dermatomyositis in patients with anti-TIF-1γ autoantibody was 41%. In the presence of anti-TIF-1γ antibodies, the overall diagnostic odds ratio of cancer was 9.37. The study confirmed that detection of anti-TIF- 1γ autoantibody is a valuable tool to identify a subset of adult dermatomyositis patients with higher risk of cancer.

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The study investigated the temporal relationship between anti-TIF1-Ab positivity and cancer onset in a large dermatomyositis cohort over a 10 years follow-up period after dermatomyositis onset. The results showed that anti-TIF1-Ab-positive-associated malignancy occurred exclusively within 3 years either side of the dermatomyositis onset, and no incident cancer cases were detected in this group within the subsequent 7.5 years.

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# Update on pregnancy complications in systemic lupus erythematosus

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**Purpose of review**
This review summarizes recent research in the field of systemic lupus erythematosus (SLE) and pregnancy with focus on clinical and biochemical predictors of adverse pregnancy outcomes (APOs), accumulating evidence for the safety and efficacy of hydroxychloroquine (HCQ) in pregnancy, and the importance of preconception counseling.

**Recent findings**
Ongoing research from PROMISSE investigators (Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus) adds to the understanding of risk factors for APOs in SLE pregnancies, including aberrant complement activation, incomplete downregulation of lupus-associated transcription factors, and lower socioeconomic status. Evidence supporting numerous advantages for continuing HCQ in pregnancy, as well as support for low-dose aspirin in preeclampsia prevention is reviewed. Practice gaps exist among rheumatologists in ensuring effective contraception when women of childbearing age are undergoing therapy with potentially fetotoxic medications. The publication of organizational guidelines provides evidence-based recommendations on lupus pregnancy management.

**Summary**
Outcomes of lupus pregnancies continue to improve with understanding of risk factors that predict APOs as well as improvements in disease management. Rheumatologists caring for women with SLE should be familiar with the most up-to-date research in order to optimize pregnancy outcomes in this population.

**Keywords**
antiphospholipid syndrome, preeclampsia, pregnancy, systemic lupus

## INTRODUCTION

Pregnancy for women with systemic lupus erythematosus (SLE) is associated with increased risks of adverse pregnancy outcomes (APOs) including prematurity, intrauterine growth restriction (IUGR), preeclampsia and fetal loss, increases in maternal morbidity and mortality, and the syndrome of neonatal lupus because of transplacental passage of autoantibodies [1–3]. Advances in the field of reproductive health and autoimmunity over the last several decades have improved outcomes significantly, and women with SLE are attempting and carrying healthy pregnancies at higher rates than in the past and conceiving at higher rates [4,5]. Rates of fetal loss in SLE pregnancies have decreased from 43% in the period of 1960–1965 to 17% in the period of 2000–2003 [6], reflecting healthier pregnancies. A recent study from a nationwide prospective observational registry of women with inflammatory rheumatic diseases revealed that women with SLE are not only more often successful in achieving pregnancy than women with rheumatoid arthritis (RA), but they also have substantially shorter time to pregnancy than women with RA, defined as the time in months between pregnancy wish and the first day of the last menstrual period before pregnancy (3 vs. 7 months, $P = 0.001$) [7]. Multiple factors likely contribute to this trend: a growing understanding of the importance of risk assessment as part of preconception counseling, increasing use of pregnancy-compatible medications to control disease throughout pregnancy and postpartum, and advances in the management of SLE in general and in high-risk pregnancies, in particular. This review summarizes

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## KEY POINTS

- HCQ should be continued in SLE pregnancy and started in anticipation of pregnancy, as it has been shown to improve pregnancy outcomes in this population and may have a role in prevention of preeclampsia and cardiac neonatal lupus.
- Up-to-date evidence-based guidelines from European and American Rheumatology organizations provide complementary recommendations on management of reproductive health issues in women with SLE and the antiphospholipid syndrome (APS).
- Aberrant complement activation has been implicated in APOs in women with lupus and APS, and is currently being targeted in a therapeutic trial aimed at improving outcomes in high-risk pregnancies in these populations.
- Low-dose aspirin is recommended by multiple professional groups to reduce rates of preeclampsia in at risk pregnancies, including SLE/APS, yet rates of use are low, particularly in black and Asian pregnancies.
- A knowledge gap persists among rheumatologists (and among patients with rheumatic diseases) regarding reproductive health issues, particularly regarding safe and effective contraception and pregnancy-compatible medications.

the most recent data on the topic of pregnancy in lupus.

## PREDICTING RISK FOR ADVERSE OUTCOMES IN LUPUS PREGNANCIES

### Clinical characteristics and biomarkers

Clinical and laboratory predictors of APOs in women with SLE and mild or inactive disease at conception have been examined in the PROMISSE study (Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Syndrome and Systemic Lupus Erythematosus), a prospective, observational multicenter, multiethnic, and multiracial cohort [8]. A 2015 study of this population identified high clinical SLE disease activity at baseline, the presence of lupus anticoagulant, nonwhite ethnicity, the use of antihypertensive medications at baseline, and thrombocytopenia as predictors of APOs. Other studies have confirmed the importance of low disease activity in predicting favorable pregnancy outcomes, and a 6-month period of SLE disease quiescence has been associated with decreased risk of flare during pregnancy and improved fetal outcomes, including fewer pregnancy losses [9–12]. A history of lupus nephritis or active nephritis at conception has also been shown to predict APOs, whereas past kidney disease and low C4 at baseline are independently associated with higher risk of developing active nephritis in pregnancy [13–15]. A recent study examined the effect of WHO lupus nephritis histologic classification on pregnancy outcomes in a Brazilian SLE cohort of majority nonwhite women [16]. Data on lupus nephritis were collected retrospectively from 2011 to 2015 and prospectively in 2016 on 137 women who had 147 pregnancies; those with current or history of proliferative lupus nephritis (III/IV; $n = 54$) experienced more disease flares ($P = 0.02$) and ongoing active SLE throughout pregnancy and postpartum than those without current or history of lupus nephritis ($n = 81$; $P = 0.006$). Women with proliferative lupus nephritis had more hospitalizations [both SLE-related ($P < 0.001$) and non-SLE related ($P = 0.04$)], and higher frequency of preeclampsia ($P = 0.01$) than women without lupus nephritis.

More recently, PROMISSE investigators found an association between aberrant activation of the alternate complement pathway and APOs [17▪▪]. Prior research in a murine model of obstetric antiphospholipid syndrome (APS) demonstrated complement activation in placental tissue, and that tumor necrosis factor (TNF)-α was a crucial intermediate between C5 activation and fetal loss. Indeed, either TNF blockade or TNF deficiency resulted in clear fetal protective effects in this model [18]. Similar findings of complement activation have been observed in women from the general population who developed preeclampsia between 10 and 20 weeks gestation, with complement split products identified in amniotic fluid of women with severe preeclampsia [19–21]. PROMISSE investigators found that in their cohort, markers of complement activation (Bb and sC5b-9) were detectable in blood early in pregnancy among SLE/APS patients who went on to have APOs, and remained elevated through 31 weeks compared with those with normal outcomes.

These results informed an open-label phase II interventional trial testing the hypothesis that blocking pro-inflammatory downstream effects of complement activation, such as TNF-α will reduce APOs in women with risk factors identified in the PROMISSE study. The IMPACT trial [IMProve Pregnancy in APS With Certolizumab Therapy (NCT03152058)] is recruiting women at less than 8 weeks of gestation with APS or lupus anticoagulant positivity on two occasions, with or without SLE, who will receive anti-TNF-α therapy with certolizumab, a PEGylated monoclonal antibody with minimal transplacental passage [22▪]. The study will assess the ability of TNF-α inhibition to improve outcomes in this at-risk population in whom only 70–80% of pregnancies

result in live births despite anticoagulation and effective disease control and in whom APOs – including preterm delivery, preeclampsia (particularly early, severe preeclampsia), and IUGR – are still observed at alarmingly high rates [23].

Another recent study using PROMISSE data examined whole blood transcription profiles obtained in early pregnancy (92 SLE patients and 43 healthy pregnant women) and found that incomplete downregulation of SLE-associated transcriptional networks, including type-l interferon and plasma-cell-related transcripts, was associated with SLE pregnancy complications – supporting a potentially pathogenic role for these signatures at the maternal–fetal interface [24▪]. The investigators further identified a list of early transcriptional changes that predicted preeclampsia, thus purporting a potential mechanism for APOs in this population that may help distinguish between preeclampsia and lupus nephritis (a common clinical quandary).

A final noteworthy and recent study from PROMISSE investigators examined the contribution of socioeconomic status (SES) and ethnic disparities to APOs in SLE pregnancies [25▪▪]. Among SLE patients negative for antiphospholipid antibodies (aPLs) in the PROMISSE study, the frequency of APO for black and Hispanic women was nearly two-fold greater than for white women. However, after additional adjustment for SES, there were no longer significant differences in APOs among black women compared with white women, implicating a potential larger role for SES in pregnancy disparities among black women with SLE.

### Cardiovascular health

A study from the Hopkins Lupus Pregnancy Cohort examined American Heart Association (AHA) guidelines for cardiovascular health (BMI, total cholesterol, and blood pressure), in women with SLE prior to conception and showed that better preconception cardiovascular health is associated with better pregnancy outcomes [26▪▪]. The study included 309 live births, 95 of which were preterm. Overweight women had a nearly 40% increased risk of preterm birth compared with low/normal BMI women, and a 74% decreased risk of small for gestational age (SGA) infant compared with women with low or normal BMI [odds ratio (OR) 0.26, 95% confidence interval (CI) 0.11–0.63], after adjustment for race and prednisone use. Unfavorable cholesterol was associated with increased odds of preterm birth (OR 2.21, 95% CI 1.06–4.62), and hypertension was associated with decreased gestational age at birth ($\beta$ −0.96, 95% CI −1.62 to −0.29), adjusted for race and renal involvement. This study highlights a potential practice gap in caring for a population in whom traditional cardiovascular risk factors associated with APOs in their own right may be overlooked, despite the fact that hypertension, dyslipidemia, and obesity affect 30–60% of SLE patients [27–29], and preeclampsia, recognized by the AHA as a cardiovascular disease risk factor, affects 22.5% of SLE pregnancies [30].

### Predictive model for fetal loss in lupus pregnancy

Although numerous risk factors have been identified for APOs in SLE pregnancies, a recent study from a tertiary hospital in Shanghai is notable for testing a prediction model and risk score for pregnancy loss in SLE using retrospective data for 338 SLE pregnancies seen at the authors' institution from September 2011 to May 2017 [31▪]. The authors identified three variables that independently predicted fetal loss after adjusting for confounders: unplanned pregnancies (OR 2.84, 95% CI 1.12–7.22), low C3 (OR 5.46, 95% CI 2.30–12.97), and 24 h-urinary protein (OR 2.10, 95% CI 2.30–15.06). The risk score was calculated by the following equation: Fetal loss risk score = 'unplanned pregnancy' score + 'hypocom- 'hypocomplementemia-C3' score + '24 h-urinary protein' score. Results were then divided into low risk (0–3) and high-risk groups (>3), with a sensitivity of 60.5%, specificity of 93.3%, positive likelihood ratio of 9.03, and negative likelihood ratio of 0.42. The risk score may prove practical and applicable to everyday practice, having the advantage of including standard-of-care testing (if spot urine protein/creatinine is substituted) and ease of calculation for identifying high-risk SLE pregnancies.

## PREECLAMPSIA

Preeclampsia, characterized by new hypertension and proteinuria after the 20th week of gestation, occurs in 2–8% of pregnancies in the general population, 17.3% of APS pregnancies, and 22.5% of SLE pregnancies, representing a14% higher risk in SLE compared with healthy women [2,32,33]. Underlying SLE as well as both lupus nephritis and aPL/APS are risk factors for preeclampsia [34–37]. The addition of low-dose aspirin (LDA) for preeclampsia prevention in SLE pregnancies is recommended by the European League Against Rheumatism (EULAR) [38▪▪]. Recommendations to start LDA at 12 weeks of gestation for women with absolute risk of preeclampsia of at least 8% from the US Protective Health Task Force (USPHTF) and the American College of Obstetrics and Gynecology (ACOG) support this practice in SLE/APS pregnancy, and are based on

a systematic review of randomized controlled trials reporting risk reduction of preeclampsia by 24% with LDA among women at high risk (RR, 0.76; CI 0.62–0.95) [39–41]. A recent analysis of the use of aspirin among pregnant women in the Systemic Lupus International Collaborating Clinics (SLICC) inception cohort revealed its use in only 25% of pregnancies [42▪]. This study identified 475 pregnancies in 300 women with SLE from 2000 to 2017, during which time aspirin use in pregnancy did not increase. Furthermore, although aspirin was used in a third of pregnancies in Caucasians and Hispanics, it was used in only 10 and 11% of black and Asian pregnancies, highlighting an important practice gap in SLE obstetric care in general and among nonwhite women in particular, in whom disparities in obstetric outcomes have been previously observed [8].

## NEONATAL LUPUS

Thirty to 40% of women with SLE are positive for anti-Ro/SSA autoantibodies, and 10–15% are positive for anti-La/SSB autoantibodies [43], putting their pregnancies at risk for neonatal lupus. This syndrome can cause transient, relatively benign cutaneous, hematologic, and hepatic manifestations in anywhere from 10 to 30% of newborns [44]. The more feared complication is cardiac neonatal lupus, of which congenital heart block (CHB) is the most common manifestation, found in about 2% of SSA/SSB+ pregnancies [45]. If a woman with SSA/SSB has had an infant with prior cutaneous or cardiac neonatal lupus, the risk for third degree heart block is increased to 13–18% for future pregnancies [45]. Approximately 20% of children affected by complete CHB will die *in utero* or in the first year of life, and up to 70% will require a pacemaker [46,47]. Involvement beyond the conduction system may also be observed in cardiac neonatal lupus, including endocardial fibroelastosis and dilated cardiomyopathy, imparts a worse prognosis [48,49].

Although use of fluorinated corticosteroids for first or second-degree CHB has revealed mixed results, a common practice is to treat with 4 mg of oral dexamethasone once a day for a period usually of no more than several weeks given the risks to mother and fetus associated with corticosteroid therapy [50–52]. Corticosteroids have not been shown to prevent extension of inflammation beyond nodal disease in advanced CHB, prevent pacemaker implant, or improve survival [51].

A retrospective study examining the role of hydroxychloroquine (HCQ) for prevention of recurrent cardiac neonatal lupus among an international registry of pregnant women with anti-SSA/SSB revealed decreased risk of CHB developing in their next pregnancy [53]. Among 257 pregnancies, 40 women were exposed to HCQ and 217 were unexposed to HCQ, with a recurrence rate of cardiac neonatal lupus in HCQ pregnancies of 7.5% (3/40), as compared with 21.2% (46/217) in the unexposed group ($P = 0.05$). An open-label, prospective trial of HCQ in women at risk for recurrent pregnancies complicated by cardiac neonatal lupus, The Preventive Approach to Congenital Heart Block with Hydroxychloroquine (PATCH) study (NCT01379573) is currently recruiting.

## MEDICATIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS PREGNANCY

More extensive discussion on maternal and paternal medication safety in SLE/APS pregnancies is available in EULAR guidelines and taskforce publication on 'points to consider on use of antirheumatic drugs before pregnancy, and during pregnancy and lactation' [38▪▪,54,55▪]. Immunosuppressive medications including cyclophosphamide, methotrexate, leflunomide, mycophenolate mofetil/mycophenolic acid, and thalidomide are contraindicated in pregnancy because of teratogenic and/or abortifacient effects, and should be discontinued within 3 months prior to conception (see Table 1) [57–61]. Azathioprine, HCQ, sulfasalazine, cyclosporine, tacrolimus, low-dose prednisone, and IVIG are generally considered well tolerated in pregnancy. Pregnancy data on the safety of belimumab, a human monoclonal antibody that inhibits the soluble form of a B-cell survival factor known as BLYs or BAFF, is being collected through pregnancy exposure registries, and no congenital abnormalities have been observed in approximately 200 exposed pregnancies from the manufacturer's registry and from pregnancies occurring incidentally during clinical trials [62,63].

HCQ has been shown to reduce SLE flares and disease activity, allowing for lower doses of glucocorticoids and reducing rates of prematurity, and likely has additional benefits in prevention of complications related to obstetric APS and cardiac neonatal lupus [53,64–67]. A 2019 retrospective single-center study at a tertiary care center also observed lower risk of preeclampsia in SLE pregnancies associated with HCQ therapy in 151 pregnancies among 122 SLE patients [68▪]. The preeclampsia rate was lower (7.5 vs. 19.7%, $P = 0.032$) and neonatal birth weight was greater ($2757.0 \pm 583.5$ vs. $2542.3 \pm 908.3$ g; $P = 0.001$) in pregnancies in which women took HCQ throughout pregnancy than in the nontreatment group (those who stopped HCQ more than 3 months before pregnancy).

**Table 1.** Safety of medications in pregnancy and breast feeding

| Medication | Safe in pregnancy | Compatible with breastfeeding |
|---|---|---|
| Immunosuppressive therapy | | |
| Azathioprine | Yes | Yes |
| Cyclosporine | Yes | Yes |
| Tacrolimus | Yes | Yes |
| Cyclophosphamide | No; discontinue within 3 months prior to conception[a] | No |
| Methotrexate | No; discontinue within 3 months prior to conception | No |
| Mycophenolate mofetil | No | No (no data) |
| Leflunomide | No; cholestyramine wash out recommended prior to conception | No (no data) |
| Antimalarial therapy | | |
| Hydroxychloroquine | Yes | Yes |
| Quinacrine | No (no data) | No (no data) |
| Prednisone | Yes; goal <20 mg/day | Yes |
| IVIG | Yes | Yes |
| NSAID | | |
| Traditional NSAID | Yes; discontinue prior to third trimester | Yes |
| COX2 inhibitors | No (less data) | No (less data) |
| Low-dose aspirin | Yes; start at 12 weeks for preeclampsia prevention[b] | Yes |
| Unknown[c] | | |
| Belimumab | | |
| Rituximab | | |

Adapted from Götestam Skorpen *et al.* [54].
[a]Contraindicated in the first trimester. Use in second or third trimesters for organ-threatening disease if benefits outweigh potential risks [54].
[b]Daily LDA not excreted in breast milk [56].
[c]Avoid prior and during pregnancy/lactation unless benefits outweigh potential risks [54].

A 2018 study from the Hopkins Lupus Cohort examining SLE disease activity and HCQ use in pregnancy found an increased incidence of flare during pregnancy and within the 3 months postpartum, but that continuing HCQ throughout that period appeared to mitigate the risk of flare [69▪▪]. Three hundred and ninety-eght pregnancies in 304 patients were observed, and SLE flare assessed by physician's global assessment (PGA) was more common during pregnancy (hazard ratio 1.59; 95% CI 1.27–1.96) compared with outside of pregnancy, but only in women not taking HCQ. The hazard ratio of flares in pregnancy compared with nonpregnant/nonpostpartum periods was 1.83 (95% CI 1.34–2.45) for patients with no HCQ use and 1.26 (95% CI 0.88–1.69) for patients with HCQ use. The risk of flare was also elevated among non-HCQ users in the 3 months postpartum, but not for women taking HCQ after delivery; hazard ratio of flares was 1.63 (95% CI 1.04–2.39) without HCQ and 1.25 (95% CI 0.71–1.87) with HCQ, suggesting a beneficial effect of HCQ on postpartum disease. This study is also noteworthy because it extends previous work of the Hopkins Pregnancy Cohort, revealing a dramatic decrease in flare rates since the initial observations published in 1991 [70], reflective of the improvements in SLE pregnancy management over the past 25 years.

A recent observational study from the Duke Autoimmunity in Pregnancy registry examined the effect of physiologic changes of pregnancy on serum HCQ concentrations and correlations with pregnancy outcomes [71]. HCQ levels from women taking the medication prior to and continuously during pregnancy were measured, and levels were categorized as nontherapeutic (≤100 ng/ml) or therapeutic (>100 ng/ml). In 145 samples from 50 patients with rheumatic disease, 56% of whom had SLE, HCQ concentrations varied widely in each trimester. Mean PGA scores in SLE patients were significantly higher in women who averaged HCQ levels 100 ng/ml or less as compared with greater than 100 ng/ml (0.93 vs. 0.32, $P = 0.01$). Among women with SLE, 83% with average drug levels 100 ng/ml or less delivered prematurely ($n = 6$), compared with only 21% with average levels greater than 100 ng/ml ($n = 19$; $P = 0.01$). These results highlight opportunity for further research into HCQ drug levels and pregnancy outcomes.

A 2018 systematic review examining data on childhood ocular outcomes after exposure to antimalarial medications during SLE pregnancy and/or lactation included 1477 infants, 789 of whom were exposed to HCQ or chloroquine, and 331 of whom underwent ophthalmologic exams [72▪]. The results were surprising in that only half of the women were taking HCQ, but reassuring in that two children with clinically evident ocular anomalies noted at birth (retinal hemorrhages) healed by the first month, and 6/21 children with abnormal electroretinography all had normal fundoscopy before the age of 4 years, leading the authors to conclude that the risk of ocular toxicity in HCQ exposed offspring appears low to nonexistent.

## PRECONCEPTION COUNSELLING AND CONTRACEPTION

SLE may be associated with disease-specific target-organ involvement for which pregnancy poses serious risks of morbidity and mortality, including severe renal disease, pulmonary artery hypertension, or interstitial lung disease [38▪▪]. Rheumatologists caring for women with SLE should be familiar with these risks, and be able to provide recommendations and education on safe and effective contraception as well as pregnancy-compatible medications for treating SLE, to help patients plan for conception during a period of well controlled or quiescent disease.

Hormonal contraception with progestin only, or combined with low-dose estrogen, appear well tolerated in quiescent or mildly active SLE (assuming the absence of aPLs) [73]. Copper intrauterine device (IUD) insertion in women with SLE has been shown in a randomized, prospective study of contraceptive methods to impact nether disease activity nor incidence of SLE flare [74]. Both copper and progestin-only IUDs are safe contraceptive options for women with SLE in general, and IUDs probably represent the best option for women with autoimmune diseases; the action of the progestin IUD is exerted mostly within the reproductive tract, and the copper-releasing device does not exert hormonal activity, making it preferred for patients with aPLs/APS [75,76].

A recent study of single-center administrative data from reproductive-age women with rheumatic diseases examined associations between the use of prescription contraception, use of potentially fetotoxic medications, and visits with rheumatologists, primary care providers (PCPs), and gynecology providers [77▪▪]. Results revealed that only 32.1% of women used any kind of prescription contraception despite the fact that 70% were taking at least one fetotoxic medication during the 2 years of the study. Furthermore, women who saw gynecologists or PCPs were more likely to use prescription contraception overall (adjusted OR 3.35, 95% CI 2.77–4.05 and aOR 1.43, 95% CI 1.18–1.73, respectively), and rheumatology visits were not associated with use of prescription contraception in any models. A separate survey study of rheumatologists' knowledge of contraception, teratogens, and pregnancy risks revealed that, among 270 respondents, 88% identified methotrexate as a teratogen, but only 69% identified cyclophosphamide and 37% mycophenolate, raising concerns about rheumatologists' ability to give accurate recommendations to their young, female SLE patients [78▪▪]. Rheumatologists in this study were aware of the high effectiveness of IUDs, but overestimated the effectiveness of injectable medroxyprogesterone and condoms. These studies highlight a gap in rheumatologists' practice of preconception counselling and family planning for their female patients of reproductive age, many of whom report the need for more information on pregnancy planning, fertility, giving birth, and breastfeeding [79]. Efforts at improving education about safe and effective contraception and reproductive health issues in general for both rheumatologists and our patients are clearly needed.

## PREGNANCY MANAGEMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

The 2017 'EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome' provide recommendations for pregnancy management in SLE [38▪▪,54,55▪], and the draft ''2019 American College of Rheumatology Reproductive Health in Rheumatic and Musculoskeletal Diseases Guideline' recommendations are consistent with previously published professional group guidelines (currently under review by *Arthritis and Rheumatology*). EULAR 2017 recommendations include risk assessment related to anti-SSA/SSB, aPLs, control of disease, and minimization of glucocorticoid exposure with pregnancy-compatible medications, and evaluation for disease flare in each trimester or more frequently if higher risk [38▪▪]. LDA should be started at 12 weeks for preeclampsia prevention, and HCQ should be started or continued during pregnancy if not contraindicated given its effectiveness in preventing SLE flares, as well as possible risk reduction for recurrent cardiac neonatal lupus and preeclampsia. Co-management with High Risk Obstetrics/

Maternal Fetal Medicine is recommended for higher risk patients, as is the involvement of nephrologists for more complicated situations such as lupus nephritis or distinguishing between preeclampsia and active lupus nephritis in which examination of urinary sediment and identification of cellular casts is diagnostic for lupus nephritis. Similarly, conditions, such as HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) or eclampsia may also be difficult to distinguish from a severe SLE flare, requiring input from a multidisciplinary team.

## CONCLUSION

With increasing understanding of risk factors that predict APOs in SLE pregnancy, rheumatologists are in a better position than ever before to help their patients plan for the optimal time to attempt pregnancy, and plan for monitoring and treatment based on a woman's individual risk profile. Optimizing pregnancy compatible medications, as well as attention to disease-specific risk factors, cardiovascular risk factors, and racial and SES disparities, should all be part of the rheumatologist's care plan for female SLE patients of reproductive age. More education for rheumatologists is needed to help improve rates of aspirin use in SLE pregnancy for preeclampsia prevention, as well as improve numbers of women on safe and effective contraception during therapy with potentially fetotoxic medications. The results of several therapeutic trials will be known in the near future, both of which have the potential to change practice: The IMPACT trial is testing the ability of certolizumab to prevent APOs in women with APS or the lupus anticoagulant, and the PATCH study is testing the ability of HCQ to prevent recurrent cardiac neonatal lupus. Finally, the publication of updated organizational guidelines from American and European groups will serve as complementary resources for rheumatologists, providing the most accurate information on managing reproductive health issues in women with autoimmune diseases.

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### Conflicts of interest

*There are no conflicts of interest.*

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Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

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# An update on the genetics of systemic lupus erythematosus

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**Purpose of review**
The aim of this study is to update on the most recent findings on the genetics of systemic lupus erythematosus.

**Recent findings**
Our overview focuses particularly on results from expression quantitative trait loci, exome sequencing, and rare variants and their impact on disease.

**Summary**
Systemic lupus erythematosus is a systemic autoimmune disease for which a significant number of susceptibility genes have been identified. Several genome-wide association studies were recently published in different populations that provide a better picture of the molecular mechanisms. It is becoming clear that the genetic architecture of lupus is quite well established but more information is required on the role of rare variants.

**Keywords**
autoimmunity, exome sequencing, genome-wide association study, low-frequency variation, rare variation, systemic lupus erythematosus

## INTRODUCTION

Systemic lupus erythematosus (SLE MIM [152700]) is a systemic autoimmune disease (SAD) with a complex multifactorial etiology and a broad spectrum of clinical manifestations [1,2]. The complexity resides in the combination of various environmental and genetic factors in the initiation and progression toward disease development that occurs with time.

In this review, we provide an update of the genetics of SLE focusing on genetic association studies and fine mapping of known genetic variants affecting gene expression, but also on rare and de-novo variants and their potential role in familial aggregation and clinical features of the disease.

## GENETIC ASSOCIATIONS AND GENOME-WIDE ASSOCIATION STUDIES

SLE is a complex SAD that affects every organ and system in the body and with varying clinical and serological manifestations. Basically, the loss of tolerance in the immune system leads to the presence of autoantibodies and the deposition of immune complexes in various tissues, causing a great diversity of symptoms [3]. SLE has a strong genetic component supported by twin and family studies [4–6]. Multiple genome-wide and candidate-gene association studies identified over 80 SLE susceptibility loci, explaining about 30% of narrow-sense SLE heritability [7–9]. Although the overall heritability of complex diseases is difficult to estimate, a classical study in European (EUR) population did estimate a heritability of 66 plus-minus 11% for SLE [10], indicating that there was still more than 50% of heritability estimations missing for SLE from current genome-wide association studies (GWAS). However, the estimates of genetic influence on SLE susceptibility vary between studies and populations, reaching up to 44% in a Taiwanese study [11]. Even so,

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## KEY POINTS

- Currently, genetic studies have allowed the identification of more than 80 risk *loci* for SLE susceptibility, more than 50 of which were independently replicated.
- In general, there is the enrichment of *loci* SLE-susceptibility lying within transcription factors.
- Majority of established SLE risk loci, identified in independent GWAS studies correspond to adaptive and innate immunity, but there several stable risk associations remain unexplained.
- Many of SLE associated loci are also expression QTLs, regulating expression of these or neighboring genes.
- Availability of large-scale population sequencing databases allowed performing more comprehensive studies, on the basis of the genotype imputation both for common and rare variant SLE genetic studies.
- The most recent progress was achieved in the role of rare and de-novo variants in SLE susceptibility, with ~100 candidate loci identified, but further research is needed for their replication, validation, and causal mechanisms uncovering.

one-third of the heritability of SLE would still remain to be explained.

Most of the genetic studies have identified common variants showing consistent and robust genetic association after the strong advent of microarray technology and despite stringent Bonferroni correction. The main genes, associated with SLE susceptibility are summarized in Figure 1 and Table 1. The first three GWAS for SLE were simultaneously published [12–14] and addressed between 100,000 and 300,000 single nucleotide polymorphisms (SNPs). Nowadays, following the genotyping of millions of SNP per individual, the combined analysis of genotyping and sequencing can be used for imputation of missing SNVs onto the experimental genotype data to increase resolution. Both new whole-genome and whole-exome sequencing and population-specific databases such as the 1000 Genomes or the EUR ancestry-centered Haplotype Reference Consortium were shown to be useful for this purpose [15–17]. In general, the majority of the genes identified as susceptibility to SLE correspond to innate and adaptive immune system pathways.

The various GWAS studies identified several genes for SLE such as *BLK*, *ITGAM-ITGAX*, and *PXK* [12–14] and confirmed the human leucocyte antigens (*HLA*) and *FCGR2A*. An updated list of all known and replicated loci for SLE is found in Table 1. New knowledge on the function of some of the genes has been attained, for instance, the role of BANK1 in MyD88-TRAF6 innate immune signaling in B cells and the importance of its exon-2 TIR-domain where the genetic association described is located [13,18]. *IRF5* remains one of the best SLE-associated loci along with other interferon regulators *IRF7* and *IRF8*. A more recent GWAS in EURs analyzed over 8274 cases and 23,579 controls and identified 10 new associations [19]. These new signals increased the explained proportion of genetic variance in SLE from 8 to 16.3%. The validity of these SLE association signals was supported by meta-analysis using Bayesian methods. Among SLE risk variants from publications prior to 2017, 45 (34% of all tested) were under applied false-positive probability estimation approaches [20]. The new analysis approaches increased efficiency even in smaller case-control datasets. Thus a study including 907 cases and 1524 EUR controls followed by genotype imputation and metaanalysis using a larger sample was able to reveal new SLE-associated loci *GRB2*, *SMYD3*, *ST8SIA4*, *LAT2*, and *ARHGAP27* and supported 51 of 52 of the previously known [21]. Machine learning was applied on a targeted ImmunoChip genotyping study providing 15 new SLE-associated candidate loci and increasing SLE risk prediction accuracy, especially for lupus nephritis (area under the curve 0.91) [22].

The *HLA* class II region is undoubtedly associated with SLE susceptibility in all populations, but the tight linkage disequilibrium that characterizes the region has made it difficult to identify independent signals. Particularly, the DR15 haplotype containing classical alleles HLA-DRB1*15:01, HLA-DQB1*06:02, HLA-DQA1*01:02 [23], and HLA-DRB5*01:01 [24] is consistently replicated. Various signals within *HLA* class I and class III were found to independently contribute to SLE genetic susceptibility [25]. Targeted next-generation sequencing (NGS) of the major histocompatibility complex (MHC) region showed a haplotype with regulatory polymorphisms associated with changes in the gene expression of the HLA class II molecules [26]. Proper analysis of the highly variable *MHC* locus across populations is challenging. Interestingly, using local ancestry analysis on an Amerindian–EUR mixed ancestries' sample, it was found that the *HLA* risk-alleles had a EUR origin, whereas protective alleles or haplotypes were Native American [27]. A combined analysis of risk alleles of the HLA performed in EURs and African Americans showed that the long-range SLE associated signals in EUR corresponded to narrow independent peaks in African Americans data. Population-specific independent associations were detected. Nevertheless the majority of risk alleles, despite the great diversity of the

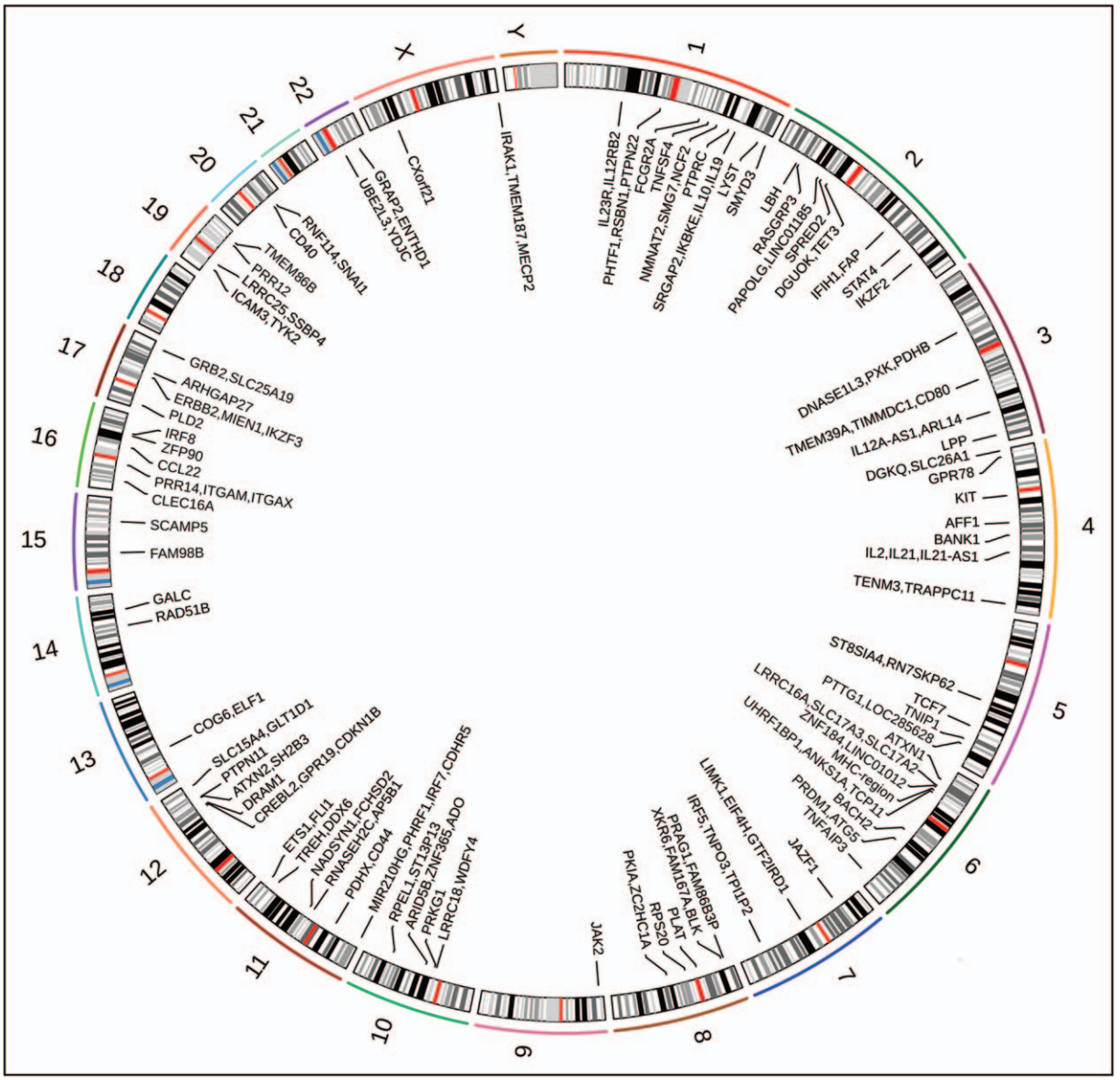


**FIGURE 1.** Schematic representation depicting genes located within the systemic lupus erythematosus risk loci (88 in total) according to their genomic position. The full set of variants and loci for this plot is summarized in Supplementary Table 1, http://links.lww.com/COR/A45. The annotation of genes and cytogenetic bands correspond to the hg19 assembly. The red block in each chromosome marks its centromere. This figure was made using R package 'Rcircos' [82].

African ancestry population, demonstrated similarity to EUR *HLA* SLE association and significant concordance in the direction of the allele effects [28]. Another study characterizing SLE risk in the HLA region across EUR, African, and Hispanic ancestries described risk-allele heterogeneity within DQA1/DQB1 and DRB1 [9]. HLA-DRB1∗15:01 and HLA-DQB1∗06:02 were associated to SLE risk in Asian ancestry populations [29,30]. The contribution of HLA alleles and specific amino-acid residues to the risk of SLE and the presence of specific autoantibodies has recently been dissected in a large East Asian sample [31].

Many of the SLE-associated genes identified in EURs have been replicated and confirmed in other populations, particularly the Chinese [8,3–38,39▪,40–43]. Targeted loci evaluation analysis confirmed *SPRED2*, *IKZF2*, *IL12A*, *TCF7*, *PLD2*, and others [39▪]. A study of a large cohort of East Asian patients (11,656 cases versus 23,968 controls) revealed five new SLE loci: *MYNN*, *ATG16L2*, *CCL22*, *ANKS1A*, and *RNASEH2C* [8]. A transancestral study of sporadic SLE in EURs and East Asian datasets of 12,280 cases and 18,828 controls further revealed *ST3AGL4*, *MFHAS1*, and *CSNK2A2*. The most pronounced results were obtained for *CSNK2A2* kinase,

**Table 1.** List of the 88 genomic regions annotated with the SLE trait which include associations with P values less than $8 \times 10^{-8}$ according to GWAS catalog (https://www.ebi.ac.uk/gwas/)

| *N* locus | CytoBand | Reported gene(s) | Ancestry | *N* studies |
|---|---|---|---|---|
| 1 | 1p31.3 | IL23R, IL12RB2 | AFR, EUR, HIS | 3 |
| 2 | 1p13.2 | PHTF1, RSBN1, PTPN22 | AFR, EAS, EUR, HIS | 5 |
| 3 | 1q23.3 | FCGR2A | AFR, EAS, EUR, HIS | 3 |
| 4 | 1q25.1 | TNFSF4 | AFR, EAS, EUR, HIS | 10 |
| 5 | 1q25.3 | NMNAT2, SMG7, NCF2 | AFR, EAS, EUR, HIS | 2 |
| 6 | 1q31.3 | PTPRC | EAS, EUR | 1 |
| 7 | 1q32.1 | SRGAP2, IKBKE, IL10, IL19 | AFR, EAS, EUR, HIS | 4 |
| 8 | 1q42.3 | LYST | EUR | 1 |
| 9 | 1q44 | SMYD3 | EUR | 1 |
| 10 | 2p23.1 | LBH | AFR, EUR, HIS | 2 |
| 11 | 2p22.3 | RASGRP3 | AFR, EAS, EUR, HIS | 1 |
| 12 | 2p16.1 | PAPOLG, LINC01185 | AFR, EUR, HIS | 1 |
| 13 | 2p14 | SPRED2 | EAS, EUR | 1 |
| 14 | 2p13.1 | DGUOK, TET3 | EAS, EUR | 2 |
| 15 | 2q24.2 | IFIH1, FAP | AFR, EAS, EUR, HIS | 3 |
| 16 | 2q32.3 | STAT4 | AFR, EAS, EUR, HIS | 15 |
| 17 | 2q34 | IKZF2 | EAS, EUR | 2 |
| 18 | 3p14.3 | DNASE1L3, PXK, PDHB | AFR, EUR, HIS | 5 |
| 19 | 3q13.33 | TMEM39A, TIMMDC1, CD80 | AFR, EAS, EUR, HIS | 3 |
| 20 | 3q25.33 | IL12A-AS1, ARL14 | AFR, EAS, EUR, HIS | 3 |
| 21 | 3q28 | LPP | EAS, EUR | 1 |
| 22 | 4p16.3 | DGKQ, SLC26A1 | AFR, EUR, HIS | 2 |
| 23 | 4p16.1 | GPR78 | EAS | 1 |
| 24 | 4q12 | KIT | EAS | 1 |
| 25 | 4q21.3 | AFF1 | EAS | 1 |
| 26 | 4q24 | BANK1 | AFR, EAS, EUR, HIS | 3 |
| 27 | 4q27 | IL2, IL21, IL21-AS1 | AFR, EUR, HIS | 2 |
| 28 | 4q35.1 | TENM3, TRAPPC11 | EAS | 2 |
| 29 | 5q21.1 | ST8SIA4, RN7SKP62 | AFR, EUR, HIS | 2 |
| 30 | 5q31.1 | TCF7 | AFR, EUR, HIS | 3 |
| 31 | 5q33.1 | TNIP1 | AFR, EAS, EUR, HIS | 8 |
| 32 | 5q33.3 | PTTG1, LOC285628 | AFR, EUR, HIS | 5 |
| 33 | 6p22.3 | ATXN1 | AFR, EAS, EUR, HIS | 1 |
| 34 | 6p22.2 | LRRC16A, SLC17A3, SLC17A2 | AFR, EUR, HIS | 1 |
| 35 | 6p22.1 | ZNF184, LINC01012 | EUR | 1 |
| 36 | 6p21.32–33 | MHC region | AFR, EAS, EUR, HIS | 12 |
| 37 | 6p21.31 | UHRF1BP1, ANKS1A, TCP11 | EAS, EUR | 4 |
| 38 | 6q15 | BACH2 | EAS, EUR | 1 |
| 39 | 6q21 | PRDM1, ATG5 | AFR, EAS, EUR, HIS | 7 |
| 40 | 6q23.3 | TNFAIP3 | AFR, EAS, EUR, HIS | 7 |
| 41 | 7q15.1 | JAZF1 | AFR, EAS, EUR, HIS | 4 |
| 42 | 7q11.23 | LIMK1, EIF4H, GTF2IRD1 | AFR, EAS, EUR, HIS | 6 |
| 43 | 7q32.1 | IRF5, TNPO3, TPI1P2 | AFR, EAS, EUR, HIS | 15 |
| 44 | 8p23.1 | PRAG1, FAM86B3P | AFR, EUR, HIS | 1 |
| 45 | 8p23.1 | XKR6, FAM167A, BLK | AFR, EAS, EUR, HIS | 15 |
| 46 | 8p11.21 | PLAT | AFR, EUR, HIS | 1 |
| 47 | 8q12.1 | RPS20 | AFR, EUR, HIS | 1 |

**Table 1** (*Continued*)

| *N* locus | CytoBand | Reported gene(s) | Ancestry | *N* studies |
|---|---|---|---|---|
| 48 | 8q21.12 | PKIA, ZC2HC1A | AFR, EUR, HIS | 1 |
| 49 | 9p24.1 | JAK2 | EAS, EUR | 1 |
| 50 | 10q11.23 | LRRC18, WDFY4 | AFR, EAS, EUR, HIS | 4 |
| 51 | 10q21.1 | PRKG1 | AFR, EAS, EUR, HIS | 1 |
| 52 | 10q21.2 | ARID5B, ZNF365, ADO | EAS, EUR, AFR, HIS | 3 |
| 53 | 10q24.33 | RPEL1, ST13P13 | EUR, HIS | 1 |
| 54 | 11p15.5 | MIR210HG, PHRF1, IRF7, CDHR5 | AFR, EAS, EUR, HIS | 6 |
| 55 | 11p13 | PDHX, CD44 | AFR, EAS, EUR, HIS | 4 |
| 56 | 11q13.1 | RNASEH2C, AP5B1 | AFR, EAS, EUR, HIS | 1 |
| 57 | 11q13.4 | NADSYN1, FCHSD2 | EUR, EAS | 2 |
| 58 | 11q23.3 | TREH, DDX6 | AFR, EUR, HIS | 1 |
| 59 | 11q24.3 | ETS1, FLI1 | AFR, EAS, EUR, HIS | 6 |
| 60 | 12p13 | CREBL2, GPR19, CDKN1B | EAS | 1 |
| 61 | 12q23.2 | DRAM1 | EAS | 1 |
| 62 | 12q24.12 | ATXN2, SH2B3 | EAS, EUR | 2 |
| 63 | 12q24.13 | PTPN11 | EUR | 1 |
| 64 | 12q24.33 | SLC15A4, GLT1D1 | AFR, EAS, EUR, HIS | 4 |
| 65 | 13q14.11 | COG6, ELF1 | EAS, EUR | 2 |
| 66 | 14q24.1 | RAD51B | AFR, EUR, HIS | 2 |
| 67 | 14q31.3 | GALC | AFR, EUR, HIS | 1 |
| 68 | 15q31.3 | FAM98B | EUR | 1 |
| 69 | 15q24.2 | SCAMP5 | EAS, EUR | 2 |
| 70 | 16p13.13 | CLEC16A | AFR, EAS, EUR, HIS | 2 |
| 71 | 16p11.2 | PRR14, ITGAM, ITGAX | AFR, EAS, EUR, HIS | 10 |
| 72 | 16q13 | CCL22 | AFR, EUR, HIS | 1 |
| 73 | 16q22.1 | ZFP90 | AFR, EAS, EUR, HIS | 1 |
| 74 | 16q24.1 | IRF8 | AFR, EAS, EUR, HIS | 5 |
| 75 | 17p13.2 | PLD2 | EUR | 1 |
| 76 | 17q12–q21.1 | ERBB2, MIEN1, IKZF3 | EAS, EUR | 2 |
| 77 | 17q21.31 | ARHGAP27 | EUR | 1 |
| 78 | 17q25.1 | GRB2, SLC25A19 | AFR, EUR, HIS | 2 |
| 79 | 19p13.2 | ICAM3, TYK2 | AFR, EAS, EUR, HIS | 3 |
| 80 | 19p13.11 | LRRC25, SSBP4 | AFR, EUR, HIS | 1 |
| 81 | 19q13.33 | PRR12 | EUR | 1 |
| 82 | 19q13.42 | TMEM86B | AFR, EUR, HIS | 1 |
| 83 | 20q13.12 | CD40 | AFR, EUR, HIS | 1 |
| 84 | 20q13.13 | RNF114, SNAI1 | EUR | 1 |
| 85 | 22q11.21 | UBE2L3, YDJC | AFR, EAS, EUR, HIS | 6 |
| 86 | 22q13.1 | GRAP2, ENTHD1 | AFR, EUR, HIS | 1 |
| 87 | Xp21.2 | CXorf21 | EUR | 1 |
| 88 | Xq28 | IRAK1, TMEM187, MECP2 | EAS, EUR, HIS | 2 |

'*N* studies' refers to the number of references compiled in GWAS-catalog describing association to SLE for each locus. These references are listed by variant in Supplementary Table 1, http://links.lww.com/COR/A45. 'Ancestry' indicates the populations in which association to SLE has been described in the GWAS-catalog reference list for each locus. AFR, African American; EAS, East Asian; EUR, European; HIS, Hispanic or Native American; MHC, major histocompatibility complex; SLE, systemic lupus erythematosus.

showing a B-lymphocyte-specific regulatory effect of the associated risk variants [42]. A GWAS in Koreans identified a new *locus* in chromosome 11q14 (*ATG16L2*, *FCHSD2*, *SIGLEC12*, and *P2RY2*) and confirmed many other [44]. Interestingly, *ATG16L2* was originally described in Crohn's disease [45], showing a tighter link across several autoimmune diseases, where some genes are implied in the tissue-specific regulation or in the systemic inflammation still needs to be investigated.

Despite the prevailing immune-relevant functions among the established SLE loci, for some of them, such as *JAZF1*, *XKR6*, *UHRF1BP1*, or *WDFY4*, there are no known studies of their role in disease development. For some described loci despite lack of known function the expression pattern confirms presence of corresponding mRNAs in relevant tissues such as spleen, lymph nodes or blood cells. In general, the enrichment of susceptibility *loci* lying within transcription factors was a major finding EURs [19]. Fig. 1 and Table 1 show the 88 genomic regions annotated with the 'SLE' trait which we include associations with $p$ value less than $8 \times 10^{-8}$ according to the GWAS-catalog (https://www.ebi.ac.uk/gwas/). All significant associations are shown in Supplementary Table 1, http://links.lww.com/COR/A45.

## GENE EXPRESSION REGULATION

Genetic variants can affect disease susceptibility through modifying genetic expression levels [expression quantitative trait loci (eQTLs)]. Genotype and gene expression data are used combined for this analysis. Publicly available eQTLs datasets help evaluate whether the detected SLE-risk SNPs influence transcript levels of genes [46–50]. These approaches initially showed that trait-associated SNPs were more likely to be eQTLs [50]. Noteworthy, the STAT4 locus, significantly associated with SLE in all studied populations, remained unexplained in the terms of expression regulation. SLE risk variants lay in intronic areas not genetically linked to the STAT4 promoter. A study based on fine-mapping and eQTL analysis, supported the importance of STAT4-located SLE risk rs11889341 variant on expression of neighboring STAT1, but not STAT4 expression increase in B cells [51]. The SLE protective role of *SIGLEC12* described in East Asians is mediated by eQTLs enhancing the expression of the gene [52]. Ten of the Immunochip-based identified variants in East Asians altered gene expression [30]. Another East Asian GWAS supported regulatory variation in 82 genes and overrepresentation of p53, MEF2A, and E2F1 transcription-factor-binding sites [8]. A large-scale exome-wide study in a Han Chinese sample identified an intergenic variant with a *cis*-eQTL effect reducing TPCN2 expression in immune cells from SLE patients [41]. Usually, eQTL-mapping studies identify variants affecting gene expression of nearby genes (*cis*-eQTLs). However, *trans*-eQTLs have also been detected. For example, the SLE SNP rs4917014 acts in *cis* on IKZF1 and in *trans* for C1QB and five type I interferon genes, both hallmarks of SLE [53]. Similarly, the major SLE-risk SNPs in BANK1 are eQTLs, and risk associated with increased gene expression, connecting the regulatory landscape of BANK1 with cotranscriptional splicing of exon-2 [54]. The use of RNA-seq to assess genome-wide transcription abundance provides information on allele-specific expression and RNA-isoform expression, which is not available from gene expression microarrays [55]. It has been shown that conventional gene expression quantification underestimated the identification of causal *cis*-eQTLs [56▪▪]. Integrating eQTL data derived from both microarray and RNA-Seq experiments with GWAS results in SLE identified new susceptibility genes (e.g., *NADSYN1* and *TCF7*). In addition, this procedure allows the identification of novel SLE associated splicing events and noncoding RNAs contributing to the better understanding of the functional consequence of regulatory variants [57▪▪].

## RARE AND LOW-FREQUENCY VARIANTS

Individual effects of common variation on susceptibility are small, with odds ratios ranging from as little as 1.01–2.5 at the most. Description of the effect of rare variation on complex phenotypes is an open field. In this regard, few examples exist of low-frequency or rare genetic variants in SLE related to the genes identified in GWAS studies. One example is *BLK*, where a low-frequency variant (a nonsynonymous change from alanine to threonine) was identified [58]. Another example is *NCF2*, where a nonsynonymous coding mutation in exon 12, a histidine to glutamine substitution in the PB1 domain of NCF2 protein reducing its binding efficiency to the guanine nucleotide exchange factor Vav1 [59].

A recent study of Sardinia SLE and multiple sclerosis patients revealed a new insertion–deletion variant in the *TNFSF13B* gene encoding the B-cell activating factor (BAFF). Serum levels of BAFF are frequently elevated in SLE patients. This variant resulted in the alternative polyadenylation of the transcript, lacking the binding site for inhibitory microRNAs and consequently BAFF upregulation [60▪▪]. This mutation propagated becoming more frequent possibly because of its protective role against malaria.

*DNASE1*-like deoxyribonucleases are well-established loci, known for their rare loss-of-function variants in familial SLE. *DNASE1L3* located in the close vicinity of *PXK* contains rare heterozygous variants enriched in sporadic SLE [61▪]. A later study performed quantitative PCR analysis of copy number variations in *DNASE1L3* and *DNASE2* and found that although the variants were universally rare in most populations with no copy loss-or-gain events, these were more frequent in EURs and Asian SLE [62].

The abundance of articles describing rare genetic variants, historically those on complement deficiencies and lately on interferonopathies where rare mutations found in monogenic diseases can be found in some SLE patients [63–66,69▪▪] suggest that such mutations may explain the relatively elevated frequency of familial cases of SLE [5] (8–12%), or alternatively, that SLE is a much more heterogeneous collection of individuals. Possibly, mutations causing forms of monogenic diseases having ill-described autoimmune manifestations are much more prevalent than previously suspected, contributing to the overall SLE phenotype. It is therefore potentially relevant to identify the possible genes involved and perform searches for autoimmunity among rare diseases to help further determine the rainbow of pathways that lead to disease. However, and relevant, whereas monogenic lupus may imply the contribution of mutations with high penetrance, in complex phenotypes such as SLE, we would expect rare variation with small effects, very hard to identify using genetic association [66].

In the first large SLE study of its kind exome sequencing of 30 parent-affected-offspring trios identified using SLE-associated burden analysis three functional de-novo loci: *DNMT3A*, *PRKDC*, and *C1QTNF4* [67▪▪]. In another study, a set of 71 SLE patients and their healthy parents was studied using whole genome sequencing and the contribution of ultrarare missense and nonsense variants in genes known to be causal for monogenic SLE was estimated. Enrichment of ultrarare variants was found for 22 genes. Interestingly, for *C1S*, *DNASE1L3*, *DNASE1*, *IFIH1*, and *RNASEH2A* disrupting rare variants were shown to be significantly associated with SLE mostly in the heterozygous state [68].

By performing exome sequencing on the most distantly related affected individuals from two large Icelandic families, multiple rare and likely pathogenic variants in 19 genes cosegregating with disease through multiple generations were identified [69▪▪]. The data was supplemented with information on coexpressed and protein–protein interacting partners. This unsupervised functional analysis showed enrichment in the gene ontology categories of immune system development, lymphocyte activation, DNA repair, and VDJ T and B-cell receptor gene recombination. Further support was found using a very stringent aggregate association analysis in sporadic cases for the *FAM71E1/EMC10* locus. The *EMC10* gene (endoplasmic reticulum membrane complex subunit 10) codes for a protein involved in endoplasmic reticulum-associated degradation and lipid transport. Another interesting gene was *DCLRE1C*. *DCLRE1C* is involved in double-strand break repair, cellular response to DNA damage stimuli, and chromosome organization protein Artemis. Recessive mutations in this gene cause Omenn syndrome, a severe combined immunodeficiency associated with increased cellular radiosensitivity because of a defect in V(D)J recombination leading to early arrest of B and T-cell maturation [70]. A recent functional study demonstrated that Artemis-deficient cells have type I and type III interferon signatures because of the chronic accumulation of DNA [71].

Owing to the difficulty in defining the role of single rare or low-frequency mutations in association analyses, a very stringent imputation-based approach for the whole genome rare variant enrichment analysis in SLE patients of EUR ancestry [72] was applied. This approach uncovered 98 top candidate loci. The loci were prioritized by two independent approaches: highly stringent sequence kernel association test and a case-control burden test. Several of these loci had immune-relevant functions, whereas for others their role in SLE remains obscure. Significant overrepresentation of Online Mendelian Inheritance in Man (OMIM) disease genes was observed, suggesting that such variants could be involved in the generation of combined SLE phenotypes, which looks reasonable considering the broad spectrum of SLE clinical manifestations and the large number of anecdotal descriptions of rare phenotypes in SLE patients [1,2]. Proper analysis of rare variants may be overcome with the numerous computational approaches being published (reviewed in [73]). In addition to the importance of avoiding sequencing errors and analysis artifacts, there is no consensus for the best strategy for rare variants analysis. Sequencing of targeted genes is an optimal procedure for detecting mutations. Thus, exome sequencing of SLE-risk genes in an European ancestry sample with 69 SLE affected and 97 healthy controls showed that rare BLK and BANK1 missense variants contributed to risk [74]. Resequencing of the SLE associated *ITGAM* gene in 73 SLE cases identified two case-specific nonsynonymous variants, F941V and G1145S that significantly impaired phagocytosis [75]. Similarly, targeted resequencing of coding

and conserved regulatory regions in a set of autoimmunity-realed loci in a Swedish SLE cohort identified a rare regulatory variant rs200395694:G>T in intron 4 of *MEF2D* associated with the presence of Raynaud's phenomenon, anti-ribonucleoprotein and anti-Sm antibodies [76]. In-depth whole-genome sequencing is the most comprehensive approach for measuring rare variation in both coding and noncoding regions. However, its application for large-scale cohorts is still limited. A tentative alternative would be to combine genotype imputation with targeted sequencing in a gene-centered strategy as a first glimpse followed by directed sequencing of the resulting best hits and a final corroboration of the association with SLE through an aggregated effect analysis. However, the reliability of the imputation of rare variation is still under discussion [72].

As in some of the first candidate gene and GWAS studies, modern rare and de-novo variant-based articles mostly analyze coding regions searching for missense and nonsense variants [60▪▪,69▪▪,76]. Several articles addressed the impact of the regulatory role of noncoding mutations showing association with extreme gene expression [77–80]. Overall, it was demonstrated that rare variants contribute to large gene expression changes across tissues providing an integrative method for interpretation of rare variants effects [81].

The heterogeneity of SLE loci identified in rare and de-novo variant studies could reside in the diversity of pathways, involved in SLE pathogenesis, without ruling out other additional explanations such as local population differences, different experimental designs and computational approaches.

In summary, rare and de-novo variant analysis has shown its applicability for SLE genetics research and has uncovered several candidate SLE loci for further studies.

## CONCLUSION

During the last decades of SLE susceptibility studies, we came from preliminary candidate genes to genome-wide scans of thousands of SLE cases and controls from different populations. Initially, EUR biased research was spread to Asian, Amerindian, and African ancestry, supporting the universal role of many SLE loci. Currently, genetic studies have focused on the identification of causal variants, their mechanisms of action, and the involvement of rare variation (see Fig. 1 and Table 1). Further progress of the field is based on multiomic studies including data on gene expression, epigenetics, gene-gene interaction studies, and the analysis of rare and de-novo variants and copy number variations. Rare variants are already identified as important components of the genetic context of SLE and future studies can clarify their functional impact and the combined role of common and rare variant in the individual SLE risk.

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### Conflicts of interest

*There are no conflicts of interest.*

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# Update on the pathogenesis of central nervous system lupus

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**Propose of review**
Neuropsychiatric systemic lupus erythematosus (NPSLE) is an emerging frontier in lupus care encompassing a wide spectrum of clinical manifestations. Its pathogenesis remains poorly understood because of the complexity of pathophysiologic mechanisms involved and limited access to tissue. We highlight recent advances in the pathophysiology of neuropsychiatric lupus.

**Recent findings**
Disruption of blood–brain barrier (BBB) facilitating entrance of neurotoxic antibodies into the central nervous system (CNS), neuroinflammation and cerebral ischemia are the key mechanisms. Disruption of the BBB may occur not only at the traditional BBB, but also at the blood–cerebrospinal fluid barrier. Certain autoantibodies, such as anti-*N*-methyl-D-aspartate receptors, antiribosomal P and antiphospholipid antibodies may cause injury in subsets of patients with diffuse neuropsychiatric disease. Activation of microglia via autoantibodies, interferon-a or other immune reactants, may amplify the inflammatory response and promote neuronal damage. New inflammatory pathways, such as TWEAK/Fn14, Bruton's tyrosine kinase, Nogo-a and ACE may represent additional potential targets of therapy. Novel neuroimaging techniques suggest alterations in brain perfusion and metabolism, increased concentration of neurometabolites, indicative of glial activation, vasculopathy and neuronal impairment.

**Summary**
NPSLE encompasses a diverse phenotype with distinct pathogenic mechanisms, which could be targeted by novel therapies or repositioning of existing drugs.

**Keywords**
autoantibodies, blood–brain barrier, microglia, neuroimaging, neuropsychiatric lupus

## INTRODUCTION

Systemic lupus erythematosus (SLE) frequently affects the central and peripheral nervous system, a syndrome collectively termed neuropsychiatric SLE (NPSLE) [1]. Up to 40% of SLE patients may experience at least one neuropsychiatric event over the course of their disease, with less than half of these manifestations directly attributed to lupus *per se* [2]. The underlying pathogenesis remains ill-defined [3], because of limited access to tissue, the diversity and complexity of clinical manifestations, and the overlap with non-SLE related neuropsychiatric events [1].

One of the early key assumptions in NPSLE was that a disrupted blood–brain barrier (BBB) allowed autoantibodies and immune components of peripheral blood to penetrate into the central nervous system (CNS), causing inflammation and damage [4]. Among autoantibodies, anti-*N*-methyl-D-aspartate receptors (anti-NMDA) and antiribosomal P (anti-RP) can become pathogenic upon entering the brain; the role of other autoantibodies remains poorly understood [5,6]. Recently, type I interferon (IFN) and microglial cells have emerged as central players in CNS disease, with recent studies substantiating their role in NPSLE [7▪▪,8].

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## KEY POINTS

- Neuroinflammation and cerebral ischemia are the two major pathogenetic mechanisms in NPSLE.
- Abnormal BCSFB may represent an additional central mechanism in NPSLE pathogenesis.
- Microglia cells emerge as central players in CNS lupus and targets of novel therapies.
- Advanced imaging techniques may dissect the multifactorial nature of CNS lupus.

## OVERVIEW AND EVOLVING CONCEPTS IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

In NPSLE, a 'mosaic' of genetic, environmental and neuroendocrine factors culminates in neuroinflammation and cerebral ischemia, the two major mechanisms operant [9]. Brain autopsies of patients with NPSLE show diffuse vasculopathy, microthrombi, microinfarction, macroinfarction and vasculitis, along with complement deposition [10▪]. The presence of 'vasculopathy' is supported by the high prevalence of white matter hyperintense lesions on brain MRI, representing microvascular disease, and the strong association of certain NPSLE syndromes with antiphospholipid antibodies (aPL) [3,11]. On the other hand, in the setting of a BBB disruption, the presence of inflammatory mediators and autoantibodies in the cerebrospinal fluid (CSF) of lupus patients highlights the role of an immune response and CNS inflammation [12]. In clinical practice, in a given patient, it is often hard to distinguish between ischemia and inflammation. When in doubt both immunosuppressive and antithrombotic agents, especially in aPL-related NP events, may be used [13].

## BRAIN–BARRIER DISRUPTION: GLOBAL VS. LOCALIZED

BBB is a highly selective semipermeable border of CNS vessels, formed mainly by brain capillaries at the level of endothelial cells with specialized tight junctions [14]. The umbrella term 'BBB disruption' denotes the impairment of any structure of the human CNS that can potentially be distorted, allowing immune and toxic components of the blood to enter [4,15]. Historically, BBB disruption was the first pathophysiological mechanism proposed to play a role in NPSLE pathogenesis. Early studies showed the presence of IgG, albumin and inflammatory cytokines in the CSF of patients with lupus and in lupus-prone mice [12,16]. Due to the complexity of BBB and inability to fully visualize the loss of integrity *in vivo*, it remains unclear whether these molecules originate from peripheral blood or are produced intrathecally.

Over the last years, more structures of the brain have been recognized as 'barriers' of the CNS, including the blood–CSF barrier (BCSFB). The choroid plexus is a plexus of modified ependymal cells located in the ventricles that produces the cerebrospinal fluid. The BCSFB – located at choroid plexus epithelial cells – is the natural 'dam' between the systemic circulation and CSF. Thus, the presence of inflammatory mediators in the CSF of NPSLE patients [12] can also be explained by a disrupted BCSFB rather than global dysfunction of BBB. Accordingly, in recent years, studies have focused on BCSFB in MRL/lpr mice, demonstrating that BCSFB is disrupted in the absence of BBB dysfunction [17]. A recent study confirmed the presence of infiltrating leukocytes through the BCSFB of MRL/lpr mice and detected CD4+ and CD8+ T cells at the level of choroid plexus. Of interest, T cells were predominantly T-follicular helper cells (Tfh) producing IFN-γ and Bcl-6, with an almost complete absence of regulatory, T cells, such as T-follicular regulatory cells and Tregs [18▪]. Together, these results suggest that the abnormal BCSFB may represent a central mechanism in NPSLE pathogenesis, although this hypothesis requires further study.

Two interesting anatomical components that potentially regulate the movement of immune mediators from the systemic circulation into the CNS, are the meningeal barrier and glymphatic system [19,20]. The former may represent another route for immune substances to move into CNS. On the other hand, the glymphatic system is a recently introduced perivascular system, which participates in the clearance of interstitial solutes out of the CNS [21] and allows the exchange of molecules between CSF and interstitial spinal fluid (ISF). In neurodegenerative diseases, such as Parkinson's and Alzheimer's, the glymphatic system inhibits the clearance of proteins, participating in the underlying pathogenesis [22]. To date, there are limited studies regarding its role in the pathogenesis of other CNS diseases.

## AUTOANTIBODIES IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS: ESTABLISHED PLAYERS AND 'NEW ENTRIES'

In addition to anti-NMDA, aPL and anti-RP, many autoantibodies have also been detected in NPSLE patients, yet they lack sensitivity and specificity [5,6]. From a clinical perspective, B-cell depletion

with rituximab may be beneficial in some NPSLE cases [23]. Of note, this has not been confirmed in murine studies, as early B-cell and/or antibody depletion did not modify or prevent neuropsychiatric disease in MLR/lpr mice [5]. The same group showed that neuropsychiatric manifestations remained unaffected after early bone marrow transplantation, whereas systemic inflammation, including nephritis, was attenuated [24]. Thus, the role of B cells and antibodies in CNS disease has not been fully elucidated [6,5].

A subset of antids DNA antibodies (termed DNRAb) recognize an extracellular domain of the NMDA receptor subunits NR2a and NR2b, and thus cross-react with the NMDA receptor, leading to neural cell apoptosis both in human and murine disease [25]. Direct injection of DNRAb in mice induced neuronal apoptosis at the level of hippocampus, leading to cognitive impairment. The effect of anti-NMDA antibodies is dose-dependent, as at high concentrations, they can induce excitotoxic cell death, whereas at lower concentrations, they do not cause neuropsychiatric manifestations [26]. Of interest, these abnormalities were detectable even when DNRAbs were no longer present in the hippocampus [27]. Anti-NMDA antibodies may damage the BBB *in vitro* and penetrate into the CNS [28]. Nevertheless, these antibodies may also be present in SLE patients without neuropsychiatric involvement [29,30], and thus these data need to be interpreted with caution.

Anti-RP antibodies are highly specific for SLE and have been associated with several NPSLE syndromes, especially psychosis and depression [30,31]. Anti-RP react with epitopes on the surface of neuronal cells, known as cross-reacting neuronal surface protein P (NSPA) [32]. González *et al*. demonstrated that NSPA is a ubiquitin ligase, which regulates the function of the NMDA receptor at the synaptic region [33]. Anti-RP bind to NSPA, which is distributed in brain regions involved in memory and emotion leading to neuronal apoptosis via intracellular $Ca^{2+}$ influx [34]. This provides a molecular link between NSPA and the NMDA receptor (NMDAR) – known to be involved in plasticity and synaptic transmission related to memory, suggesting a possible pathogenic role for anti-RP. Importantly, injection of these antibodies through the limbic system or peripheral circulation leads to cognitive impairment and depression in mice [35,36].

aPL antibodies are major risk factors for NPSLE, especially for focal syndromes like cerebrovascular disease [11,37]. aPL carriers may also be at increased risk for subclinical atherosclerosis, although this has not been firmly established [38]; aPL may also affect the small vessels creating a microthrombotic environment within the CNS and consequent cerebral microangiopathy. This local vascular injury to small vessels may disrupt the BBB [39,40]. Intracerebroventricular injection of aPL induced a hyperactive behavior in mice implying a direct pathogenic role [41].

## THE ROLE OF THE ACTIVATION OF MICROGLIA IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS PATHOGENESIS

Microglia, the resident macrophage cells of the brain, account for 10–15% of all neuronal cells. They act as the first and main form of active immune defense in CNS, responding to pathogens and injury by changing morphology and migrating to the site of infection/injury, where they destroy pathogens and remove damaged cells [42]. As part of their response, they secrete various cytokines, chemokines, prostaglandins and reactive oxygen species.

Accumulating evidence support an active role for microglial cells in the pathogenesis of NPSLE. Lupus-prone mice lacking estrogen receptor alpha experienced a significant reduction in memory errors, which correlated with decreased number of activated microglial cells and an accompanying reduction of CNS inflammation [43]. Administration of colony stimulating factor-1 receptor (CSF-1R) kinase inhibitor – which crosses the BBB causing microglia depletion [44] – in MRL/lpr mice improved depression [45▪]. Microglia are activated by sera of patients with SLE *in vitro*, but the actual factors responsible for this activation are unknown [46]. More recently, robust evidence for the role of microglia in CNS lupus came from a study by Bailas *et al.* who documented an IFN-driven microglia-dependent synapse loss pathway, using the 564Ig mouse model [7▪▪]. In this article, peripheral type I IFN was found to enter the brain and activate the IFNαR and microglia. The latter then engulfed synaptic material leading to synapse loss and subsequent cognitive impairment. Mice treated with IFNαR blocking antibody (anifrolumab) exhibited attenuation of CNS disease.

Another study [47▪▪], used the DNRAb+ mouse model (immunization with the DWEYS peptide) to explore the role of microglia in autoantibody-mediated CNS lupus. DNRAb+ mice exhibited increased microglia activation and a decrease in dendritic complexity, which was reversed when microglia was depleted. This decreased spine density and dendritic complexity were dependent on C1q. The latter binds to dendrites using high mobility group box 1 protein as mediator, with C1q serving as a bridge to NMDARs. Importantly, administration of captopril

[an angiotensin-converting enzyme (ACE) inhibitor, which crosses the BBB] significantly reversed the activation of microglia and improved the cognitive function of mice [47▪▪].

In MLR/lpr mice, reactive microglia may be activated through the nuclear factor κB (NF-κB) pathway, highlighting the role of TNF-α as mediator; inhibition of NF-κB led to decreased CD68 expression (activation marker) in microglia [48]. In another study, treatment with fingolimod (a modulator of sphingosine-1-phosphate, which sequesters lymphocytes within lymph nodes) attenuated the depressive behavior and cognitive impairment of MLR/lpr mice. RNA-sequencing analysis of fingolimod-treated microglia revealed downregulation of multiple immune-mediated pathways, including NF-κB signaling and IFN response with negative regulation of type I IFN-mediated signaling; this was associated with increased IFNβ expression [49▪]. Finally, lipocalin-2 (LCN2), a protein, which promotes microglial M1 polarization [50] was detected at increased levels in the serum of NPSLE patients. Lupus-prone mice with LCN2 deficiency performed better in neuropsychiatric tests exhibiting decreased microglia activation and brain apoptosis. LCN2 directly regulates immune microglia-associated pathways suggesting yet another pathogenic mechanism [51]. Overall, these data indicate that microglia cells are central players in CNS lupus and may serve as targets for novel therapies.

## INTRACELLULAR SIGNALING PATHWAYS IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS: A ROLE OF KINASE INHIBITORS?

Tumor Necrosis Factor-like Weak inducer of apoptosis (TWEAK), a TNF superfamily member, promotes the activation of NF-κB and mitogen-activated protein kinase via its receptor, fibroblast growth factor-inducible 14 (Fn14) [52]. Evidence towards the involvement of the TWEAK/Fn14 pathway in NPSLE is growing. TWEAK displays a dual role in both neuroinflammation and cerebral ischemia [53]. Increased expression of TWEAK/Fn14 was detected within the cerebral cortex of MRL/lpr mice; knocking-out Fn14 improved depression and cognitive function [54]. Importantly, this finding was accompanied by a reduction of immune infiltrates, fibronectin, IgG deposition and complement activation in brain histology [55]. Intracerebroventricular injection of TWEAK in wild-type mice induces cognitive dysfunction and depression-like behavior through increased BBB permeability and accelerated neuronal cell death [55,56].

Bruton's tyrosine kinase (BTK) is essential for the function of B cells and macrophages. Inhibition of this pathway by use of a specific inhibitor (BI-BTK-1) in MRL/lpr mice, resulted in decreased accumulation of macrophages, T cells and B cells in the choroid plexus and improved cognitive function [57▪]. In view of the recent promising data of baricitinib in SLE [58], ibrutinib, a selective BTK inhibitor, could potentially prove useful in neuropsychiatric disease. Of interest, evobrutinib, another BTK inhibitor, was evaluated in patients with multiple sclerosis in a phase 2 trial with promising results [59].

Neurite outgrowth inhibitor-A (Nogo-a) with its respective receptor, NgR1, form a signaling pathway, which mediates inhibition of neuron generation. Compared with other autoimmune or neurological diseases, patients with NPSLE overexpress Nogo-A in the CSF [60]. Increased levels of Nogo-α/NgR1 were also observed in MLR/lpr mice; administration of Nogo-66(1-40), an antagonist, improved cognitive function, decreased expression of proinflammatory components and reduced axonal degeneration and demyelination [60] (Table 1).

## TYPE I INTERFERON AND NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

SLE is characterized by a robust IFN molecular signature in most patients. A link between NPSLE and IFN has been proposed based on clinical and molecular findings of monogenic interferonopathies, such as Aicardi–Goutières syndrome (AGS). AGS is an inflammatory disorder mainly affecting the skin and brain, characterized by aberrant secretion of type I IFN and lupus-like systemic features [61]. Among the responsible mutated genes for AGS, is the three prime repair exonuclease 1 (*TREX1*) [61], a susceptibility gene for SLE [62] and, more specifically, CNS lupus [63]. Brain pathology of patients with AGS shows small vessel disease, including aneurysmal dilation, vasculitis and thrombotic microangiopathy [61] findings also seen in SLE [10▪].

Of note, IFN-α causes endothelial cell damage promoting abnormal angiogenesis in SLE patients, which may also involve CNS vessels [64]. Whether IFN *per se* causes cerebrovascular disease, frequently manifest in patients with increased IFN levels, or is merely an epiphenomenon, remains to be defined. Patients with various diseases treated with IFN-α or IFN-β, developed thrombotic microangiopathy suggesting a possible role of IFN on vascular damage [65]. Monogenic interferonopathies could serve as a model to study the role of IFN in NPSLE pathogenesis.

**Table 1.** Therapeutic targets in neuropsychiatric systemic lupus erythematosus

| Target | Evidence and rationale | Experimental setting | Potential drugs | References |
|---|---|---|---|---|
| Type I IFN pathway | Type I IFN activates microglia, which then engulfs synaptic material leading to cognitive impairment. Mice treated with IFNαR blocking antibody, exhibited attenuation of CNS disease | 564Igi lupus-prone mice | Anifrolumab (Type I IFN receptor inhibitor) | [7▪▪] |
| ACE | Microglia and C1q are essential in neuronal damage process. ACE inhibitors can prevent microglia activation preserving cognitive status and neuronal function | BALB/c mice immunized with DWEYS peptide, leading to DNRAb+ production | Captopril, other ACE inhibitors | [47▪▪] |
| BTK | Treatment with BI-BTK-1 (a novel inhibitor of BTK) significantly attenuated the neuropsychiatric disease along with decreased accumulation of macrophages, T cells and B cells within the CNS | MRL/lpr mice | BTK inhibitors (BI-BTK-1, ibrutinib, evobrutinib) | [57▪] |
| Nogo-a/ NgR1 pathway | Nogo-a/ NgR1 pathway is involved in NPSLE. Treatment with Nogo-66(1–40) antagonist improved cognitive function and myelin repair | MRL/lpr mice | Nogo-66 (1–40), an antagonist of NgR1 receptor | [60] |
| S1P signaling pathway | Modulation of the S1P signaling pathway may serve as a novel therapeutic target in CNS lupus | MRL/lpr mice | Fingolimod, a S1P receptor modulator that sequesters lymphocytes within lymph nodes | [49▪] |
| LCN-2, a protein, which promotes microglial M1 polarization; a major regulator of innate immunity | Increased levels of LCN-2 were detected in the serum of NPSLE patients. Cognitive impairment and depression-like behavior were attenuated in lupus-prone mice lacking LCN-2 | Sle1,3 lupus-prone mice | - | [51] |
| Activated microglia cells | Lupus-prone mice treated with CSF-1R (microglia depletion) exhibited improvement in the depression-like behavioral deficit | MRL/lpr mice | GW2580, a small CSF-1R kinase inhibitor; depletion of microglia | [45▪] |
| TWEAK/Fn14 pathway | TWEAK/Fn14 interactions promote the loss of BBB integrity and increase neuronal damage and the accumulation of inflammatory cells in the choroid plexus | MRL/lpr mice | Monoclonal antibodies (hIgG1) against Fn14 | [54] |
| Complement cascade | Complement deposition was increased in brain tissue of SLE patients suggesting an underlying pathogenic role | Human brain autopsies | Eculizumab (inhibitor of complement factor C5 | [10▪] |

ACE, angiotensin-converting enzyme; BKT, Bruton's tyrosine kinase; CNS, central nervous system; CSF-1R, colony stimulating factor-1 receptor; IFN, interferon; IFNaR, interferon-a receptor; LCN-2, lipocalin-2; NPSLE, neuropsychiatric systemic lupus erythematosus; S1P, sphingosine-1-phosphate; TWEAK/Fn14, Tumor Necrosis Factor-like Weak inducer of apoptosis/fibroblast growth factor-inducible 14.

## TRANSCRIPTOMIC ANALYSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS: BRAIN AS A CAUSAL TISSUE

Transcriptomic analysis of SLE by RNA sequencing has revealed novel molecular signatures for disease susceptibility and severity [66▪]. These studies have also shown that brain is not only a target tissue but also a causal tissue in SLE. More specifically, using SLE GWAS signals and eQTLs from 44 tissues, we found that SLE-associated polymorphisms regulated gene expression not only in the blood but also in other tissues, including the basal ganglia – suggesting that SLE genetic susceptibility may affect multiple tissues including CNS [66▪]. These findings

provide additional evidence that the brain may also be a causal tissue in SLE corroborating earlier data linking the nervous and the immune system.

## NOVEL BRAIN IMAGING TECHNIQUES AND CLUES FOR NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS PATHOGENESIS

Approximately 40% of SLE patients with established neuropsychiatric disease do not show abnormalities on conventional brain imaging. Furthermore, no consistent association exists between any neuroimaging finding and specific neuropsychiatric syndrome or severity. To this end, a number of advanced imaging techniques have been tested in order to increase sensitivity and detect more subtle abnormalities. Indeed, imaging techniques have provided additional evidence for microglial activation. A recent study [67] demonstrated intracellular changes in glia with increased diffusivity of choline and creatine. The authors suggested that this finding could serve as an imaging marker for glial activation in response to inflammation; of note, this correlated also with disease activity. Microglia activation has also been shown in NPSLE by PET and [11C] DPA-713 using a radiopharmaceutical substance that targets mitochondrial translocator protein, a protein upregulated during glial cell activation [68].

Regarding cerebral perfusion, our group examined whether dynamic susceptibility contrast-enhanced perfusion MRI (DSC-MRI), a minimally invasive and widely available method of cerebral perfusion assessment, may assist the diagnosis of NPSLE. We found decreased cerebral blood flow in the semioval center bilaterally in normal-appearing white matter region of NPSLE patients [69▪]. Importantly, the combination of DSC-MRI-measured blood flow in the semioval centre with conventional MRI was found to improve the attribution of neuropsychiatric events to SLE. Another technique, magnetization transfer imaging (MTI), uses the magnetization transfer ratio – histogram peak height (MTR-HPH) as a marker of the integrity of tissue microstructure; the latter was found decreased in individuals with inflammatory NPSLE manifestations compared with patients with presumed ischemic ones [70]. Decreased MTR-HPHs values were reversed with immunosuppressive treatment, pointing towards an inflammatory process rather than ischemia. Proton magnetic resonance spectroscopy (1H-MRS), which measures the concentration of several types of neurometabolites, has also been used in NPSLE. These studies have shown increased levels of myoinositol and choline [71,72], consistent

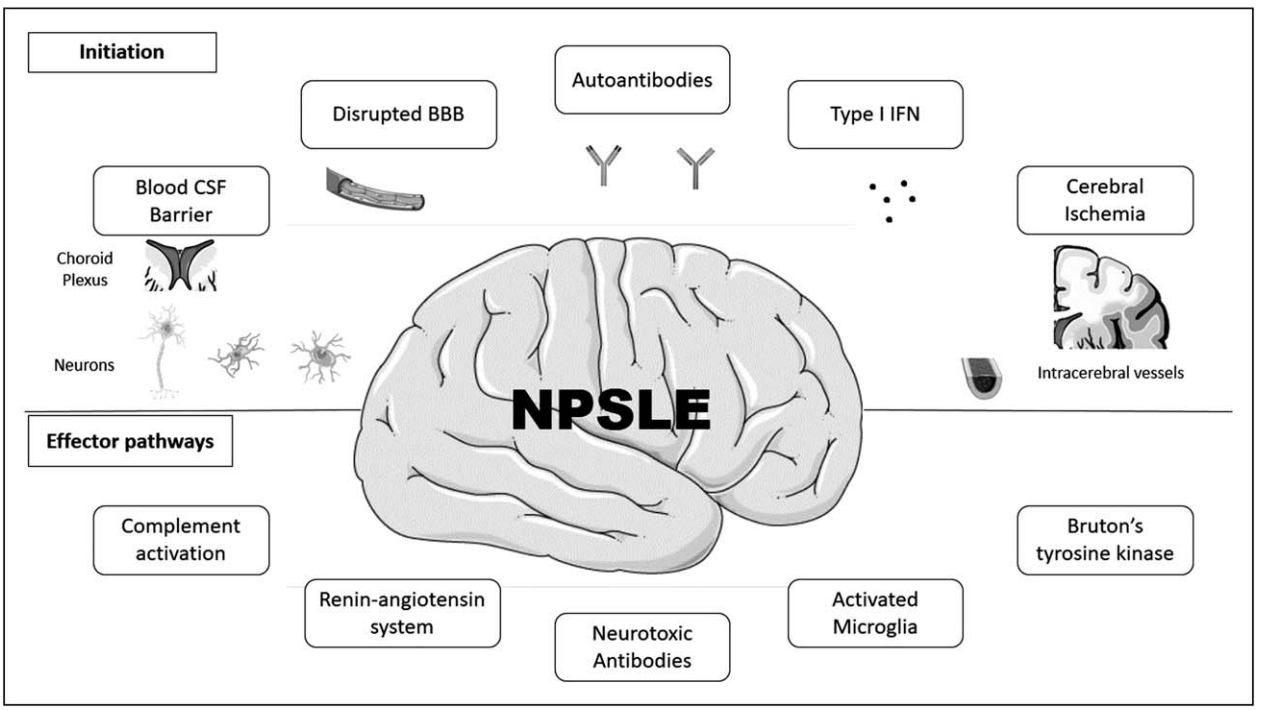


**FIGURE 1.** Pathogenesis of central nervous system lupus. Proposed pathophysiologic mechanisms in NPSLE. Collectively these mechanisms target various components of the CNS including neurons (synapse, myelin sheath), astrocytes, microglia and the cerebral vasculature. CNS, central nervous system; NPSLE, neuropsychiatric systemic lupus erythematosus.

**Table 2.** Research agenda

| |
|---|
| Further definition of the molecular signature of NPSLE by transcriptomic analysis including single-cell RNA sequencing |
| Correlation of molecular subphenotype with clinical subgroups of NPSLE |
| Exploration of the brain not only as a target tissue but also as a causal tissue in the pathogenesis of lupus |
| Development and testing of molecular markers for neuroinflammation, ischemia and demyelination |
| Exploration of the glymphatic system and its role in NPSLE |
| Delineation of the relative importance of interferon pathways in intracerebral vascular beds |
| Improved biomarkers for disease activity, prognosis and response to therapy |
| Repositioning of drugs inhibiting pathways found to be relevant for lupus |

NPSLE, neuropsychiatric systemic lupus erythematosus.

with glial activation and vasculopathy, along with decreased *N*-acetylasparate [71,72], compatible with neuronal impairment in patients with neuropsychiatric manifestations.

Recently, functional MRI in SLE patients with cognitive dysfunction revealed structural and functional brain changes and an inflammatory process pointing out the multifactorial nature of NPSLE [73▪]. Finally, PET studies in NPSLE have shown both increased (hypermetabolism) and decreased (hypometabolism) FDG uptake, consistent with inflammation and tissue loss, respectively. The most common finding was hypermetabolism in the parieto-occipital grey matter [74], even in the absence of MRI lesions. Collectively, these neuroimaging findings suggest that both inflammation and tissue loss may be operant in NPSLE.

## CONCLUSION

NPSLE remains only partly understood, both in terms of pathophysiology and management, the latter remaining largely empiric [2]. Most evidence derives from studies in animal models, which interestingly do not manifest the full spectrum of human NPSLE (e.g. severe manifestations, like seizures or myelopathy are not seen in mice); rather they exhibit more subtle abnormalities, and as such, may not completely model the human disease [75]. Notwithstanding this limitation, advances have certainly been made in our understanding of disease pathogenesis (Fig. 1). With regards to treatment, recent findings suggest new potential therapeutic opportunities, such as type I IFN blockade, ACE inhibition and kinase inhibitors [7▪▪,47▪▪,57▪] (Table 2). We anticipate that some of these pathways may serve as targets for the development of new drugs or for repositioning of already existing ones.

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### Conflicts of interest

*There are no conflicts of interest.*

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Papers of particular interest, published within the annual period of review, have been highlighted as:
- ▪ of special interest
- ▪▪ of outstanding interest

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# Malignancies in systemic lupus erythematosus: an update

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**Purpose of review**
Patients with systemic lupus erythematosus (SLE) have altered incidences of certain malignancies as compared with the general population. This review summarizes the recent literature on risk of malignancy in SLE and proposed mechanisms for these altered susceptibilities.

**Recent findings**
Recent studies have confirmed previous data showing an increased risk of hematological, lung, thyroid, liver, cervical and vulvovaginal cancers, while demonstrating a decreased risk of breast and prostate cancer. Lymphomagenesis in SLE has been linked to increased activity of multiple inflammatory cytokines as well as possible viral causes. The decreased rates of hormone-sensitive cancers, such as breast and prostate is speculated to be related to the presence of lupus autoantibodies and downregulation of certain proteins in SLE. This knowledge has been utilized to investigate new therapeutic modalities for these malignancies.

**Summary**
Recent data confirm previously reported altered malignancy rates in SLE. There has been some elucidation of mechanisms underlying cancer development in SLE, although additional work is yet to be done.

**Keywords**
breast cancer, lymphoma, malignancy, systemic lupus erythematosus

## INTRODUCTION

A growing number of studies have investigated the malignancy risk associated with systemic lupus erythematosus (SLE), and our review will focus mainly (though not exclusively) on studies published in the past year. Numerous potential risk factors have been investigated including immunosuppressive therapy, genetics, clinical factors and environmental factors, such as smoking exposure. The objective of this article is to review the most recent evidence regarding cancer risk (and risk factors) in SLE.

## MALIGNANCIES WITH INCREASED RISK IN SYSTEMIC LUPUS ERYTHEMATOSUS

### Hematologic malignancy risk

The literature has consistently reported an increased risk of hematologic malignancies in SLE [1,2▪,3▪]. Specifically, the risk of lymphoma is increased about four-fold in SLE compared with the general population, and patients are at a higher risk not only of developing lymphoma but also of increased mortality related to it [4▪]. Recently Tallbacka *et al.* [5] published a follow-up study of 205 SLE patients from Finland, who had been studied in 1992. The authors updated the findings of these patients, who ultimately were followed an average of 25.7 years [2▪]. As has been seen in other studies, the risk of developing non-Hodgkin lymphoma (NHL) in SLE was increased, with diffuse large B-cell lymphoma (DLBCL) being the most frequent NHL type [4▪]. A

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## KEY POINTS

- SLE is associated with an overall increased risk of malignancy, particularly NHL, lung, liver, vulvar/vaginal, and thyroid, and a decreased risk of breast and prostate cancer.
- The increased risk of NHL is likely related to multiple mechanisms and cytokine pathways.
- There are recent Canadian guidelines on cancer screening specifically for SLE patients suggest that patients should adhere to general population screening recommendations, aside from cervical cancer, where more stringency may be in order.

limitation of this study was that it focussed solely on patients who had been recruited over 30 years ago (as early as 1967), and thus their findings are likely more relevant for SLE patients of very long duration, not necessarily of the general SLE population. Another bias is that this is a survivor cohort unless they also reported mortality from the original study, specifically cancer.

Several authors have utilized administrative health data (claims data from physician visits and hospitalizations) to study cancer risk in SLE. This was the case of a recent study from Taiwan [6▪]. Despite the methodologic limitations with claims data (related to case ascertainment of both SLE and cancers), the results were consistent with an increased risk of NHL in SLE, although the point estimate was slightly higher than that seen in clinical studies, suggesting the possibility of some SLE diagnoses being actually paraneoplastic presentations. Another study tackled the question differently, estimating the risk of developing SLE in patients with NHL, and finding a two-fold increased risk in SLE onset, compared with the general population. The risk was highest within the first year after the diagnosis, suggesting common potential risk factors or pathophysiology. These findings again suggest that some early cases of SLE onset could have been paraneoplastic. Hodgkin's lymphoma also seems to be increased in SLE, as has been highlighted in the most recently published meta-analysis [3▪].

Some potential cytokine pathways have been hypothesized as driving the increased risk of NHL in SLE. Higher levels of circulating IL-6 and IL-10 are associated with NHL risk in the general population [7▪▪], and thus it may be that elevated IL-6 [8▪] and IL-10 described in SLE could play a role in driving NHL risk. Huiying *et al.* found a higher expression of IL-10 especially in nongerminal centre lymphomas [9], which is the most frequently diagnosed DLBCL subtype of NHL in SLE [10▪▪,11▪]. Both IL-6 and IL-10 may play specific roles in DLBCL, as they are associated with more aggressive DLBCL [12▪] Recently, authors have hypothesized that epigenetic regulation by microRNA abnormalities may be a mechanism underlying the increased risk of NHL in SLE [13]. Additionally, based on previous analyses of data from general population genome-wide association studies, our group has identified that Tumor Necrosis Factor (TNF) T Alpha Induced Protein 3, TNF Ligand Superfamily Member 4 and possibly interferon pathways are of high interest as potential mediators of the risk of DLBCL (particularly nongerminal centre type) in SLE. [14].

Epstein–Barr virus (EBV) is believed to be implicated in DLBCL in the general population. A recent study demonstrated lower virus-specific T-cell responses to EBV in patients with SLE, which was independent from immunosuppressant medications. This led to the hypothesis of a causal relationship between EBV and DLBCL in SLE. However, studies have failed to demonstrate the presence of EBV in lymphoma cells specifically in SLE [4▪].

### Lung malignancy

Studies have consistently reported an increased risk of lung malignancy in SLE and lung cancer is the second most frequent cancer to occur in SLE [15▪▪]. These results were confirmed in a recent metanalysis, which reported a 60% increased risk of lung cancer in SLE [3▪]. Data from a multicentre cohort study reported that the most important risk factors associated with lung malignancy risk in lupus were male sex, older age at SLE diagnosis and smoking history [15▪▪].) The findings also suggested that the immunosuppressive treatments used in SLE are not associated with an increased risk of lung cancer. In particular, none of the SLE patients diagnosed with lung cancer had a history of exposure to cyclophosphamide [15▪▪]. There are currently no lung cancer screening recommendations specific for SLE.

### Thyroid malignancy

A recently published study of administrative data from Korea suggested an increased risk of thyroid cancer in SLE versus persons without SLE [16▪]. A systematic review and metanalysis assessing the risk of thyroid cancer in lupus reported consistent evidence of an almost two-fold increased risk of thyroid cancer [3▪].

### Hepatobiliary malignancy

The recently published systematic review and metanalysis reported consistent evidence of a more than

three-fold increased risk of hepatobiliary cancers in SLE [3▪]. The reasons for this are not fully understood, but a decreased ability to clear viral infections in SLE could play a role. As mentioned earlier, T-cell responses to EBV may be impaired in SLE, independent from immunosuppressant medications [4▪,17▪]. Interestingly, chronic EBV infection has been suggested as a trigger for hepatobiliary cancer in nonlupus populations [18▪]. On the other hand, infection with hepatitis B virus is a more established risk factor for liver cancer, but it is not clear if SLE patients are at increased risk of chronic hepatitis B infection. The most recent recommendations from the Canadian Rheumatology Association state that SLE patients with a high-risk behavior for hepatitis B infections should be screened as per the general population [19▪▪].

### Cervical and vulvar/vaginal malignancy

Cervival dysplasia, which is the precursor to cervical cancer, is known to be more common in SLE than in the general population. Although the increased risk of cervical dysplasia in patients with SLE has consistently been documented in many studies, whether there is an increased risk of cervical cancer itself is unclear. The recent meta-analysis by Song *et al.* [3▪] reported a 50% increased risk of cervical cancer, but some of the included studies may have had methodologic issues related to outcome ascertainment. A study by Bae *et al.* [16▪] (based on Korean administrative healthcare data) estimated a much higher, three-fold increased risk of cervical cancer. Recent Canadian SLE guidelines suggest annual cervical screening in every patient who has ever been sexually active, until the age of 69 [19▪▪]. The main risk factor for developing cervical dysplasia or cancer is human papillomavirus virus (HPV) infection, and a recent meta-analysis suggests that SLE patients are more at risk for persistent HPV. SLE patients are also at increased risk of vagina/vulva cancer [3▪]. This cancer is also associated with HPV infection in the general population, and thus the increased risk of cancers of the vagina/vulva in SLE may be because patients tend not to eradicate HPV. The vaccine against HPV has been shown to be well tolerated and effective in SLE, and a recent study reported a maintained immunogenicity at 5 years in most patients [20▪].

### Other cancers

A recent meta-analysis suggested an increased risk of nonmelanoma skin cancer, but a decreased risk of melanoma [2▪,3▪]. The same meta-analysis confirmed an increased risk of cancers of the oropharynx and larynx [3▪]. Although the cause of an increased risk of head and neck cancers in SLE remains unknown, the increased susceptibility to EBV and HPV in SLE may theoretically increase the risk as both these infections are associated with head and neck malignancy in the general population [21▪]. SLE patients may also have an increased risk of esophageal and gastric cancers [3▪]. According to the most recently published meta-analysis, SLE patients may also be at higher risk than the general population for renal and bladder cancer [3▪]. The reasons for this are not clear, but one hypothesis (not clearly proven) is that cyclophosphamide could be responsible for at least some of the bladder cancers.

## MALIGNANCIES WITH DECREASED RISK IN SYSTEMIC LUPUS ERYTHEMATOSUS

### Breast, ovarian and endometrial cancers

The very large multicenter cohort study from the Systemic International Collaborating Clinics (SLICC) reported a decreased risk of breast cancer in SLE [1]. The results from the recent meta-analysis were consistent with a decreased breast cancer risk, but with a wide confidence interval (CI) that barely included the null value [3▪]. However, it should be noted that some of the studies included in that meta-analysis (and other earlier meta-analyses) were subject to ascertainment bias (e.g. using administrative data without clinically confirming SLE diagnoses or cancer cases) or selection bias. This may have led to a bias towards the null. Several studies have suggested a tendency toward a higher prevalence of triple-negative breast cancers in SLE than in the general population [22▪], although this could be in part because of the relatively young age of typical SLE patients. One hypothesis (yet unproven) for a decreased risk of hormone-sensitive cancers in SLE is that these patients tend to have higher age at menarche and lower age at menopause versus the general population. This may result in reduced total endogenous estrogen exposure, which theoretically could decrease risk of hormone receptor-positive breast cancer. Ovarian and endometrial cancers have also been reported to be decreased in female patients with SLE; the aforementioned recent meta-analysis reported pooled results, which were consistent with a decreased risk for these cancers in SLE versus the general population, though again the 95% confidence interval (CI) just barely included the null value (possibly because of the methodological considerations indicated above) [3▪]. Like breast cancer, endometrial and ovarian cancers are also partially driven by estrogen

exposures, so decreased lifetime exposure to estrogen in women with SLE might also explain the reduced incidence of these cancers in SLE.

### Prostate cancers

SLE may also have a protective effect on prostate cancers, as has been shown in previous meta-analyses, and confirmed in the most recent meta-analysis [3▪]. A reduced prostate cancer rate in SLE might be attributed to hormonal causes, as male SLE patients are known to have low levels of androgens, which can contribute to a decreased risk of prostate cancer.

## CONCLUSION

This review has discussed the literature on cancer risk in SLE. Evidence consistently show an overall increased risk in certain cancers especially hematologic, lung, thyroid, liver, and vulvovaginal cancers. Lung cancer is the second most frequent cancer in SLE and is highly linked to smoking. Clinicians, thus should emphasize smoking cessation in SLE. Recent guidelines recommend that SLE patients follow the same cancer screening recommendations as the general population, aside from advising more stringent screening for cervical dysplasia.

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### Conflicts of interest

*There are no conflicts of interest.*

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- ▪ of special interest
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# Lupus nephritis: challenges and progress

*Anne Davidson, Cynthia Aranow, and Meggan Mackay*

**Purpose of review**
The management of lupus nephritis remains unsatisfactory due to insufficiently effective treatment regimens and the dearth of reliable predictors of disease onset or progression to guide individualized therapeutic decisions. This review summarizes new findings related to lupus nephritis over the last 18 months and discusses clinical needs that should be considered to advance trials of mechanism-based therapeutic strategies.

**Recent findings**
Collaborative teams are addressing how to improve disease definitions and are developing predictive models for disease onset, disease response and risk of flare in individual patients. More attention is being paid to clinical trial design. Advanced technologic approaches are allowing the analysis of small amounts of human tissue and urine in unprecedented detail so as to discover new pathogenic mechanisms and identify disease biomarkers. Novel therapies continue to be tested in disease models and include new strategies to protect renal tissue from cell damage and fibrosis.

**Summary**
The collaborative efforts of patients, clinical and translational researchers, the pharmaceutical industry and funding sources are needed to advance therapies for lupus nephritis. Specialized clinical centers can then deliver optimal and more personalized patient care that will improve patient outcomes.

**Keywords**
lupus nephritis, pathogenesis, systemic lupus erythematosus, treatment

## INTRODUCTION

Lupus nephritis is a severe complication of Systemic Lupus Erythematosus that progresses to end stage renal disease (ESRD) in ≈10% of patients within 5 years of onset. Current standards for diagnosis and treatment of lupus nephritis are unsatisfactory and it is not possible to accurately predict responsiveness to therapy or the long-term outcome of individual patients. Although there has been a recent decrease in the severity of lupus nephritis in European patients [1], perhaps reflecting a more comprehensive approach to lupus management, the risk of lupus nephritis-related ESRD has remained unchanged in the US population since the 1990s [2]. Immune-mediated inflammation is a major initiator of lupus nephritis, but pathogenic mechanisms leading to ESRD are poorly understood and cannot be therapeutically addressed in a patient-specific manner. There is as yet no successful biologic therapy for lupus nephritis and many unsolved problems in clinical trial design impact the interpretation of trial outcomes. In this article, we will review recent advances in clinical and mechanistic approaches to lupus nephritis and consider what is needed for translation of new information into successful clinical trials.

## DIAGNOSIS AND OUTCOME MEASURES

Renal biopsy is the gold standard for diagnosis of lupus nephritis. Treatment decisions are based on the International Society of Nephrology/Renal Pathology Society classification of glomerular involvement [3] and indices for active inflammation and chronicity. Importantly, although long-term renal outcomes are worse for proliferative disease, better predictive models for risk and outcomes are needed to direct therapeutic decisions. It is still unknown whether preemptive treatment of serologic flare will prevent subsequent lupus nephritis onset [4▪▪] – this needs larger controlled studies and the development of models that predict the risk of renal flare [5,6]. Predictive models are also needed to

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## KEY POINTS

- Lupus nephritis remains a significant unmet need that causes morbidity and mortality in patients with systemic lupus erythematosus.
- There is a pressing need for the development of noninvasive predictors of lupus nephritis risk and prognosis that can only be addressed using clinical registries with optimal collection of patient data and biospecimens.
- Using advanced technologies, human tissue can now be examined in unprecedented detail, yielding new insights into disease mechanisms.
- The successful development of new therapies will need more attention to clinical trial design and the consideration of individual patient features including genetic polymorphisms and tissue characteristics.
- Improvement of disease outcomes will need universal patient access to specialized clinics that monitor disease progression and medication adherence and deliver optimal access to both standard care and new therapies.

define a uniform composite short-term treatment response that can predict long-term outcomes and be used either as a surrogate endpoint in clinical trials or as a guide for decreasing maintenance immunosuppression. As a start in this direction, a proteinuria cutoff of less than 0.7–0.8 g/dl at 12 months after lupus nephritis onset has been confirmed as a biomarker of good long-term outcome in several studies [7▪▪,8,9] and a set of hazard index tools incorporating clinical data from the first 12 months of treatment, have been shown to predict long-term outcomes [7▪▪]. A longitudinal study comparing the accuracy of spot urines to 24-h collections strongly advocates use of 24-h collections for accurate results [10]. Because large patient numbers are needed to test and develop predictive models, the establishment of lupus nephritis registries with prospectively collected data and biospecimens will be essential to refine current models (Table 1). An international lupus nephritis registry would also address the problem of multiple small studies in distinct ethnic and racial groups that lack generalizability.

**Table 1.** Outcome prediction

| |
|---|
| Predictors of poor outcome at disease onset |
| Clinical [1,7▪▪] |
| Male sex |
| Younger age |
| Arterial hypertension |
| Increased serum creatinine |
| African-American race |
| Histologic [11,12] |
| Proliferative disease |
| High activity and chronicity index |
| Glomerulosclerosis and crescents |
| Interstitial inflammation |
| Tubular injury |
| Interstitial fibrosis |
| Biomarkers that need to be further evaluated [13▪,14] |
| Markers for tubular injury |
| Nonalbumin proteinuria |
| Predictors of poor outcome after treatment |
| Lack of access to a specialized center [2] |
| Absence of maintenance immunosuppressive therapy [1] |
| Failure to reach proteinuria threshold of <0.7–0.8 g/dl at 12 months [7▪▪,8,9] |
| High activity index on second biopsy at 12 months [15▪▪] |

## CONTROVERSIES IN LUPUS NEPHRITIS MANAGEMENT AND CLINICAL TRIAL DESIGN

Mycophenolate (MMF) or cyclophosphamide combined with high doses of prednisone are standard of care treatment for lupus nephritis [16,17]. Clinical questions remain about the optimal management of lupus podocytopathy, renal microangiopathy and membranous nephritis [18–20]. Because remission rates of lupus nephritis are low even with optimal management, studies using combinations of standard immunosuppressives [21], or the introduction of a calcineurin inhibitor are being considered. Controlled trials in Asian populations suggest that the combination of low-dose MMF with tacrolimus is more effective than MMF alone, but safety and long-term efficacy remain to be established in other populations [16,22]. The new calcineurin inhibitor voclosporin, modified to confer enhanced potency and decreased toxicity, has shown efficacy in combination with low-dose MMF and a rapid steroid taper in two phase 2 trials [23,24▪] – phase 3 studies are in process. Combinations of immunosuppressive agents with biologic drugs have not yet been successful in randomized clinical trials in lupus nephritis patients. The addition of belimumab to cyclophosphamide or rituximab or both is a rational approach to prevent the expansion of autoreactive B cells by high levels of B cell activating factor resulting from B-cell depletion. However, data from the CALIBRATE trial showed no improvement in outcome at 24 or 48 weeks of a regimen of cyclophosphamide, rituximab, prednisone and belimumab compared with the same regimen without

belimumab [25]. A study of belimumab and either MMF or cyclophosphamide is ongoing. Given the increasing reports of decreased general systemic lupus erythematosus (SLE) flare rates over the long term in patients treated with belimumab [26], longer term follow-up will be needed to determine its benefit in lupus nephritis.

MMF is the preferred treatment for remission maintenance [27,28] but there are no data regarding the optimal duration of treatment and no definition of a low disease activity state predicting safe treatment withdrawal [29,30]. Two recent studies have addressed this question by performing repeat biopsies at 6–12 months after induction and have shown an alarming discrepancy between clinical and histologic response. A small prospective study showed that activity on a second biopsy performed 12 months after induction predicted subsequent renal flare following maintenance withdrawal, regardless of clinical parameters [15▪▪]. A second study showed that chronicity at 6 months after induction predicted long-term renal outcome regardless of clinical or histologic remission status [31▪]; larger studies are needed to define the utility of second biopsies for guiding personalized treatment based on pathologic or molecular findings. Unfortunately, adherence to maintenance therapy among lupus nephritis patients is unacceptably low and new approaches are needed to address the complex contributors to this behavior [32▪▪]. In those patients who develop ESRD, timely referral for transplant is associated with a survival benefit by reducing deaths from comorbidities such as infections and cardiovascular disease [33▪].

Because of the continuing failure of clinical trials of rational therapies for lupus nephritis [16], much thought is being given to clinical trial design [34▪▪]. Limited duration phase 3 trials allow evaluation of remission induction but do not address subsequent flare prevention or long-term renal outcomes. Defining response for clinical care and endpoints in clinical trials remains problematic as there is no consensus on definitions of complete and partial renal response or remission and the utility of second renal biopsies is still unknown. Doubts about the inclusion of high doses of prednisone in lupus nephritis clinical trials and about the robustness of outcome measures have led to difficulty in the interpretation of lupus nephritis trial outcomes. Most biologic agents are tested with MMF, without considering whether each drug targets the same or different immune pathways. Design features that consider the mechanism of action of each drug in the context of genetic polymorphisms or biomarkers have not yet been incorporated into lupus nephritis clinical trials (Table 2).

**Table 2.** Considerations in trial design

| |
|---|
| Problems |
| Confounders |
| High placebo response to standard of care therapy leads to small effect sizes, necessitating large patient cohorts |
| Use of high-dose background steroids may mask the effects of new immune therapies |
| Enrolment criteria do not always reflect patients seen in clinical practice |
| Optimal outcome measures are still not defined and are not uniform across trials |
| Long-term outcomes are currently not measured |
| In the absence of informative biomarkers, diagnosis of residual activity may require a second biopsy |
| Solutions |
| When to treat |
| Current approach |
| Based on clinical evidence of nephritis e.g. proteinuria onset |
| Future approaches |
| Prevention based on evaluation of risk |
| Preclinical initiation of treatment triggered by biomarker change |
| Medication choice based on risk stratification (genetic, biomarker or OMICs driven) |
| Drug mechanism should drive trial design |
| Treatment of active disease vs. flare prevention |
| Rational choice of standard of care therapy |
| Stratification for individual patient differences should replace post-hoc analyses e.g. ethnicity, genetic polymorphisms, sex |
| Uniform trial design may help to compare the effects of agents with similar mechanisms of action |
| Measurements of compliance need to be incorporated, especially when multiple drugs are being used |
| Improved understanding of disease pathogenesis should result in development of better diagnostic and therapeutic tools |

OMICs, genomics/proteomics/metabolomics.

## MECHANISMS FOR RENAL DAMAGE IN LUPUS NEPHRITIS

An increased understanding of disease pathogenesis may expand treatment strategies beyond global immunosuppression.

### Genetics of lupus nephritis

Genetic risk factors for lupus nephritis are only beginning to be described. In SLE patients of European descent, polymorphisms of platelet derived growth factor receptor A, sodium glucose cotransporter Slc5a11, hyaluronan synthase 2, TNFAIP3 interating protein 1 and major histocompatibility complex Class I and II alleles are associated with lupus nephritis [35]. Identification of lupus nephritis-associated variants of intergrin subunit alpha M

that increase its proinflammatory properties and genetic polymorphisms that decrease renal kallikrein expression have led to the development of therapies that specifically target these pathways [36,37]. A polymorphism that increases the expression of the adapter Dab2 that mediates transforming growth factor (TGF)β signaling in epithelial cells is associated with chronic kidney disease (CKD) in humans [38▪▪]. Epigenetic studies have identified differential methylation of genes regulating the response to tissue hypoxia and interferon-mediated signaling in women with lupus nephritis [39]. European ancestry protects against lupus nephritis [40] and it is therefore important to study genetic risk factors in patients of other ethnicities. Apolipoprotein L1 (APOL1) risk genotypes are associated with poor outcome of most forms of CKD in individuals with African ancestry, with the risk being intrinsic to the kidney. Several pathogenic mechanisms have been suggested, but the relative role of each mechanism is still not known, making the APOL1 risk alleles difficult to target therapeutically. Consideration of APOL1 status in the kidney transplant setting is now being prospectively studied by the APOLLO Consortium [41▪,42].

### Cellular composition and gene expression in lupus nephritis kidneys

The kidney harbors multiple cell types and infiltrating immune cells add to the complexity of the microenvironment in the lupus nephritis kidney. Two new technologies are being used to understand the heterogeneity of the renal microenvironment in lupus nephritis patients so as to develop better diagnostic tools and individualized therapy. The first is two-photon microscopy together with cell distance mapping (CDM) to determine relationships between infiltrating renal cells [43▪]. The combination of CDM with staining of more than 20 different antigens using only small amounts of frozen tissue will yield insights into inflammatory responses by revealing how various cell types interact in the kidneys.

The second technology is single-cell transcriptome analysis of renal biopsies [44▪▪,45▪▪]. Although the sequencing depth using this approach is relatively shallow, it allows a full description of the cell types present in individual kidneys and some understanding of cell functions that can be correlated with histologic and clinical variables and outcomes. Phase 1 studies of infiltrating renal cells in 24 lupus nephritis patents and 10 controls revealed multiple subtypes of B, T and myeloid cells in the lupus nephritis kidneys. Natural killer cells and CD8 T cells with cytotoxic activity are the major proliferating immune populations; despite the identification of exhausted CD8 T cells in kidneys from MRL/lpr mice [46], no CD8 T-cell exhaustion phenotype was identified in lupus nephritis biopsies although it was readily identified in peripheral blood mononuclear cells. T follicular helper cells and activated B cells were present, with the accumulation of plasma cells and B cells with an age-associated B-cell phenotype. These studies failed to show a predominance of IL-17 or IFNγ-producing CD4 T cells with the caveat that most patients had already been treated at the time of biopsy. There is also evidence for activation of the resident macrophage population and for renal infiltration with $CD16^+$ inflammatory macrophages that then appear to transition to a M2-like phenotype that may orchestrate migration of other inflammatory cell subsets [45▪▪]. Dysregulated repair function of these cells may contribute to their pathogenic potential [47].

A similar single cell transcriptomic analysis of renal stromal cells from 21 lupus nephritis patients revealed both an interferon signature and a fibrotic signature in the tubular cells, both of which may be associated with poorer response to therapy [44▪▪]. Analyses of other tissues such as urine and unaffected skin that are more amenable to repeat sampling may yield information that reflects changes in the kidneys [44▪▪,45▪▪].

### Gut microbial diversity and lupus nephritis

An alteration in the composition of the gut microbiota has been associated with the production of antibodies to a particular species, *Ruminococcus gnavus* only in patients with active lupus nephritis [48▪] but not in inactive lupus patients. T and B-cell tolerance to the gut microbiota may be lost if disturbance of the gut epithelial barrier allows bacterial translocation to sites where they may elicit an inflammatory response [49,50]. Alternatively, a change in the composition of the microbiota may induce pathogenic cross-reactive antiself/anticommensal immune responses. It remains to be determined whether the dysbiosis of lupus nephritis is causative or reflects homeostatic disturbances associated with inflammation and immunosuppressive medications. Longitudinal studies are now needed to examine the course of gut dysbiosis in lupus nephritis patients and to test the therapeutic applicability of approaches that restore commensal homeostasis and gut barrier integrity.

## LUPUS NEPHRITIS BIOMARKERS

Biomarker discovery in lupus nephritis has progressed from analysis of individual candidate

markers to unbiased high-throughput methods such as mass spectrometry [51▪], multiplexed immunoassays, renal imaging [52] and modular transcriptome analyses. Proteomic studies indicate that small panels of biomarkers can distinguish lupus nephritis from healthy control urine and that of active from inactive disease [13▪,53–56]. A set of six biomarkers, Renal Activity Index for Lupus (RAIL) is associated with nephritis in children and to a lesser extent in adults in cross-sectional studies [57,58]. However, a recent longitudinal study using RAIL and additional biomarkers failed to identify a panel that outperformed glomerular filtration rate (GFR) or predicted renal functional decline in individual patients [59▪]. Nevertheless it may be possible to develop a home-based assay to be performed between visits to improve early detection of nephritis [60]. Transcriptomic analyses have identified a peripheral blood neutrophil signature as a risk factor for lupus nephritis, although it may not be a robust biomarker of disease response [61,62]. Finally there is still debate about the significance of renal deposition of the terminal Mac complex as a biomarker for complement activation that could be targeted by anti-C5 drugs [63,64].

## NEW APPROACHES TO THERAPEUTIC TARGETING

Mining of existing databases has revealed pathways that could be targeted by available drugs - repurposing of IL12/23 inhibitors, proteasome inhibitors and Jak inhibitors are examples of this approach. Although the concordance of mouse and human interventions for lupus nephritis has historically been poor, a number of new therapeutic targets have been recently tested in murine models. Allogeneic mesenchymal stem cells prevent lupus nephritis in mouse lupus models and multiple mechanisms for their efficacy have been reported (reviewed [65]). Results in humans are conflicting and await randomized trials. Defective production of IL-2 by conventional T cells in patients with lupus and lupus nephritis favors the generation of inflammatory T cells. Correction of this defect by low-dose IL-2 improves survival in lupus mouse models and approaches to enhance IL-2 are now being developed for human use [66]. Immune activation is associated with metabolic changes that favor cell proliferation and differentiation. Inhibition of both glycolysis and oxidative phosphorylation can effectively treat established nephritis in mouse models [67]. Other approaches include targeting of the cholinergic inflammatory reflex with galantamine [68], targeting of renal macrophages by introducing deficiency of IL-34 [69], targeting of inflammatory cytokines by introducing deficiency of inactive rhomboid 2 [70], targeting of Th17 cells and podocyte injury by inhibition of CAMK4 [71▪▪] and targeting of inflammasome mediated renal injury by inhibition of NLRP3 [72,73]. Because some of these therapies may have unacceptable systemic consequences, new approaches are needed to direct such therapies only to the inflamed site. Nanogels are specially formulated drug-containing liposomes that can be targeted to specific cells using antibodies. In a recent proof-of-principle study a nanogel containing a CamK4 inhibitor was directed to podocytes using antinephrin or antipodocin antibodies and prevented podocyte injury and proteinuria in a mouse lupus model [71▪▪]. Development of such site-directed therapeutic agents may enhance our current ability to prevent flares while decreasing the need for chronic global immune suppression.

It is increasingly evident that nonimmune pathways contribute to renal injury in lupus nephritis. One recent question is whether the kidneys can be protected from inflammatory damage by altering the resilience of resident renal cells to oxidative stress [74]. Tubular cell injury is a common feature of all forms of CKD. Tubular epithelial cells rely on mitochondrial fatty acid oxidation for their energy supply and can be protected in mice by strategies that conserve this pathway [75▪]. Tubule-specific deletion of the TGFβ signaling effector Dab2 in mice also protects from renal injury and fibrosis, suggesting another therapeutic approach especially in genetically susceptible individuals [38▪▪]. Since multiple cell types are affected during renal injury, it is expected that multi-OMICs (genomics, proteomics, metabolomics) discovery experiments will uncover new pathways for targeting.

## CONCLUSION

Coordinated approaches that involve all stake holders are needed to prevent and treat lupus nephritis, especially in those ethnic groups whose outcome is historically poor. Collaborative interactions can help to identify knowledge deficits and clinical needs so as to design appropriate multicenter studies. Registries and uniform biospecimen collection will allow testing of hypotheses that can only be addressed with longitudinal data. Mechanistic studies involving individual renal cell types from human samples will allow us to further unravel precise mechanisms in appropriate mouse models and to predict new and/or synergistic therapeutic approaches. Because it is easier to prevent than to treat established lupus nephritis, identification of patients at risk and at home monitoring for early

renal changes may improve outcomes. Providing access to care in specialized lupus centers can facilitate early detection, encourage and monitor patient compliance, improve management of ancillary morbidities and enable access to renal transplant so as to improve the long-term outcome of all patients with lupus nephritis.

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## Conflicts of interest

*There are no conflicts of interest.*

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REVIEW

OPEN

# Update on lupus epidemiology: advancing health disparities research through the study of minority populations

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**Purpose of review**
The current review focuses on recent population-based studies that have examined the burden of lupus, disease outcomes, and gaps in quality of care, with an emphasis in research addressing health disparities.

**Recent findings**
The Centers for Disease Control and Prevention National Lupus Registries underscored higher susceptibility of both systemic lupus erythematosus (SLE) and primary cutaneous lupus among people of color, compared with whites. Not only does SLE disproportionately strike people from racial and ethnic minorities, those individuals are also at increased risk of developing severe manifestations following SLE diagnosis. Mortality is higher and death occurs at a younger age among blacks, compared with whites. Furthermore, ongoing Centers for Disease Control and Prevention-supported population-based lupus cohorts, along with research by other groups, have provided new insight into the role of social determinants on outcomes and opportunities to improve care in diverse lupus populations.

**Summary**
While descriptive epidemiological efforts have been critical to providing more accurate estimates of the burden and mortality of lupus across diverse demographic groups, emerging research suggests a significant influence of psychosocial and healthcare system factors on disease outcomes. These current efforts represent important steps toward the development of clinical and public health interventions aimed at eliminating health disparities in lupus populations.

**Keywords**
epidemiology, lupus, minorities, racial disparities, systemic lupus erythematosus

## INTRODUCTION

In the past years, the Centers for Disease Control and Prevention (CDC) have supported a variety of national lupus activities (https://www.cdc.gov/lupus/funded/index.html), including five registries designed to study US-based populations diagnosed with systemic lupus erythematosus (SLE) or cutaneous lupus. Providing more accurate estimates of the burden of lupus, the CDC lupus registries continued to underscore substantial racial disparities across US populations. As SLE severity is worse and mortality remains high among minority groups, new epidemiological efforts, including three longitudinal population-based cohorts formed from the CDC-funded registries, are providing novel insights into the natural history of both SLE and cutaneous lupus across racially and ethnically diverse populations. This review focuses on studies that have examined the burden, mortality, outcomes, and quality of care in US populations with lupus, with emphasis on those that provide insights into health disparities.

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## KEY POINTS

- We reviewed population-based studies on the burden of lupus, disease outcomes, and gaps in quality of care, with an emphasis in research addressing health disparities.
- Descriptive epidemiological studies in the United States have provided more accurate estimates of the burden and mortality of lupus across diverse demographic groups.
- Emerging research suggests a significant influence of psychosocial and healthcare system factors on disease outcomes and lupus health disparities.

## ADVANCING LUPUS HEALTH DISPARITIES RESEARCH IN THE UNITED STATES THROUGH THE CENTERS FOR DISEASE CONTROL AND PREVENTION NATIONAL LUPUS REGISTRIES

The CDC National Lupus Registries were created from an unprecedented national lupus surveillance project aimed at addressing the limitations of previous epidemiologic data, particularly in racial/ethnic minority populations in the United States. Established in selected counties of Georgia, Michigan, California, New York, and at selected Indian Health Service regions, the five sites used similar methods while taking advantage of novel federal, state, and local partnerships with academic institutions (Table 1). The public health surveillance exemption to the Health Insurance Portability and Accountability Act Privacy Rule (https://www.hhs.gov/hipaa/for-professionals/privacy/index.html) allowed investigators to obtain protected health information without written patient consent, which enabled investigators to maximize case ascertainment, determine if potential cases met case definition criteria, and provide enough information to prevent duplicate counting of patients examined in multiple facilities. Primary sources of cases included queries of administrative databases from hospitals, physician offices, as well as laboratories and population databases. The primary aims of the registries were to obtain updated and more accurate incidence and prevalence rates of SLE, which have been published in the past 5 years [1–5,6▪,7,8].

Racial and ethnic differences were recently analyzed in lupus manifestations and in the timing and risk of developing severe manifestations in the California Lupus Surveillance Project (CLSP) [9▪]. This was the first epidemiologic study of lupus comparing manifestations among the four-major racial/ethnic groups in the United States: blacks, whites, Asian/Pacific Islanders (APIs), and Hispanics. Among 724 patients with SLE; the authors found substantial differences in the prevalence of several clinical manifestations between groups. Data indicated that blacks, APIs, and Hispanics are at increased risk of developing severe manifestations following SLE diagnosis. Blacks and APIs had a higher prevalence of lupus nephritis (20 and 52%, respectively), compared with 13–14% among the other groups, and thrombocytopenia (24 and 39%, respectively), compared with 17–19% among the other groups. Neuropsychiatric lupus was less

**Table 1.** The National Lupus Registries supported by the Centers for Disease Control and Prevention in the United States

| Registry (partner) | Public health authority | Surveillance area or health system | Population at risk | Type of lupus | Main racial/Ethnic group | Surveillance period |
|---|---|---|---|---|---|---|
| GLR (Emory University) [1,6▪] | GA Department of Public Health | Fulton and DeKalb Counties, GA | 1552 970 | SLE, CCLE | White and Black | 2002–2004 |
| MILES (UM) [2,7] | MI Department of Community Health | Wayne and Washtenaw Counties, MI | ~2400 000 | SLE | White and Black, Arab and Chaldean Americans | 2002–2004 |
| MLSP (NYU) [4] | NY City Department of Health | New York County, NY (Borough of Manhattan) | 1585 873 | SLE | White, Asian/Pacific Islander, Hispanic, Black | 2007–2009 |
| CLSP (UCSF) [5] | CA Department of Public Health | San Francisco County, CA | 790 582 | SLE | White, Asian/Pacific Islander, Hispanic, Black | 2007–2009 |
| IHS Lupus Registry (ANTHC) [3,8] | Indian Health Service | IHS Data Warehouse of Alaska, Phoenix & Oklahoma City Areas | 211,916 | SLE, MCTD | American Indian/ Alaska Native | 2007–2009 |

ANTHC, Alaska Native Tribal Health Consortium; CA, California; CCLE, chronic cutaneous lupus erythematosus; CLSP, California Lupus Surveillance Project; GA, Georgia; GLR, Georgia Lupus Registry; IHS, Indian Health Service; MCTD, mixed connective tissue disease; MI, Michigan; MILES, Michigan Lupus Epidemiology & Surveillance Program; MLSP, Manhattan Lupus Surveillance Project; NY, New York; NYU, New York University; SLE, systemic lupus erythematosus; UCSF, University of California, San Francisco; UM, University of Michigan.

common among Hispanics, and antiphospholipid syndrome was more common among APIs. Blacks, APIs, and Hispanics were at increased risk of developing lupus nephritis, thrombocytopenia, and antiphospholipid syndrome earlier than whites following SLE diagnosis.

Given the methodology of the CDC registries, other forms of lupus and associated conditions have been evaluated, including mixed connective tissue disease in the American Indian/Alaska Native population [8]. More recently, minimum estimates of the incidence of primary chronic cutaneous lupus erythematosus (CCLE) from the Georgia Lupus Registry (GLR) have been published [6▪]. Findings uncovered striking racial disparities in the susceptibility of CCLE, with blacks having a three-fold to five-fold increased risk, compared with white people, depending on the case definition. Black/white disparities in the incidence of CCLE were analogs to those described thorough the GLR for SLE in the same geographic area [1]. Moreover, data suggest that black/white disparities may also occur concerning the age at CCLE diagnosis, with blacks tending to develop CCLE at earlier ages compared with whites, as noted with SLE in the same population [1].

## MORTALITY IN LUPUS

The power of the CDC registries can be extended beyond the initial surveillance periods through matching with other population-based databases and leveraging identification of nearly all validated SLE cases in an area without reliance on administrative data for case ascertainment. Incident and prevalent SLE patients from the GLR were matched to the National Death Index through 2016 [10▪]. During 2002–2004, 336 incident SLE cases were identified, of whom 86.9% were female, 73.8% blacks, and 22.9% whites. In 2002, 1353 prevalent SLE cases were 89.9% female, 75.7% black, and 23% white. Among prevalent and incident SLE, 401 and 97 deaths, respectively, occurred through 2016. Standardized mortality ratios (SMRs) using 2002–2016 data were 2.3–3.3 times higher for persons with prevalent SLE relative to expected deaths in the general population. Cumulative mortality was significantly higher among blacks than among whites for both incident and prevalent SLE. Black females with prevalent SLE were three times more likely to die than were black females in the general population (SMR = 3.38). Death occurred at a younger age among incident and prevalent black SLE cases than it did among whites. Mortality among blacks was markedly higher in the years immediately following SLE diagnosis compared with mortality among whites. There were no significant differences by sex.

There were other significant studies of mortality in SLE. In 2017, a nationwide population-based study of the United States from the 1968 through 2013 used death certificate data from the CDC's WONDER (Wide-ranging Online Data for Epidemiologic Research) database and found age-standardized mortality rates over time decreased in SLE, but remained high relative to non-SLE mortality, with higher mortality rates in females, blacks, and residents of the South and West [11]. The same authors utilized the death certificate data from the CDC's WONDER database and the leading causes of death data from the Web-based Injury Statistics Query and Reporting System database from 2000 to 2015 to rank organic causes of deaths of females of reproductive age by race/ethnicity and age [12]. After excluding the external injury causes of death, namely, unintentional injury, homicide, and suicide from the analysis, the study showed that SLE is among the leading causes of death in young females. For females of all races/ethnicities, SLE ranked seventh as the leading cause of death among females ages 15–24 years and eleventh among both those ages 25–34 years and those ages 35–44 years. Among black and Hispanic females, the rankings for SLE were higher: fifth, sixth, and eighth or ninth among females' ages 15–24, 25–34, and 35–44 years, respectively.

Using the 2014 National Center for Health Statistics multiple causes of death database, a population-based electronic medical recording of all death certificates issued in the United States and its territories, sex-stratified demographic characteristics and the most commonly listed comorbidities in decedents with and without SLE were compared [13]. Out of 2036 decedents with SLE, 86.2% were females, who were 22 years younger than non-SLE female decedents. The difference was 12 years among male decedents. These data continue to underscore the disproportionate impact of female sex on premature mortality in SLE. There were differences in the most frequently listed causes of deaths between female and male SLE decedents. Septicemia (4.32%) and hypertension (3.04%) were the most common in females. Heart disease (3.70%) and diabetes mellitus with complications (3.61%) were the most common in males. Though these are the same leading comorbid conditions observed in the general population, the sex differential in SLE may help focus efforts to minimize premature SLE mortality.

A prior study observed concurrent poverty and persistent poverty were associated with damage accrual, while exiting poverty was associated with lower levels of accumulated damage [14▪]. Building on the same data source, the Lupus Outcomes Study, the authors evaluated 807 completed interviews

 

from 2009 for the effect of poverty on mortality from 2009 to 2015 [15]. Cox proportional hazards regression was used to estimate the impact of poverty on other variables on risk of all-cause mortality. The association of mortality risk with poverty adjusted for age was significant (hazard ratio 2.14; 95% confidence interval 1.18–3.88) but lost significance when level of damage was introduced. This suggested that poverty resulted in higher mortality in SLE by increasing damage accumulation. In addition to improving medical care, the authors suggested that potential strategies to reduce damage must include reducing stress associated with poverty, improving access to affordable food and house, improving coping abilities, and aiding transition to better neighborhoods.

## THE CENTERS FOR DISEASE CONTROL AND PREVENTION POPULATION-BASED LUPUS COHORTS

The CDC National Lupus Registries established a strong foundation to advance our understanding of lupus outcomes in racially/ethnically diverse populations. Those efforts have been lately galvanized by the creation of three CDC-supported longitudinal cohorts of adults with diagnosed SLE and cutaneous lupus, which are primarily derived from the five national registries:

*The Michigan Lupus Epidemiology & Surveillance (MILES) Program Cohort and Biobank* has been developed from the CDC-supported population-based lupus registry, which has been established in Detroit and Ann Arbor, encompassing a large white and black population of individuals with SLE. The overarching goal of the cohort is to prospectively collect data and biospecimens to conduct investigations related to risk factors for lupus onset, progression, and comorbidities. Major thematic areas of the MILES Cohort & Biobank include epigenetics, environmental epidemiology, and renal lupus.

*The Georgians Organized Against Lupus (GOAL) Cohort* was born out of the efforts of the GLR to create a population-based prospective cohort of validated SLE and cutaneous lupus patients, reflecting 'real world' lupus in and around metropolitan Atlanta, Georgia, where half of the population is African-American or black. The ongoing GOAL Cohort encompasses over 1000 individuals with a validated diagnosis of SLE and nearly 130 with a dermatologist-documented diagnosis of chronic cutaneous lupus confined to the skin. Through the longitudinal collection of a broad battery of patient-reported outcomes and biospecimens and matching of participants with other population databases (e.g., US Renal Data System; US National Death Index; Georgia Comprehensive Cancer Registry; Georgia Hospital Discharge Database; Georgia Birth Records), GOAL is exploring how social determinants of health interact with biologic factors to influence natural history, treatment, and healthcare access through the overarching lens of racial disparities.

*The California Lupus Epidemiology Study (CLUES)* is a racially and ethnically diverse longitudinal cohort of over 400 patients with physician confirmed SLE derived from the population-based CLSP. A unique contribution of CLUES is the ability to study the natural history and outcomes of SLE among Asian and Hispanic individuals, as these groups currently comprise 34 and 22% of the cohort, respectively. As with the other CDC-funded lupus cohorts, comprehensive longitudinal data are collected, ranging from clinical and patient-reported outcomes to genetic, epigenetic and environmental exposures.

The most recent contributions of the ongoing CDC-supported cohorts are summarized below.

### Depression in systemic lupus erythematosus and primary chronic cutaneous lupus erythematosus

Depressive symptoms have been recognized in 10–75% of individuals with SLE or cutaneous lupus [16,17]. Compared with whites, African-Americans with SLE have worse mental health, which in turn can lead to adverse health-related behaviors, such as poor medication adherence [18]. However, African-American patients with SLE have been underrepresented in studies of depression. Moreover, recent data suggest that African-Americans with SLE are less likely to be diagnosed with depression than their white counterparts [19]. Findings from the GOAL cohort underscored that among 635 African-American individuals with SLE, 35% reported moderate to severe depressive symptoms and 54% reported low medication adherence [20]. Moreover, the severity of depressive symptoms had an increasingly negative impact on treatment adherence. Moderately severe-to-severe depressive symptoms versus minimal depressive symptoms rendered the highest odds ratios (ORs) for low medication adherence (OR 4.2, $P < 0.0001$), followed by moderate (OR 3.3, $P < 0.0001$), and mild depressive symptoms (OR 2.7, $P < 0.0001$). Depression was also found to shape an individual's perceptions of physician–patient interaction in the African-American GOAL population with SLE [21]. Specifically, African-Americans patients with greater disease activity and those with more severe depressive symptoms reported poorer communication and less personable involvement

by their doctors. Moreover, African-American women with depressive symptoms were more likely to accumulate more organ damage and report lower emotional support, compared with those without depression [22].

In another study, depression was also found to be highly prevalent in GOAL participants with lupus confined to the skin [23]. Among 106 participants with primary CCLE, over one-quarter reported moderate to severe depression, a rate three to five times higher than those previously described in the general population from the same metropolitan Atlanta area. In this predominantly African-American cohort of patients with primary CCLE, depression was directly associated with a patient's perceptions of staff disrespect and inversely associated with emotional support.

GOAL data suggest that routine mental health screening should be considered in lupus patients, particularly in those from minority groups who do not adhere to their medications. In addition, provider-based interventions on communication and interpersonal style, as well as public health programs that foster social networks and promote resilience may help to reduce the burden of depression in high-risk lupus populations.

## Psychosocial stressors and lupus outcomes

Genetic and socioeconomic factors do not fully explain racial disparities in SLE outcomes. Compared with whites, African-Americans are more likely to experience psychosocial stressors, which can potentially aggravate or exacerbate SLE. Three recent publications have addressed the impact of psychosocial stressors on outcomes among SLE participants of the GOAL and CLUE cohorts.

### Racial discrimination and vicarious racism

The interpersonal experience of racial discrimination is a source of stress that can activate inflammatory pathways and lead to poor SLE outcomes. A study conducted among 427 African-American women with SLE recruited from the GOAL cohort revealed that 80% of participants reported experiencing racial discrimination in at least one of nine domains (e.g., at school; getting a job; at work; getting housing; medical care; service at a store/restaurant; obtaining a credit/loan; on the street/public setting; from the police or in the courts), with 40% experiencing racial discrimination in five or more [24▪]. Greater racial discrimination correlated with both higher disease activity and organ damage after adjusting for socioeconomic and health-related factors. The same group of investigators further examined the relationship between vicarious racism and disease activity in the GOAL sample of African-American women with SLE [25]. Vicarious racism is a 'secondhand' type of exposure to racism (e.g., hearing about or observing acts of racism or discrimination) that causes psychosocial stress and may contribute to health disparities. Vicarious racism stress was found to be associated with SLE activity after adjusting for socioeconomic and health-related covariates, as well as for everyday discrimination.

As people of color are disproportionately stricken by lupus and also more frequently exposed to racial discrimination, these experiences can lead or accentuate health disparities. The authors suggested that public health interventions directed to eradicate racial discrimination across multiple societal levels, along with policies aimed at combating the structural systems that perpetuate racism are needed to reduce racial disparities in US populations, including those afflicted by SLE.

### Childhood trauma

Another psychosocial stressor that has been linked to chronic conditions is childhood trauma [26]. In a racially/ethnically diverse sample of 269 individuals with SLE, the CLUES cohort underscored a significant association of increasing levels of adverse childhood experiences (ACEs) with higher depression, higher disease activity, and worse physical function [27]. Moreover, women, Latinos or African-Americans, older participants, those without a college degree, and those with lupus nephritis were more likely to reported ACEs. As these subgroups have worse SLE outcomes, these findings support the need for ACE screening and psychological interventions among high-risk patients with SLE.

## Quality of lupus nephritis care

Renal involvement occurs in up to 60% of SLE patients and the 5-year cumulative incidence of end-stage renal disease was estimated to be 6.4 and 2.5% among black and white SLE patients, respectively [28]. As early diagnosis and treatment are critical to reducing morbidity and mortality associated with lupus nephritis, the CLUE cohort was used to assess the quality of lupus nephritis care in patients with and without lupus nephritis. Findings indicated that the largest quality gap across 25 different clinical sites was in the screening of SLE patients for renal involvement [29▪]. Of 148 patients without lupus nephritis, the overall performance across lupus nephritis screening measures was 54%. While the majority (81%) had the blood pressure checked every 6 months, only 42 and 38% had

 

nephritis screening labs and serology to test lupus activity, respectively. The overall performance for lupus nephritis screening was significantly better at academic (63.4–73%) versus community clinics (37.9–38.4%). Similarly, among those with lupus nephritis, higher performance in academic (84.1–85.2%) versus community clinics (54.8–60.2%) was observed for treatment measures.

### Impact of dietary omega fatty acid intake on health-related quality of life domains

Omega-3 ($n-3$) polyunsaturated fatty acid (PUFA), which is found in fatty fish, oils, nuts, and seeds has anti-inflammatory effects. However, it is consumed at relatively low levels in the US diet. In contrast, omega-6 ($n-6$) PUFA, including linoleic and arachidonic acids, tend to be proinflammatory and are ubiquitous (e.g., soybean and corn oils) in the US diet. A cross-sectional study of 456 SLE participants (51% whites, 45% blacks, 3% Asian, or other races) of the MILES Cohort & Biobank demonstrated a significant association between higher dietary intake of $n-3$ FAs and lower $n-6:n-3$ ratios with lower self-reported lupus activity and better sleep quality [30]. A nonsignificant association was also found between higher $n-3$ intake and less depressive symptoms, fibromyalgia, and higher quality of life, whereas results for the $n-6:n-3$ ratio trended in the opposite direction. The authors suggested that promoting a better balance of FAs from dietary sources, with a higher intake of $n-3$ PUFA might positively impact the quality of life of SLE individuals through immunomodulatory and anti-inflammatory effects.

## OTHER RESEARCH TARGETING HIGH-RISK POPULATIONS WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN THE US

### Quality of systemic lupus erythematosus care in the US American-Indian/Alaska Native population

Using data abstracted from medical records through the CDC Indian Health Service lupus registry, differences in the diagnosis and management of SLE by primary care and specialist physicians in the American Indian/Alaska Native population were investigated [31]. Among 320 individuals with SLE, 78% had the diagnosis documented by a specialist and 22% by a primary care provider. Individuals with a specialist diagnosis were more likely to have documentation of fulfilling a variety of validated sets of criteria for SLE diagnosis. Moreover, specialist diagnosis was significantly associated with documentation of antidouble-stranded DNA antibody and low complement testing. Individuals with documentation of specialist diagnosis were also more likely to ever receive hydroxychloroquine. These data support the need to increase specialist access for American Indian/Alaska Native individuals with suspected SLE and to provide lupus education to primary care physicians serving this population.

### Treatment adherence in systemic lupus erythematosus

Given the growing body of evidence indicating low treatment adherence in lupus [20,32,33], recent efforts have examined large Medicaid data to assess disparities in lupus medication adherence [34,35,36]. Among over 10 000 US Medicaid beneficiaries who met the case definition of SLE and initiated hydroxychloroquine, only 15% were classified as adherent [34]. Adherence was lower in geographic areas with higher percentages of black individuals [highest tertile OR 0.81 (0.69–0.96) versus lowest]. This association remained significant after controlling for zip code, education, poverty, urbanicity, and healthcare resources. Moreover, blacks and Hispanics were less likely to be persistent adherers than whites [36]. Black race and Hispanic ethnicity also increased the odds of azathioprine nonadherence; however, no significant associations were reported between race/ethnicity and mofetil mycophenolate adherence [35]. The authors suggested that further studies of contextual and social factors are warranted to inform effective interventions directed to improve treatment adherence within racial minorities with lupus in the United States.

### Kidney allograft survival in US minorities with systemic lupus erythematosus

A consistent finding of epidemiological studies is the higher incidence of lupus nephritis and end-stage renal disease in Blacks and Hispanics with SLE [1,9▪,28,37,38]. A US group analyzed records in the United Network for Organ Sharing program and Standard Transplant Analysis and Research files to compare kidney allograft survival in African-American and Hispanic individuals who had SLE and received kidney transplants between 1987 and 2006 [39]. Data from a cohort of 478 pairs of recipients that matched for 16 confounders, including sociodemographic, type of donor, human leukocyte antigen (HLA) mismatch, cold ischemia time, and follow-up time, showed significantly lower allograft survival, higher rates of rejection, and higher allograft failure attributed to rejection in

African-Americans, compared with Hispanics. The overall mortality was similar between African-Americans and Hispanics in the matched cohort (2.7 and 2.3/100 patient-years, respectively). However, the unmatched cohort ($n = 1816$ African-Americans and $n = 901$ Hispanics) revealed that African-Americans were older, had lower frequency of both private insurance and college or technical education, primarily received kidney from deceased donors with higher frequency of kidneys from expanded criteria donors, longer cold ischemia time and higher HLA mismatch level, and had a significantly higher mortality (2.8 deaths/100 patient-years), compared with Hispanics (1.7 deaths/100 patient-years).

## CONCLUSION

Recent epidemiological studies have made significant contributions to our understanding of the population burden and natural history of individuals with SLE and cutaneous lupus from diverse race and ethnic backgrounds. While ongoing research is providing new insight into the social and healthcare system factors that shape outcomes in lupus minorities, future studies addressing causal pathways, biologic mechanisms, and mitigating factors will be critical in guiding multilevel interventions to confront the problem of lupus health disparities in the United States, as well as the rest of the world.

Note by the authors: Black and African-American terms are used in this article as in the original article each section is referencing.

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### Conflicts of interest

*There are no conflicts of interest.*

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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# Porphyromonas gingivalis and rheumatoid arthritis: Erratum

In the article, ''Porphyromonas gingivalis and rheumatoid arthritis'' [1], an author name was listed incorrectly. The author name ''Saccucci Matteo'' should instead read ''Matteo Saccucci'', and the correct author list should read as follows:

In the HTML version: Perricone, Carlo; Ceccarelli, Fulvia; Saccucci, Matteo; Di Carlo, Gabriele; Bogdanos, Dimitrios P.; Lucchetti, Ramona; Pilloni, Andrea; Valesini, Guido; Polimeni, Antonella; Conti, Fabrizio

In the PDF version: Carlo Perricone, Fulvia Ceccarelli, Matteo Saccucci, Gabriele Di Carlo, Dimitrios P. Bogdanos, Ramona Lucchetti, Andrea Pilloni, Guido Valesini, Antonella Polimeni, and Fabrizio Conti

On PubMed: Perricone C, Ceccarelli F, Saccucci M, Di Carlo G, Bogdanos DP, Lucchetti R, Pilloni A, Valesini G, Polimeni A, Conti F.

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1. Perricone C, Ceccarelli F, Matteo S, *et al.* Porphyromonas gingivalis and rheumatoid arthritis. Curr Opin Rheumatol 2019; 31:517–524.