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

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 myosis and Sjogren 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.

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Dr Assassi's research focuses on the correlation of clinical features of systemic sclerosis with genetic and gene expression data to identify novel therapeutic targets and to develop clinically useful biomarkers. His laboratory works with mouse fibrosis models, as well as primary human samples to elucidate the molecular basis of systemic sclerosis. He is the principal investigator of several National Institute of Health and foundation funded research projects.

Dr Assassi has co-authored over 185 peer-reviewed manuscripts and has received multiple awards including a Clinical Investigator Fellowship Award from the American College of Rheumatology and University of Texas System STARs Award. He is a member of Medical Scientific Advisory Board of National Scleroderma Foundation and the president of Scleroderma Clinical Trial Consortium.

Biomarkers of disease activity in dermatomyositis

Xin Lu, Qinglin Peng and Guochun Wang

Purpose of review
This review provides updates regarding biomarker studies that address key clinical unmet needs, which relate to the evaluation of the disease activity in patients with dermatomyositis.

Recent findings
Increasing evidence supports that the serum levels of dermatomyositis-specific antibodies (DM-MSAs), which include anti-Mi-2, anti-NXP2, anti-MDA5, anti-TNF1-γ, and anti-SAE, are correlated with the disease activity. Moreover, serial measurements of DM-MSA levels may help to predict the disease status. Beyond the MSA, macrophage activation-related biomarker-soluble CD163, CD206, neopterin, and galectin-3/9 are the most currently talked biomarkers for disease activity in dermatomyositis; new circulating T-cell subsets CD4+CXCR5+CCR7loPD-1hi and TIGIT+CD226+ CD4 T cells can potentially harbor biomarkers of disease activity in dermatomyositis. In addition, LDGs and NETs were also shown to be correlated with the disease activities of dermatomyositis.

Summary
Promising candidate biomarkers are now available for evaluating disease activity in dermatomyositis. These biomarkers need external validation in other large cohort studies.

Keywords
biomarkers, dermatomyositis, disease activity

INTRODUCTION

Dermatomyositis is a heterogeneous group of auto-immune disease characterized by chronic inflammation of multiple tissues including skin, muscles, and lung. It has been proposed to subclassify into six different subtypes: anti-Mi-2 dermatomyositis, anti-MDA5 dermatomyositis, anti-TIF1-γ dermatomyositis, anti-NXP2 dermatomyositis, anti-SAE dermatomyositis, and myositis-specific autoantibodies (MSA)-negative dermatomyositis according to the presence of different dermatomyositis-specific antibodies (DM-MSA) [1,2]. Different subtypes of dermatomyositis have distinct clinical characteristics and vary to the treatment response. Therefore, how to evaluate the disease activity of dermatomyositis is an important issue for clinicians. The International Myositis Assessment and Clinical Studies Group (IMACS) scoring system is the most common tool used to evaluate the disease activity in current clinical studies [3]. However, the IMACS core set measures are more complex and often cannot discriminate between the effects of chronic damage and active of disease. It is of great clinical significance to find out more simple and sensitive biomarkers that reflect the disease activity of dermatomyositis. In this article, we review the recent literature on the biomarkers, which could be used to assess the disease activity in the patients with dermatomyositis.

SERUM LEVELS OF MYOSITIS-SPECIFIC AUTOANTIBODIES CORRELATED WITH DISEASE ACTIVITY

Many studies have documented that MSA could serve as a serum biomarker not only for clinical classification and the diagnosis but also for the evaluating disease activity of dermatomyositis.

Anti-Mi-2

Anti-Mi-2 antibodies are present in about 4–20% of dermatomyositis patients who have typical skin lesions, such as Gottron sign and heliotrope rash [4▪]. Although most anti-Mi-2-positive patients with

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KEY POINTS

- Increasing evidence supports that the serum level of DM-MSA (anti-Mi-2, anti-NXP2, anti-TNF1-γ, and anti-MDA5) could be severed as biomarkers for predicting the disease activity.

- Macrophage activation-related biomarkers, such as soluble CD163, CD206, neopterin, and galectin-3/9, are the most currently talked biomarkers for disease activity in dermatomyositis.

- New circulating T-cell subsets CD4+CXCR5+CCR7loPD-1hi and TIGIT+CD226+ CD4 T cells can potentially harbor biomarkers of disease activity in dermatomyositis.

- LDGs and NETs are correlated with disease activities of dermatomyositis.

dermatomyositis have severe muscle weakness and significantly elevated serum levels of creatine kinase, they usually response well to treatment and have a favor prognosis. Previous study founded that the serum anti-Mi-2 level of dermatomyositis patients decreased after rituximab (RTX) treatment, and the decline of serum anti-Mi-2 level over time was related to the decrease of creatine kinase level and Physician Global Assessment (PGA) score and the increase of manual muscle testing (MMT8) but not related to the Patient Global Assessment score, extra-muscular score, and HAQ [5]. In a large Chinese cohort, the serum anti-Mi-2β levels of dermatomyositis patients were positively correlated with the creatine kinase level and PGA score and negatively correlated with MMT8. The antibody level can decrease but will not be negative after treatment [6].

Anti-NXP2

Anti-NXP-2 appears in 3–24% dermatomyositis patients [4■]. Anti-NXP2-positive patients present distinct clinical phenotype. It has an increased risk of calcinosis in children and young adult patient, whereas malignancy in elderly patients. Few studies investigated the relationship between the antibody levels and disease activity. Yang et al. developed an ELISA kit in-house and confirmed by immunoprecipitation assay to determine the serum levels of anti-NXP2 and its clinical association in a single adult IIM cohort [7]. They reported that the serum anti-NXP2 level was closely associated with creatine kinase levels, muscle weakness, and PGA score in the patients without calcinosis. After treatment, the antibodies of these patients can decrease to normal when disease remission and increase again when

disease relapse. However, no correlation between the antibody levels and disease activity was observed in those patients with calcinosis. These findings suggested that the anti-NXP2 antibodies may play different roles in the pathogenesis of dermatomyositis with and without calcinosis, and monitoring antibody levels may help for evaluating the efficacy of treatment.

Anti-MDA5

Anti-MDA5 antibody can be detected in about 13–30% of dermatomyositis patients [4■]. It is highly correlated with interstitial lung disease (ILD), especially with rapid progression of ILD (RP-ILD) in Asian patients, which was associated with high mortality and poor prognosis. Early studies from Japan have showed that the serum antibody levels of dermatomyositis patients at onset of disease were not associated with mortality rate. However, the serum anti-MDA5 levels in survived patients would decline to normal after treatment. Antibody levels in dead group did not decrease with time, which indicated that monitoring the serum anti-MDA5 level can be a useful tool for predicting the prognosis of dermatomyositis patients during follow-up [8]. Another study reported by Shirakashi et al. also showed that the serum anti-MDA5 levels of dermatomyositis patient with exacerbation of respiratory function were strikingly reduced after 6 months of plasma exchange therapy, and these patients had a higher survival rate than those without PE treatment [9]. A recent study from a Chinese cohort revealed that the increased levels of anti-MDA5 IgG1 and IgG3 isoforms in dermatomyositis patients were associated with high risk of mortality, which suggested that more than one isotype of anti-MDA5 antibody may be involved in the pathogenesis of this condition [10].

Anti-TIF1-γ

Anti-TIF1-γ antibody appears in about 10–20% of dermatomyositis patients [4■]. Previous consistent studies have identified that anti-TIF1-γ antibody was the strongest predictor of malignancy in adult dermatomyositis patients. Early study to investigate the serum levels of anti-TIF1-γ in small sample of adult and juvenile patients with dermatomyositis found that antibody levels can decline after treatment [11]. In a clinical trial of RTX in the treatment of refractory myositis demonstrated that the serum levels of anti-TIF1-γ of dermatomyositis patients before treatment were associated with MMT8, PGA score, and HAQ of patients. The change of antibody levels after RTX treatment was positively

correlated with the improvement of disease activity [5]. A subsequent study found that among four dermatomyositis patients, the serum anti-TIF1-γ levels of two patients without cancer declined to normal but did not decrease or even increased in two patients with cancer after myositis treatment [12]. A recent study involving 30 cancer-associated myositis (CAM) and 50 dermatomyositis patients without cancer (non-CAM) demonstrated that the serum anti-TIF1-γ levels could decrease in both CAM and non-CAM groups after myositis treatment, and the decrease of antibody levels was positively correlated with the change of MMT8, creatine kinase level and PGA score [13■■]. In addition, the serum antibody levels were correlated with histopathological score of muscle biopsy in CAM patients but not in non-CAM patients. Non-CAM patients present typical dermatomyositis pathological pattern more such as perifascicular atrophy, whereas CAM patients had polymorphological features including perifascicular atrophy, predominant necrotic muscle fibers, nonspecific myositis, and even normal muscle tissue. However, although CAM patients had higher creatine kinase levels and more severe muscle weakness than non-CAM patients, no significant difference of serum anti-TIF1-γ levels was observed between patients with and without cancer in this study. Interestingly, this study found that the survival rate of patients with significantly increased anti-TIF1-γ level (three times higher than cut-off value) was much lower than those with mildly increased antibody level in CAM patients. However, no statistically significant difference of survival rate between anti-TIF1-γ levels and prognosis of patients was observed in non-CAM group [13■■]. Another cohort study involved 36 patients with CAM also showed that the anti-TIF1-γ levels can decline and tend to be negative when disease is in remission after myositis treatment [14]. These findings suggested that monitoring of serum anti-TIF1-γ levels is useful to estimate the disease activity and predict the prognosis of dermatomyositis patients with or without cancer.

Anti-SAE

Anti-SAE is a rare DM-MSA that occurs in less than 10% of the adult IIM patients [4■]. Only a few cases reported the relationship between the serum levels of anti-SAE and disease activity. A cohort study from the Chinese population showed that among six anti-SAE positive patients with dermatomyositis, the antibody levels of four patients continued to decline after treatment, and the disease activity including PGA muscle score decreased at the same time. The serum anti-SAE levels of the other two patients increased again during the follow-up [15]. The relationship between anti-SAE

levels and disease activity needs to be further clarified in a large cohort.

MACROPHAGE ACTIVATION-RELATED BIOMARKERS IN DERMATOMYOSITIS

Earlier studies have revealed increased serum levels of a macrophage activation marker, soluble CD163, in dermatomyositis patients [16,17]. Serum levels of soluble CD163 was found to be associated with disease activity of polymyositis and dermatomyositis patients in cross-sectional study and decrease with disease activity scores after treatment in longitudinal study [16]. Recently, additional macrophage activation markers, including CD206 [18,19] and neopterin [20■■], were also found to be significantly increased in the sera of dermatomyositis patients. Of note, high levels of serum neopterin was found to be associated with pulmonary function impairments and disease activity scores in dermatomyositis patients. Further prognosis study identified high-serum neopterin concentration as an independent risk factor for poor prognosis in dermatomyositis [20■■]. Another study conducted by our group revealed elevated serum levels of galectin-9 in dermatomyositis patients compared with IMNM patients and healthy controls; and histologically, galectin-9 was found to be mainly expressed by macrophages in the lung tissue of dermatomyositis patients [21]. Vitro studies uncovered that galectin-9 may promote the release of inflammatory mediator CCL2 by lung fibroblast, suggesting a role of galectin-9 in the pathogeneses of dermatomyositis-associated ILD [21]. Interestingly, another member of galectin family, galectin-3, was also found to be significantly increased in myositis sera compared with healthy controls, especially in myositis patients who are complicated with ILD and correlated with radiological lung disorder and treatment response [22]. These findings indicate that macrophage activation markers may serve as promising biomarkers for disease activity and prognosis of dermatomyositis patients, and the findings also highlight the potential role of macrophage activation in the pathogenesis of dermatomyositis.

CIRCULATING T-CELL SUBSETS AS DISEASE ACTIVITY IN DERMATOMYOSITIS

In the peripheral blood of polymyositis and dermatomyositis patients, CD28null T cells were more frequently compared with healthy controls, and T-cell infiltrates in myositis muscles were dominated by CD28null T cells [23]. Further studies have demonstrated that CD28null T cells contribute to muscle fiber damage through perforin-dependent

and HLA-regulated mechanisms [24]. These studies imply that T-cell subsets with specific phenotypes may be involved in the pathogeneses of myositis.

Moreover, several studies attempted to identify new T-cell subsets in myositis patients and correlated the subsets' abnormalities with disease activities. Zhang *et al.* [25] investigated a Tfh cell-like circulating T-cell subsets, CD4+CXCR5+CCR7loPD-1hi T cells, in polymyositis and dermatomyositis patients and found that this subset of T cells was significantly increased in polymyositis/dermatomyositis patients. It has also been revealed that higher percentages of CD4+CXCR5+CCR7loPD-1hi T cells were associated with higher disease activity scores assessed by PGA and higher serum creatine kinase levels. In addition, the percentage of circulating CD4+CXCR5+ CCR7loPD-1hi T cells decreased significantly after successful treatment [25]. These findings indicate that CD4+CXCR5+CCR7loPD-1hi T cells may be a disease activity marker in polymyositis/dermatomyositis patients.

More recently, our group examined the expression of TIGIT and CD226 on T cells of dermatomyositis patients and identified a subset of CD4 T cells with co-expression of TIGIT and CD226, showing enhanced effector function and proliferating ability [26■]. The portion of the TIGIT+CD226+ CD4 T cells was significantly expanded in dermatomyositis patients, especially in those with ILD, and they were closely related to disease activity and decreased significantly in remission after treatment. Of note, the effector function of this T-cell subpopulation could be successfully suppressed by CD226 antibody blocking [26■]. Therefore, these results suggested that TIGIT+CD226+ CD4 T cells may be a disease activity marker in dermatomyositis patients. Further studies on this subset of T cells may uncover therapeutical potential of dermatomyositis targeting TIGIT/CD226 axis.

LOW-DENSITY GRANULOCYTES AND NEUTROPHIL EXTEACELLULAR TRAPS

Neutrophil extracellular traps (NETs) play a significant role in immune defense against pathogens, and they could also result in inflammation because of the proinflammatory nature of NET components. Low-density granulocytes (LDGs) are a subpopulation of neutrophil with lower density compared with normal neutrophils. LDGs have increased capacity to synthesize NETs and type I IFNs [27,28], and thus display significant pathogenic features. It has been observed that enhanced formation and impaired degradation of NETs in polymyositis and dermatomyositis patients are associated with ILD [29], and the percentage of LDGs in peripheral blood mononuclear cells is correlated with lung disease activity in dermatomyositis patients [30]. Peng *et al.* [31] also reported higher levels of cfDNA and LL-37 in dermatomyositis patient with ILD, and a positive correlation between cfDNA and KL-6 was also revealed. Torres-Ruiz *et al.* [32] demonstrated higher levels of LDGs and NETs in myositis patients with active disease. Furthermore, the correlations of the presence and levels of LDGs and NETs with myositis disease activities were confirmed in a United States cohort [33]. Collectively, current literature reports suggest that LDGs and NETs-related markers may serve as biomarkers for dermatomyositis disease activities. In addition, Zhang *et al.* [34] provided evidence showing that NETs could induce myositis-associated ILD by promoting the proliferation and differentiation of lung fibroblasts. Interestingly, Seto *et al.* [33] found that NETs isolated from myositis patients could cause skeletal myotube cell death, which is attributed to a toxic effect mediated by citrullinated histone H4, indicating that NETs may also contribute to the direct tissue damage in myositis.

MYXOVIRUS RESISTANCE PROTEIN A EXPRESSSION AS A MUSCLE DISEASE ACTIVITY MARKER IN DERMATOMYOSITIS

A prominent feature of dermatomyositis patients is the overactivation of the type 1 interferon (IFN) pathway [35]. The type 1 IFN signature score was found to be highly correlated with disease activity [36–38]. *Myxovirus resistance protein A (MxA)* as a gene specifically regulated by type-1 IFN was reported to be highly expressed in the muscle of dermatomyositis patients, especially in the perifascicular myofibers [39]. More recent studies evaluating the diagnostic value of MxA expression in muscle biopsy for dermatomyositis and resulting data suggested that sarcoplasmic MxA expression examined by immunohistochemistry is a sensitive diagnostic marker of dermatomyositis [40,41]. In addition, studies have also revealed that the expression of MxA in dermatomyositis is correlated with muscle disease activities in juvenile dermatomyositis patients [42,43]. It requires further investigations to clarify whether MxA expression levels could reflect the severity of muscle involvement in adult dermatomyositis.

CONCLUSION

Advances in diagnosis and treatment for dermatomyositis have underlined the unmet clinical needs in evaluating the disease activity. Several promising biomarkers addressing the assessment of disease activity have been identified in preliminary studies. They provide clinicians with new ways to differentiate disease activity from the damage and assist

with management in dermatomyositis. Moreover, further clinical studies are required to confirm the validation of these biomarkers in large prospective cohorts.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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Recent advances in elucidating the genetic basis of systemic sclerosis

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Purpose of review

Systemic sclerosis (SSc) is a complex autoimmune disorder that affects the connective tissue and causes severe vascular damage and fibrosis of the skin and internal organs. There are recent advances in the field that apply novel methods to high throughput genotype information of thousands of patients with SSc and provide promising results towards the use of genomic data to help SSc diagnosis and clinical care.

Recent findings

This review addresses the development of the first SSc genomic risk score, which can contribute to differentiating SSc patients from healthy controls and other immune-mediated diseases. Moreover, we explore the implementation of data mining strategies on the results of genome-wide association studies to highlight subtype-specific HLA class II associations and a strong association of the HLA class I *locus* with SSc for the first time. Finally, the combination of genomic data with transcriptomics informed drug repurposing and genetic association studies in well characterized SSc patient cohorts identified markers of severe complications of the disease.

Summary

Early diagnosis and clinical management of SSc and SSc-related complications are still challenging for rheumatologists. The development of predictive models and tools using genotype data may help to finally deliver personalized clinical care and treatment for patients with SSc in the near future.

Keywords

genetics, genomic risk score, personalized medicine, polymorphisms, systemic sclerosis

INTRODUCTION

Systemic sclerosis (SSc) is a chronic immune-mediated disease (IMD) characterized by persistent immune imbalance, severe vascular damage and progressive fibrosis of the connective tissue [1,2]. Patients suffering from SSc are mostly women (approximately in a ratio of four women to one man) and show heterogeneous manifestations of the disease. These patients can be classified as suffering from limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc) depending on the extent of their fibrosis [3] or according to their serological status as anticentromere (ACA), antitopoisomerase (ATA) or anti-RNApol-III (ARA) auto-antibody positive [4,5]. The SSc clinical and serological subtypes are known to be correlated with disease complications and prognosis [6]. Although the cause of SSc is unknown, it is clear that genetic susceptibility factors have an essential role in the onset and the evolution of the disease [7–9]. In the last 15 years, in addition to the known associations of classical HLA class II haplotypes, several multicentre studies have interrogated the genome of thousands of

patients with SSc to identify genetic risk factors and up to 32 non-HLA loci have been clearly pinpointed as relevant for SSc [10–15]. Nevertheless, the actual causal variants and the functional mechanisms responsible for the majority of the reported association signals are yet to be defined. Furthermore, there has been no implementation of genetic markers into the clinic so far.

Recent studies have applied innovative analyses to the available genomic information or generated multiomic datasets to advance in the molecular

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causes of SSc and to better characterize the clinical or serological subtypes of SSc patients. In this review, we will analyse the new insights into SSc pathogenesis provided by these novel approaches and the promising chances of future advances in early diagnosis and patient-care that they hold.

DATA-MINING OF GENOME-WIDE ASSOCIATION STUDIES IN SYSTEMIC SCLEROSIS

Genome-wide association studies (GWAS) analyse the frequency of common genetic variants in hundreds of thousands of genomic positions located throughout the whole genome and compare large cohorts of cases and controls [16]. This information is analysed in a hypothesis-free fashion and the identified genetic association signals need to be later explained in the context of the disease [17,18]. The GWAS strategy is a very powerful tool to study the genetic basis of complex diseases, in which tens of different loci contribute very modestly to increase the risk of developing a specific condition [16]. GWAS have been fruitful in the analysis of IMDs, and they have not only greatly contributed to clarify the genetics of immune-imbalance and loss-of-tolerance, but also to identify possible drug targets [19–21]. However, despite the value of the GWAS findings, it has been proven hard to implement genetic susceptibility markers into medical routine [16,17]. Therefore, we will address recent studies that have made the most out of the available SSc genomic datasets by using novel analysis methods to identify

individuals at a high risk, to help differential diagnosis and to predict disease progression.

GENOMIC RISK SCORE AND CELLULAR PARAMETERS IDENTIFY INDIVIDUALS AT A HIGH RISK FOR SYSTEMIC SCLEROSIS

Genomic or genetic risk scores (GRS), also known as polygenic risk scores (PRS), estimate the risk of a specific individual to suffer from a disease based on the cumulative effects of different genetic risk variants that contribute modestly to disease susceptibility [22,23].

A number of GRS or PRS have been developed for immune-mediated diseases during the last years. Several reports have shown relevant advances in the estimation of disease risk, clinical manifestations or treatment response in different IMDs, such as rheumatoid arthritis [24–27], systemic lupus erythematosus [28,29], psoriasis [30], Kawasaki disease [31], Takayasu arteritis [32] and sarcoidosis [33].

In 2021, our group developed the first GRS for SSc [34▪]. The allelic effect estimates were obtained thanks to a previously published meta-analysis of several SSc GWAS studies (meta-GWAS) [13], which included 9095 SSc patients and 17 584 nonaffected controls from 14 populations with European ancestry. Thousands of GRS models were tested in a totally independent cohort integrated by 400 SSc cases and 571 controls. Finally, the best-fitting model, which included 33 independent single-nucleotide polymorphisms (SNPs), was able to correctly separate SSc cases from nonaffected controls in 67% of the instances (specificity = 0.76, sensitivity = 0.51, accuracy = 0.66). As expected, patients with SSc showed the highest GRS values and the individuals who ranked in the 95th GRS percentile had a five times higher relative risk than the average to be diagnosed with SSc.

Despite these positive results, the most common scenario in clinical practice is to provide individuals with SSc-related symptoms with a diagnosis or to manage an already diagnosed condition. This task can be very challenging due to the overlapping symptomatology between multiple IMDs or even between the different SSc subtypes. Regarding differential diagnosis, the SSc GRS showed interesting results, as it was useful to discriminate between patients with SSc and others suffering from Sjogren's syndrome [area under de curve AUC = 0.59 (0.55–0.6)] or rheumatoid arthritis [AUC = 0.57 (0.53–0.61)] and to marginally differentiate SSc from systemic lupus erythematosus cases [AUC = 0.55 (0.51–0.59)].

Unfortunately, this model was not able to correctly classify patients with SSc into the dcSSc or lcSSc subsets or to predict the presence of SSc-related

autoantibodies or pulmonary fibrosis. Therefore, the new GRS models using the allelic effect estimates from the comparisons between dcSSc and lcSSc or between ACA+ and ATA+ patients were generated. These clinical or serological subtype specific GRSs involved thousands of SNPs and showed an increased power to correctly classify ACA+ and ATA+ patients [AUC = 0.69 (0.61–0.78)], probably due to the strong effects of the HLA region. On the contrary, the clinical subtype GRS did not represent a significant improvement in the classification of lcSSc versus dcSSc.

It should be highlighted that the patients recruited for the GRS development cohort also participated in a deep phenotyping initiative [Reference PRECISESADs], which allowed us to combine the SSc GRS with tens of peripheral blood cell composition parameters into a multivariate model. Interestingly, this multivariate model, which integrated the GRS with other variables, such as age and the frequency of different immune cells [memory B cells, resting natural killer (NK) cells, M0 macrophages and activated dendritic cells] in the blood, increased the predictive power to encouraging AUC values [AUC discovery = 0.85 (0.81–0.88) and AUC test = 0.79 (0.73–0.84)].

The proposed model still needs to be validated in a different cohort with genotype information for the selected variants and, if possible, with cytometry-based peripheral blood cell estimates. Moreover, the performance of the model varied slightly depending on the geographical origin of the patients, which suggests that it might not be directly applicable to different ancestry groups. Nevertheless, we are confident that this work represents a key step in the process of translating genetic findings into early diagnosis, disease progression prediction or pharmacogenomics for patients with SSc (Fig. 1).

NEW INSIGHTS INTO THE HUMAN LEUKOCYTE ANTIGEN REGION REVEAL SUBTYPE-SPECIFIC ASSOCIATIONS AND A NOVEL ASSOCIATION OF HUMAN LEUKOCYTE ANTIGEN CLASS I WITH SYSTEMIC SCLEROSIS

The human leukocyte antigen (HLA) *locus* was the first genetic risk factor to be firmly identified as a risk factor for SSc [35–37]. The HLA genetic region encodes the proteins that form the antigen presenting complex, which has a key role in immune-mediated diseases. Reassuringly, all the SSc GWAS have
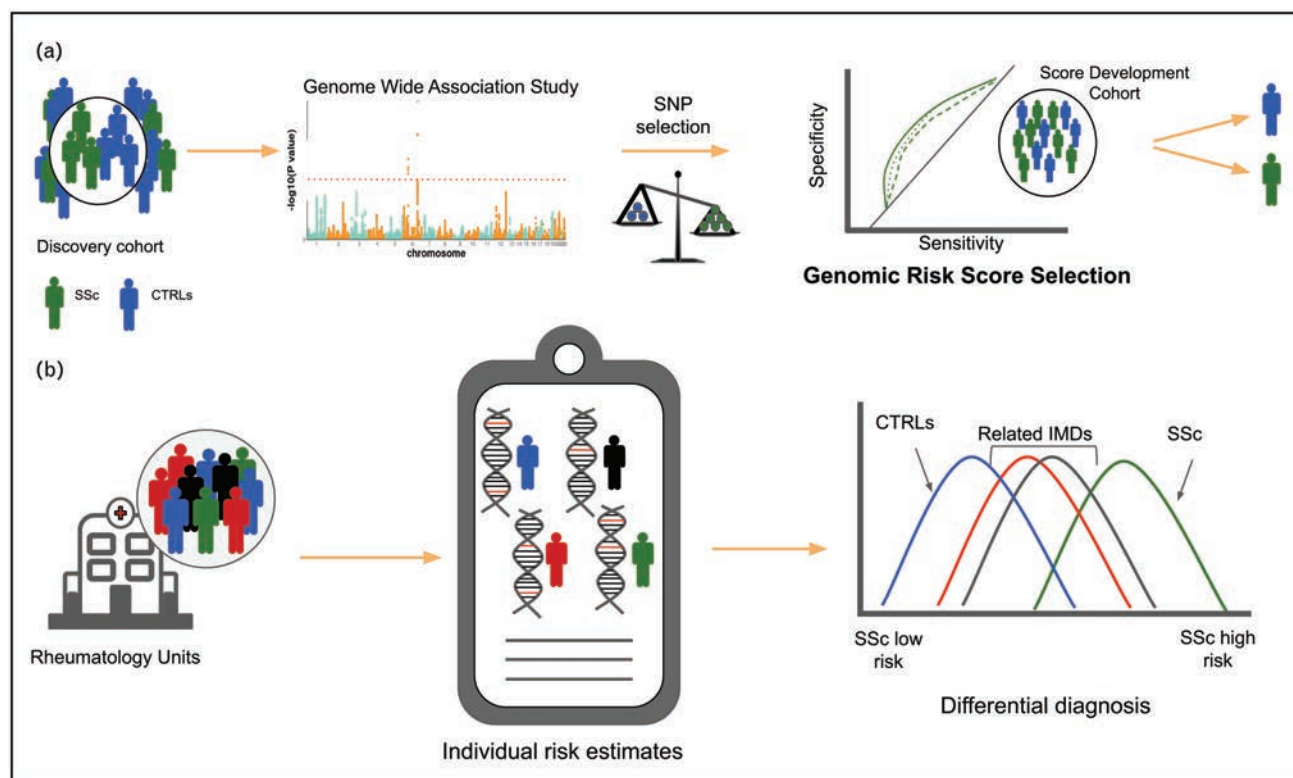
FIGURE 1. Schematic representation of the development of a genomic risk score and its implementation in the clinical management of systemic sclerosis patients. a) Selection of independently associated SNPs from a GWAS in a discovery cohort, followed by the selection of the best-fitting model in a score development cohort. b) Score calculation for each patient and classification into high or low risk for SSc.

confirmed the initial genetic findings and the most significant genetic associations with the disease correspond to specific HLA class II haplotypes [10,12,13]. Moreover, recent studies confirmed that the inheritance of HLA alleles is key in the sharing of serological and disease characteristics in familial SSc cases [38]. Nevertheless, the HLA *locus* is the most polymorphic region in the human genome and shows intricate disequilibrium patterns that require a complex methodology for analysis and is often ignored in GWAS analyses.

In order to provide a renewed insight into the HLA region, our group revisited the genetic information of the HLA region [39■] from the previously mentioned SSc meta-GWAS [13]. The genetic associations in the extended HLA region were analysed under logistic regression models, but they singled-out the independent association signals in this *locus* by iteratively conditioning the models on the most associated variants. The analysis including all the patients with SSc yielded a total of nine independent SNPs that explained the association of the complete locus. Two of these SNPs corresponded to synonymous changes in the *HLA-DQA1* gene and one to a stop mutation in the *HLA-DPB1* gene. Moreover, up to eight classical HLA class II alleles and one allele in the HLA class I were confirmed as genetic susceptibility factors at the genome-wide significance level ($P < 5 \times 10^{-8}$). Moreover, when the clinical and serological subsets were considered, two HLA class II alleles, *HLA-DQA1∗02:01* and *HLA-DQA1∗05:01*, were associated exclusively with lcSSc and dcSSc, respectively. Similarly, the *HLA-DRB1∗08:0*1 and *HLA-DRB1∗07:01* alleles acted as risk alleles only for patients with ACA+ serology, while the associations of the *HLA-DPA1∗02:01* and *HLA-DQB1∗03:01* alleles were restricted to dcSSc. Finally, it was shown that *HLA-DRB1∗11:04* was only associated with the ARA+ disease. Taking into account differences in the disease progression between the different SSc subsets, these novel findings open the way for further implementation of the HLA alleles as noninvasive biomarkers of disease severity.

Despite previous suggestive association reports, Acosta-Herrera *et al.* [39■] showed a firm association of the HLA class I region with SSc, for the first time. The reported risk allele, *HLA-B∗08:01*, is highly correlated with the presence of an Asp residue in the 9th position of the HLA-B molecule, inside the peptide-binding groove, and it might have relevance in the antigen presentation. The association of HLA class I alleles with SSc points towards a new insight of the pathogenesis, as HLA class I molecules are not only recognized by CD8+ T cells [40] but also by killer immunoglobulin-like receptors (KIR) expressed by CD8+ T cells and by NK cells [41–43].

In this line, according to a recent meta-analysis, the highly polymorphic KIR gene cluster is not likely to largely contribute to SSc on its own [44]. However, Hanson *et al.* [45■] very recently conducted a large analysis of KIR-HLA epistatic interactions in SSc. This analysis confirmed several associations of HLA class II and class I alleles (*HLA-B∗44:03-HLA-C∗16:01*) with SSc risk, a novel with ATA+ disease (*HLA-DRB1∗15:01*). In addition, the authors explored the coinheritance of KIR alleles and HLA class I alleles. They found that patients with SSc who were carriers of the *HLA-C∗16* allele inherited *KIR2DL3* (a strong inhibitor of NK cell degranulation) less than controls. However, *KIR2DL3* was more frequent in patients with SSc if they carried the *HLA-C∗04* allele, which binds the KIR2DL3 receptor less efficiently. Moreover, the coinheritance of *HLA-Bw4* and a compatible inhibitory receptor, *KIR3DL1*, had a protective effect. In contrast, the inheritance of *HLA-C1* alleles but not its inhibitory receptor, *KIR2DL3*, increased the risk of developing SSc.

Altogether, these recent evidences have extended the scope of the HLA research towards a more sophisticated estimation of the contribution of this complex *locus* to SSc risk and further support the HLA as a highly valuable locus to predict disease prognosis.

ANALYSIS OF EXPRESSION QUANTITATIVE TRAIT LOCI SUGGEST 10 DRUG TARGETS FOR SYSTEMIC SCLEROSIS

Next-generation sequencing (NGS) costs have constantly decreased over the years and, finally, it is possible to characterize different layers of genetic, epigenetic and transcriptomic information for cohorts of patients with SSc of considerable size.

On this front, we combined GWAS genotyping data and whole transcriptome information of peripheral blood immune cells, obtained using RNA-sequencing (RNA-seq) techniques, from 333 patients with SSc and 524 controls [46■]. Our group exploited this unique dataset to identify genetic variants that controlled the expression of nearby genes, that is to define expression quantitative trait loci (eQTLs) in SSc. By these means, 4539 SNPs with a suggestive allelic association with SSc ($P < 1 \times 10^{-5}$) had an impact on the expression of 565 nearby genes (cis eQTLs) in immune cells. Furthermore, 105 (45%) of the eQTL genes showed altered expression patterns in the blood cells, skin or lung tissue of SSc patients. This approach was supplemented with a drug target prioritization amongst approved medications for immune-mediated diseases, which brought up nine druggable targets. Remarkably, only three of them

have been part of SSc clinical trials, which opens the door for future drug repurposing for patients with SSc.

The characterization of a SSc-specific transcriptomic profile and the relation between the identified genetic risk factors and these altered gene expression patterns is a necessary progression towards prediction and monitoring of disease progression and response to treatment.

STUDYING PATIENTS WITH DISEASE COMPLICATIONS AND NON-EUROPEAN ANCESTRY TO IDENTIFY NEW GENETIC RISK FACTORS FOR SYSTEMIC SCLEROSIS

Following the goal of identifying patients who are prone to develop severe disease complications, the researchers in the SSc field have done tremendous efforts to recruit and to comprehensively characterize hundreds of patients with specific disease symptoms or complications. These sets of patients are essential to study specific genetic markers that will help to personalize the monitoring and management of SSc in each patient.

SSc-associated interstitial lung disease (SSc-ILD) is one of the most dangerous consequences of SSc and several SNPs have been reported as genetic markers of SSc-ILD (*IRF5, STAT4, CD226* and *IRAK1*), although most of them remain controversial. A recent study, which included 394 SSc patients with SSc-ILD and 218 SSc patients without SSc-ILD and 503 nonaffected controls, reported that only one SNP located in the *STAT4 locus*, rs7574865, showed significant differences between patients with and without SSc-ILD [47]. In addition, rs2235611, a SNP located in the promoter of the mRNA-binding protein serine/arginine protein 55 (SRp55 or SRSF6) locus, was found to increase the risk to develop SSc-ILD and nailfold videocapillaroscopy abnormalities in an Italian cohort (414 SSc patients and 458 controls) [48]. Two loss-of-function rare mutations in *TNIP2* and *TRAF2* were identified using whole-exome sequencing (WES) on families with patients affected with pulmonary arterial hypertension (PAH) with interatrial communication and SSc [49]. Finally, a pharmacogenomic study in the context of SSc-ILD was performed to check if a SNP in *MUC5B*, rs35705950, influences the response to immunosuppression with cyclophosphamide and mycophenolate [50]. Nevertheless, no effect of this genetic variant on pulmonary parameters was observed.

Another well known SSc complication is the scleroderma renal crisis (SRC), which is more frequent in ARA+ patients [51]. A GWAS of an ARA+ cohort of 99 SSc patients affected and non-affected by SRC showed suggestive associations of one SNP in the *POU2F1 locus* (rs2093658), one in *CTNND2* (rs1859082), one in *HECW2* (rs16849716) and one in *GPATCH2L* (rs935332) [52]. The former association was confirmed in a replication cohort comprising 256 additional ARA+ patients and an increased expression of the GPATCH2L and CTNND2 proteins during SRC was also reported [52].

An interesting finding was also found through the characterization of patients with both IMDs and sex-chromosome trisomies. A study by Scofield *et al.* [53] revealed an association of the Klinefelter syndrome (47, XXY) with SSc and idiopathic inflammatory myopathies.

Apart from these analyses of specific SSc-related phenotypes, to identify the missing heritability of the disease and to narrow down known genetic association signals, it is very important to extend the study of SSc to non-European populations. Last year, Liu *et al.* [54] analysed a Chinese Han cohort (343 patients with SSc and 694 controls) and replicated the previously reported associations of rs117026326 and rs73366469, located in the *GTF2I locus*, with SSc in this Asian population. Moreover, they described a suggestive association of *NFKB1* rs1599961 with SSc and found no association between *TYK2* rs2304256 and SSc in Chinese Han.

These novel or phenotype-specific associations still need to be replicated in independent and statistically powerful studies, but they will be key to understanding the genetic basis of patient heterogeneity, the interpopulational differences and the observed diversity of the clinical manifestations of SSc.

CONCLUSION

The characterization of the genetic basis of SSc, and other IMIDs, started with the identification of genetic regions associated with the disease. The characterization of the associated *loci* provided priceless knowledge about the physiopathology and biological processes involved in SSc. Nevertheless, the majority of the identified susceptibility factors did not have a straightforward explanation. Therefore, they could not be directly used to inform diagnosis or to help in the clinical management of SSc.

Recent reports made the most out of the available genomic datasets and moved forward the translation of genetic biomarkers into real clinical tools to help early and differential diagnosis of SSc. Moreover, in the last months, we have observed significant contributions of genetic analyses to the identification of relevant biological processes for SSc and to suggest possible drug targets.

Nonetheless, there is still a lack of integration of multiple layers of genomic, epigenomic and transcriptomic information into a systems biology setting to characterize each SSc patient and address their care and treatment in a personalized way. Novel technologies, such as nuclear DNA conformation analysis [55], characterization of alternative RNA splicing, noncoding RNAs or single cell RNA-sequencing [56–61] are being successfully implemented in SSc. The combination of all these sources of information with the SSc genetic risk factors will certainly contribute to breaking down the events and cellular settings that lead the SSc. Therefore, we are positive that future genetic research will contribute importantly to diagnosis and treatment of SSc, with the ultimate goal of relieving suffering in patients affected by this disease.

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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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Scleroderma autoantibodies in guiding monitoring and treatment decisions

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Purpose of review
One of the key clinical challenges of systemic sclerosis (SSc) is diversity in clinical presentation, organ involvement and disease progression. Antinuclear autoantibodies (ANA) are central to the diagnosis of SSc. ANA specificities associated with distinct clinical patterns of organ and skin involvement. Understanding of the molecular differences and pathogenesis of scleroderma has helped further inform clinical acumen. Here, we provide an update on ANA on clinical profiling, management and future direction of SSc.

Recent findings
There has been further development in delineating clinical patterns in ANA, genetic susceptibility and antigen triggers predisposing to ANA subtypes. Sub-group analysis of recent clinical trials shows differing treatment responses to novel therapeutics.

Summary
ANA subtyping is likely to be firmly embedded into future classification systems. Beyond informing current management and monitoring of scleroderma patients, ANA subsets have implication on future research and clinical trial design.

Keywords
antinuclear autoantibodies, connective tissue disease, systemic sclerosis

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune condition with substantial clinical and serological heterogeneity. Antinuclear autoantibodies (ANA) are a spectrum of autoantibodies that react with various nucleolar and cytoplasmic components of normal human cells. They are integral to scleroderma the diagnosis, subtype classification, and prognostic evaluation. ANA are present in 90% of scleroderma patients [1].

The 'classical' ANA subtypes in SSc are the anticentromere antibodies (ACA), antitopoisomerase-1 antibodies (ATA; anti-Scl-70), anti-RNA polymerase III antibodies (ARA). Collectively, these antibodies are found in 50–80% of scleroderma patients [2,3■]. ANA associated with SSc are mutually exclusive and specific for SSc. Antibodies associated with scleroderma overlap syndromes, such as anti-Pml/Scl and anti-Ku are less specific for scleroderma but remain mutually exclusive [3■]. Patients do not switch ANA subset type throughout their disease duration.

Over the recent years, advances in collaborative practice and genetic analysis have further improved our understanding of these distinct clinical patterns.

This review focuses on the principal differences in ANA profiles, mechanisms of pathogenicity, and impact on management.

CLINICAL PHENOTYPE BY ANTINUCLEAR AUTOANTIBODIES SUBTYPES

The clinical phenotypes of antibody subtypes have been summarized in Table 1.

Anticentromere antibodies

ACA, targeting centriole proteins are the most common autoantibodies found in SSc [1]. ACA seropositivity is a positive prognostic marker with an overall

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increased survival 5–20 years postdiagnosis and reduced incidence of scleroderma renal crisis (SRC), cardiac scleroderma and scleroderma associated interstitial lung disease (SSc-ILD) [3■,4]. ACA positivity is associated with calcinosis, digital ischemia with digital tip ulcerations and esophageal dysmotility (80%) [3■,4,5]. The most serious complication of ACA positivity is increased incidence of pulmonary arterial hypertension (PAH) [3■,6].

ACA is typically associated with limited cutaneous scleroderma (lcSSc). However, a small percentage of ACA positive patients (5–7%) are within the diffuse cutaneous subset (dcSSc) [7]. Comparing ACA positive dcSSc to ACA negative dcSSc, ACA positivity was associated with lower incidence of organ-based complications and improved survival, evidencing its protective effect on phenotype [7]

Antitopoisomerase antibodies

ATA are the second most common ANA and are associated with poor prognosis [3■]. ATA have a propensity towards diffuse cutaneous involvement and higher incidence of significant SSc-ILD (80%) regardless of cutaneous subtype [3■,8]. PAH incidence is decreased compared to overall scleroderma population [3■,6]. In dcSSc, ATA positivity is a negative prognostic factor with dcSSc ATA-positive patients having the worst prognosis and lowest survival rate of all SSc patients. A large cohort study found that ATA positive lcSSc patients have the second highest survival rate behind ACA-positive patients [3■]. Although incidence rates of SRC are not as pronounced relative to ARA, ATA seropositivity is associated with higher mortality rates in SRC scleroderma [9].

Anti-RNA polymerase 3 antibodies

ARA positivity occurs almost exclusively in the diffuse cutaneous subtype and associated with severe skin involvement and a 10-fold increase in SRC [3■]. Modified Rodnan Skin Score (MRSS) peak occurs earlier and in higher values relative to ARA but is also associated with faster improvement [3■,10■]. ARA seropositivity is one of the strongest risk factors for Gastric antral vascular ectasia (GAVE) with a 4–5 greater fold risk of GAVE in ARA positive patients compared to overall SSc [11–12]. ARA positivity is associated with lower prevalence of cardiac scleroderma and SSc-ILD [3■]. ARA positive patients have a 4–7-fold increased risk of developing cancer within 6 months to 5 years after SSc onset, the highest amongst all ANA subsets [13,14].

Antifibrillarin (anti-U3RNP)

Anti-U3RNP positivity is associated with the highest incidence of both PAH and cardiac involvement in SSc [3■]. A distinct feature of Anti-U3RNP is non-inflammatory skeletal myopathy [15]. Anti-U3RNP is associated with poor prognosis mainly due to its association with early severe organ involvement [16]. In early scleroderma, this antibody is associated with very high mortality rates, however, long-term survival rates in anti-U3RNP positive patients were higher compared with anti-U3RNP negative SSc [3■]. Anti-U3RNP is also strongly associated with severe gastrointestinal (GI) involvement that includes gut malabsorption and pseudo-obstruction [16].

Anti-Th/To antibodies

Anti-Th/To antibodies are associated with limited cutaneous involvement and esophageal dysmotility [8]. Diagnosis delay is usually reduced due to shorter duration between Raynaud's and first non-Raynaud's symptom onset [3■]. Anti-Th/To is associated with significant SSc-ILD and PAH, which occur early in disease course [8]. LcSSc patients with anti-Th/To positivity have higher pulmonary involvement compared to overall lcSSc [17,18]. A recent case–control study of Th/To SSc, the largest to date, showed a PAH incidence rate of 38% in Th/To positive SSc patients [18].

Anti-U11/U12RNP antibodies

Anti-U11/U12RNP is associated with high incidence of PF (>80%) and severe gastrointestinal involvement [9,19]. SSc-ILD in anti-U11/U12 positive patients is severe and rapidly progressive with a 2.25-fold greater risk of death or lung transplant in SSc-ILD patients [19]. Interestingly, overall survival rates are

Table 1. Clinical phenotype of antinuclear antibodies associated with systemic sclerosis [3■,4–9,10■,11–33]

Antibody	ANA pattern	Intracellular target	Prevalence in SSc patients [9,30]	Cutaneous subtype propensity	GI involvement	PAH	Lung involvement	Oncology	Other
ACA	Speckled centromere	Centromeric nucleoproteins	28–37%	Limited (98%)	High prevalence of esophageal dysmotility (80%)	Increased risk	Reduced incidence	–	Associated with calcinosis, DU
ATA	Nucleolar/speckled or homogenous	Type I topoisomerase	20–30%	Diffuse sustained skin fibrosis	–	Moderately decreased risk	80% develop ILD of which up to 30–50% progress to severe ILD	3–5-fold increased risk of synchronous cancer	DU in early stages
ARA	Nucleolar/homogenous	RNA polymerase type 3	4–19%	Diffuse phenotype Severe Early skin progression followed by rapid improvement	Highest prevalence of GAVE		Lower risk of SSc-ILD	4–7-fold increased risk of cancer	10-fold increased risk of SRC Decreased rate to cardiac scleroderma
Antifibrillarin	Nucleolar/homogenous	Fibrillarin	1–8% (16–19% in AA)	Diffuse	Severe GI involvement	High risk			High risk of cardiac scleroderma Increased risk of myopathy
Anti Th/To	Nucleolar	Nucleolar 7–2/8–2 RNA–protein complex	2–5%	Limited	Esophageal dysmotility	Increased risk	50% develop of which 30% progress	Reduced risk	Less DU
Anti-U11/U12	Speckled	U11/U12 RNA polymerase complex	1–3%	Limited/diffuse	Severe GI involvement		80% develop often severe and rapidly progressive	3–5-fold increased risk of synchronous cancer	–
Anti-PM/Scl	Nucleolar	Nucleolar PM/Scl macromolecular complex	3–6% (25% of SSc-myositis overlap)	Limited can present without skin involvement		Decreased risk	35–87% develop Good functional outcome	–	Decreased risk of cardiac scleroderma and SRC Increased risk of myositis, inflammatory arthritis, calcinosis
Anti Ku	Speckled	Ku complex (p70/p80 heterodimer)	2% (15% of SSc-myositis overlap)	Limited	Decreased risk of GAVE	–	Up to 76% develop Good functional outcome	–	Lower incidence of Raynaud's, telangiectasia
Anti-U1 RNP	Speckled	Small nuclear ribonucleoproteins	5–35% (100% in mixed CTD)	Limited	–	Increased risk	35% develop 20% progress	–	Increased risk of inflammatory arthritis, myositis
Anti-EIF2B	ANA negative Cytoplasmic staining	Eukaryotic initiation factor-2B	<1%	Diffuse	–	–	High incidence Up to 100% develop	–	–

AA, Afro-American population; ACA, anticentromere antibodies; ARA, anti-RNA polymerase III; ATA, antitopisomerase I; CTD, connective tissue disease; DU, digital ulcer; GAVE, gastric antral vascular ectasia; GI, gastrointestinal; ILD, interstitial lung disease; SRC, scleroderma renal crisis; SSc, scleroderma.

equivalent to anti-U11/U12 negative SSc patients [3■,19]. Anti-U11/12 SSc patients have significantly increased rates of synchronous cancer diagnosis [13].

Anti-PM/Scl antibodies

Anti-PM/Scl antibodies are associated with scleroderma-myositis overlap syndrome [20]. This antibody is associated with a good prognosis with low incidence rates of SRC, PAH, and cardiac scleroderma [3■,20–22]. In contrast to other subsets the overall mortality rate of anti-PM/Scl in early stages of SSc is low but starts to increase after 10–15 years from onset [3■]. Pml/Scl antibodies are associated with increased incidence of ILD with good functional preservation [8]. The classical phenotype for anti-Pm/Scl SSc includes mild muscle involvement ILD, calcinosis and cutaneous dermatomyositis [20–22]. Anti-PM/Scl SSc is usually associated with limited cutaneous involvement and may often present without any skin involvement [22,23]. Analysis of the EUSTAR database has shown presence of muscle involvement is associated with more severe scleroderma with higher incidence of cardiac involvement, SSc-ILD, GI involvement, joint contractures, and tendon friction rubs [20,21]. Although a recent single center cohort suggested association of anti PM/Scl with increased sold organ malignancy and SRC, reminiscent of some cases of ARA SSc, this association was not confirmed in the multicentric EUSTAR analysis [20,21].

Anti-Ku antibodies

Anti-Ku antibodies are also associated with scleroderma myositis overlap with a lower incidence compared to anti-Pm/Scl (<2% overall SSc) [24,25]. They present similar to Pm/SCl positive patients with strong associations with myositis, limited phenotype, dermatomyositis skin rashes, and inflammatory arthritis [23]. Anti-Pm/Scl, anti-Ku is strongly associated with SSc-ILD with a good functional outcome, and they have a lower incidence of vascular manifestations (Raynaud's, telangiectasias, GAVE) [8,25]. Multiple case studies report Anti-Ku antibodies are associated with immune thrombocytopenic purpura and thrombocytopenia may be a precursor to anti-Ku antibody-related scleroderma–polymyositis overlap syndrome [26]

Anti-U1RNP antibodies

Anti-U1RNP phenotype is a mix of SSc, systemic lupus erythematosus (SLE) and polymyositis [8]. Patients with this antibody are usually classified as having mixed connective tissue disease (MCTD) but if a patient exhibits predominantly scleroderma symptoms than they are classified as scleroderma. Anti-U1RNP SSc is associated with younger onset, limited cutaneous subset, inflammatory arthritis, myositis and ILD [9]. Anti-U1RNP-SSc patients who develop PAH have worse prognosis than anti-U1RNP-SLE/MCTD patients [27]

Antinuclear autoantibodies negative extractable nuclear antigen negative scleroderma

ANA−extractable nuclear antigen (ENA)− SSc patients expectedly have a heterogenous clinical phenotype. AN−ENA− SSc is associated with male gender, diffuse cutaneous subset, widespread pigmentation, and lower incidence of: GI involvement, vasculopathy and SRC [28]. As diagnostic tests continue develop, newer antibodies within this group are being identified.

AntielF2B is a novel anticytoplasmic antibody found in ANA−ENA− SSc patient, which is associated with diffuse cutaneous involvement and SSc-ILD [29,30]. The association with ILD is extremely high with two independent studies reporting a 100% ILD incidence rate with anti-eIF2 [8,29,30]. Anti-RuvBL1/2 in ANA−ENA− SSc is associated with overlap myositis and diffuse cutaneous subset [31].

MECHANISMS UNDERLYING MUTUAL EXCLUSIVITY

Both genetic and environmental factors contribute to the risk of SSc. Genomic studies have shown clear genetic risk factors in scleroderma, however, familial occurrence of SSc is uncommon accounting for <2% of overall cases [32]. A recent case report detailed three cases of systemic sclerosis within one family all of whom had different ANA subtypes (ACA, ATA, ARA) [32]. This case report feeds the upcoming hypothesis that the predisposition to SSc is genetic, however the phenotype and ANA subtype is variable and more influenceable by environmental factors. However, it should also be noted that a larger cases series showed that the observed SSc-specific antibody concordance within each multicase SSc family was statistically more common than expected by chance alone [33]

A recent genomic risk score tool utilizing 33 alleles can accurately differentiate patients with SSc and healthy controls [34]. The genetic risk score was not able to differentiate between ANA subtypes once again displaying factors beyond genetics account for SSC phenotype/ANA subtype.

Genetics of systemic sclerosis

Immune tolerance breakdown is key to scleroderma pathogenesis. In particular, the dendritic cell (DC)−T

Table 2. Summary of HLA associations of scleroderma [42,43]

Gene	Variation	Association
HLA-B	08*01	Overall SSc
HLA-DPA1	HLA-DPA1*02:01	ATA positive SSc
HLA-DPB1	HLA-DPB1*08:01	ACA positive SSc
	HLA-DPB1*13:01	Overall SSC (1.2 OR) ATA positive SSc (4.3 OR)
HLA-DQA1	HLA-DQA1*02 :01	Limited SSc
	HLA-DQA1*04:01	ACA positive SSc (2.7 OR)
	HLA-DQA1*05:01	Exclusive for DcSSc ATA positive SSc (2.1 OR)
HLA-DQB1	HLA-DQB1*02:02	Overall SSc
	HLA-DQB1*03:01	ATA positive SSc
	HLA-DQB1*05:01	ACA positive SSc (2.0 OR)
	HLA-DQB1*06:09	Antifibrillan positive SSc (3.8 OR)
HLA- DRB1	HLA- DRB1*07:01	ACA positive SSc (0.1 OR)
	HLA- DRB1*08:04	Overall SSc (3.2 OR) AntiFibrillan SSs (7.4 OR)
	HLA- DRB1*11:02	Overall SSc (2.2 OR)
	HLA- DRB1*11:04	Overall Ssc ARA positive [45] ATA positive SSc (6.5 OR) [46]

ACA, anticentromere antibodies; ARA, anti-RNA polymerase III; ATA, antitopisomerase I; HLA, human leukocyte antigens; OR, odds risk; SSc, scleroderma.

cell axis is integral to the development of autoantibodies in SSc.

Numerous studies have illustrated multiple human leukocyte antigens (HLA) alleles that confer with increased risk of SSc, In particular within the HLA class II peptide binding groove [34,35▪,36]. Known HLA associations have been summarized in Table 2.

The largest genome-wide-association study to date by Accosta-Herrera *et al.* [35▪] found a novel association of increased scleroderma risk and HLA class I locus HLA-B*08:01, which suggests novels mechanisms of pathogenesis involving CD8+ T helper cells.

Twenty-seven non-HLA GWAS level associations have been identified. Six gene loci have been highlighted with SSc susceptibility (ARHGAP31, BLK, CD247, TNIP1, CSK, STAT4-a) [37]. The genes affected suggest that most non-HLA genetic variations are related to transcriptional regulatory mechanisms.

It is notable that genetic factors are likely to underlie some of the observed differences in autoantibody frequency across different racial groups. For example, varying prevalence of autoantibodies based on race. For example, antifibrillarin antibodies are the second most common SSc related antibody in African American patients, most probably due to high rate of HLA-DRB1*08:04 positivity in this population [38]. Recent analysis suggests that this may be explained by molecular mimicry [39].

Antigen triggers

Human cytomegalovirus (CMV) infection is associated with increased incidence of SSc [40]. CMV associated antibodies anti-UL83 and anti-UL44 have been associated with ARA and ACA seropositivity [41]. These two CMV associated antibodies have also been associated with higher incidence of anti-Ro52 antibodies, a supplemental SSc antibody associated with progressive ILD [42,43]. The process underlying CMV and SSc is likely molecular mimicry leading to generation of autoantibodies.

Several case studies link silicone breast implants with increased incidence of ARA positive scleroderma and silicone breast implant rupture has been implicated in induction of ARA positive SSc [44,45].

Molecular basis of pathogenic mechanisms of antinuclear autoantibodies

ANA subtypes have a direct role in altering gene expression through immune-complexes (IC) [10▪,40,46,47]. ANA-IC have been shown to modulate pro-inflammatory and pro-fibrotic pathways in healthy control fibroblasts and endothelial cells thought to be mediated via toll-like receptors [46]. Distinct differences in between ANA-IC subset and gene expression with ATA-ICs influencing Interferon mRNA signatures whilst ARA-IC activating nuclear factor-κB (NFκB) signaling [46].

The BIOPSY and GENISOS studies both showed differing gene expression patterns between ANA subtypes with differences noted in IL-6 signaling, adhesion cascade activation and angiogenesis [10■,47]. The GENISOS study reported ACA enriched keratinocyte differentiation, ATA enriched cellular stress response pathways and ARA upregulated pathways of NFKB signaling and tumor growth factor-beta signaling [47].

MANAGEMENT IMPLICATIONS OF ANTINUCLEAR AUTOANTIBODIES

Interstitial lung disease

ILD is the leading causes of death in scleroderma patients. 50–80% of SSc patients develop ILD during the disease [8,48,49]. Disease behavior is highly variable with <30% of SSc-ILD patients progressing to respiratory insufficiency [8].

Most SSc-ILD patients are diagnosed within the first 5 years after onset with a peak incidence at 2 years from SSc onset [3■].

The current gold standard of diagnosis is high resolution computerized tomography (HRCT), however the use of this is limited due to its high radiation dose and access [48]. ANA status helps detect patients more at risk of developing SSc and, after diagnosis, risk of progression.

Diffuse cutaneous subset is strongly associated with higher incidence and severity of SSc-ILD [3■,50]. ACA is protective against ILD whereas ATA antibodies are associated with the highest incidence of ILD independent of cutaneous subset [3■]. In limited scleroderma, alongside ATA, ANAs that are associated with high incidence rates of SSc-ILD are anti-Th/To and anti-U11/U12RNP [8].

ATA seropositivity in multiple studies has been associated with faster and more severe progression [8]. A large cohort single-site study demonstrated patients ATA positivity was predictive of forced vital capacity (FVC) decline >70% within 5 years of onset in SSc-ILD [48].

Anti-U11/U12 RNP antibody in SSc-ILD patients is associated with increased risk of progress to end stage respiratory disease and death [19]. Conversely, anti-PM/Scl and anti-Ku antibodies are associated with nonsevere ILD [8,20–26].

Pulmonary hypertension

Second to SSc-ILD, PAH is one of the leading SSc-related causes of mortality [52,53]. The overall incidence of PAH is 5–10% and remains a serious clinical challenge [52,53]. Mortality rates remain high in this cohort of patients with 3-year survival for SSc patients with PAH estimated at 56% compared with 94% in those without PAH [53].

Earlier detection of PAH has been found to improve clinical outcomes. Organ surveillance using echoes and pulmonary function tests at regular intervals help detect PAH. Gold standard of diagnosis remains through right heart catheter studies which can be costly and difficult to access [53]. The DETECT study devised a two-step risk stratification tool (named DETECT) to help diagnose PAH at earlier, milder stages. Of note, this tool uses ACA status within its algorithm [53].

In contrast to SSc-ILD, incidence is lowest in early stages of scleroderma and equivalent across dcSSc and lcSSc [3■]. Incidence is low in the first 10 years (1–2%/year) after which incidence gradually increases [3■]. ACA and Th/To are associated with higher incidence. U3RNP+ (antifibrillarin) antibodies confer highest risk of PAH whilst ATA and anti-PM/Scl have lowest risk [3■].

Scleroderma renal crisis

SRC is a life-threatening complication of SSc characterized by malignant hypertension and acute renal failure. Despite the revolutionary impact of ACE-inhibitors on SRC survival, SRC is still associated with high mortality with a 5-year survival rate of 50–90% [54].

Early detection and management is integral to reducing mortality rates. ARA holds the highest risk of developing SRC with a 10-fold increased risk of SRC [10■]. Other antibodies with increased risk are anti-U1RNP and ATA [9].

A single-site Japanese study showed ATA seropositivity was associated with worst outcomes with significantly higher 1-year mortality risk 6 times greater than ATA-negative SRC patients [9].

For patients at high-risk, it is recommended regular blood pressure checks, sparring use of prednisolone, regular monitoring of urine protein creatinine ratios at clinic appointments.

Malignancy

Malignancy is the most common cause of non-SSc-related mortality accounting for 38% of non-SSc-related deaths, and third leading cause of overall death in scleroderma patients overall [13]. Scleroderma is associated with a 41–75% increased risk of malignancy on observational studies compared to the general population [13].

ARA positive patients have been found to have a marked increase in incidence of cancer across multiple studies with a 4–7-fold increase in odds of cancer within 6 months to 5 years [13]. 9–18% of cancer

diagnoses in ARA positive patients were synchronous (diagnosed between 6 months and 12 months after SSc onset) [13,14].

Other antibodies associated with increased risk of cancer are ATA and U1RNP with a 3–5-fold increase in cancer diagnosis within the first 2 years of SSc onset compared to general SSc population in both subtypes [13]. Cancers with generally increased incidence with scleroderma include lung, hematological, esophageal and breast cancer [55].

There is no agreed guideline on cancer screening with scleroderma patients. In SSc patients with high-risk ANA cross-sectional imaging may be warranted.

Differential therapeutic response

Reviewing data from recent clinical trials shows ANA subtypes have different treatment responses to therapeutic agents.

Riociguat, soluble guanylate cyclase stimulator, was trialed in dSSc in the RISE-SSc study. Overall, the study found no significant impact in reducing skin thickening compared to placebo. However, subgroup analysis showed a substantial decrease in skin fibrosis progression in ARA-positive patients but not ATA-positive [56].

In contrast, the faSScinate study that explored the use of tocilizumab in dcSSc showed highly significant decrease in rates of lung function decline in ATA positive patients but not in ATA negative patients in phase 2 and 3 studies [57].

There is difficulty in retrospective subgroup analysis as clinical trial design is often underpowered to explore these relations. This is illustrated with the SENESCIS trial of nintedanib on SSc-ILD, which showed a numerically greater preservation of lung function in ATA-negative SSc, but no significant differences [59,60].

FUTURE CONSIDERATIONS

Need for reclassification

Separation of SSc patients into limited and diffuse subsets based on their extent of skin involvement incompletely reflects the distinct clinical patterns within each group. Conversely, categorizing patients only based on their serological profile does not produce replicable clinical patterns [3▪,7].

Currently, most SSc experts use systems of subtyping SSc patients in their practice [58]. Enriching our classification system to include cutaneous subset with serological status provides a robust categorization. Hybrid classification system offers the best predictor of clinical outcome and prognosis to help aid risk management and organ surveillance [3▪,52,62].

Efforts have been initiated to update the SSc classification system and are most likely to involve a hierarchical approach.

Standardizing antinuclear autoantibodies testing

A substantial limitation in focusing clinical acumen on autoantibodies is the lack of standardization in diagnostic lab techniques and interpretation [62]. In scleroderma, there are numerous commercial diagnostic assays that utilize different methodology. For the two most predominate ANA subtypes, ACA and ATA, there is high concordance of results across differing assays, commercial platforms and laboratories [62,63]. However, despite reported concordance for anti-Scl-70 testing among the different testing methods some concerns remain about the specificity of Scl-70 antibody testing based on multiplex methods [64,65]. Moreover, other ANA have high discordance rates, in particular, anti Pm/Scl, antifibrillarin, and Th/To [66]. Further work needs to be implemented to achieve greater harmonization between centers.

Incorporating antinuclear autoantibodies into clinical trial design

As aforementioned, ANA subgroups may respond differently to therapeutic agents. Despite this knowledge, majority of clinical trial designs do not account for ANA subset and broadly divide patients into lcSSc and dcSSc. This results in multiple potentially useful therapeutic agents being labeled as ineffective when they may have a significant impact if used on the correct ANA subtype.

Stratification strategies based on ANA and cutaneous subtype offer the opportunity of selecting and identifying the best candidates most likely to achieve the greatest magnitude of treatment benefit for each targeted therapy.

Limitations of subgrouping by ANA status include the relatively small sample sizes of clinical trials due to the rarity of disease itself.

CONCLUSION

As in some other Immune-mediated inflammatory disease such as idiopathic inflammatory myopathies and ANCA-associated vasculitis, in scleroderma there are important and disease specific 'ANA-clinical phenotype links. These have important implications for management, including monitoring, risk stratifications and treatment decisions (especially targeted therapies) and because of this are also important for clinical trial design to optimize informative subject

enrolment and minimize is across treatment arms in parallel group trials. Finally, the ANA associations are giving powerful insight into disease mechanism [51,61].

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Conflicts of interest

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Imaging techniques for assessment of vascular involvement in systemic sclerosis

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Purpose of review
Vascular assessment in systemic sclerosis (SSc) is included in classification criteria for this disease, thus routinely used in the evaluation of patients in which this diagnosis is being considered. In this review, imaging techniques for assessment of vascular involvement in SSc hands and skin are discussed.

Recent findings
Longitudinal use of imaging techniques has important implications for understanding the progressive vasculopathy and fibrotic transition in SSc. Nailfold and oral capillaroscopy as well as laser speckle contrast analysis are established techniques for vascular functional assessment, but longitudinal use is challenged by equipment costs and clinical time constraints. Ultrasound techniques are well described but require technical training. Advances in mobile infrared thermography and optical coherence tomography could potentially provide a point-of-care, quantitative outcome measure in clinical trials and practice.

Summary
The equipment cost, technical training, data standardization, and invasiveness of vascular assessment techniques that quantify morphological (microangiopathy) and functional (blood flow reduction) are critical for implementation into SSc clinical trials and practice to understand progressive vasculopathy, such as wound development.

Keywords
imaging, systemic sclerosis, vasculopathy

INTRODUCTION

Systemic sclerosis (SSc) is characterized by early microvascular changes with endothelial cell dysfunction, followed by the activation of mechanisms promoting their transition into myofibroblasts with subsequent fibrosis. The complex interplay of autoimmunity, ischemia, and fibrosis in SSc involves both skin and visceral organs resulting in irreversible damage [1]. Endothelial dysfunction, microvascular and macrovascular damage are the hallmarks of SSc [2]. In fact, while extent of skin thickening and SSc-specific autoantibodies are recognized to have important prognostic implications and are included in the classification criteria for this disease, most classification criteria represent vasculopathy manifestations, highlighting the importance of vascular assessment [3]. Although classification criteria are not designed for diagnostic purposes, in the absence of diagnostic criteria, the classification criteria are often used to by clinicians during the work-up of patients with concern for SSc as the diagnosis [4]. Assessment of vascular involvement is similarly important for SSc patient management, as the disease is a progressive

self-amplifying process, which first involves the microvascular/endothelial damage, followed by autoimmune response and inflammation, and finally fibrosis [1]. Vascular based therapeutics, even in the absence of a primary preventive action, might help in slowing disease progression and postponing the onset of major vascular events [5∎,6]. There is a need for routine, cost-effective, and noninvasive imaging techniques of vascular involvement in SSc.

In SSc, correlations between morphological (microangiopathy) and functional (blood flow reduction) evaluations are established as a progressive process that results in vascular damage, insufficient repair, and ultimately loss [7,8]. Although pulmonary

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KEY POINTS

- Clinical vascular assessment is critical for systemic sclerosis patient care and clinical trials.

- Nailfold and oral capillaroscopy, thermography, and laser speckled contrast analysis are useful tools for vascular assessment.

- Techniques such as mobile thermography and optical coherence tomography are promising but require prospective clinical studies to determine the validity, reliability, sensitivity, and specificity of these measurements for routine use in systemic sclerosis patients who are at risk for vasculopathy progression.

arterial hypertension (PAH) is diagnosed by right-sided heart catheterization according to standard definitions, there are screening algorithms to assist in diagnosis in a cost-effective manner [9,10,11]. The fingers often first exhibit the early signs of SSc, thus, the most straightforward method for early detection is to assess the functional and structural changes through appropriate imaging technologies of the hand and skin. This review covers vascular assessment of the skin and hands in SSc to highlight the importance of developing standardized approaches.

HAND AND MOUTH VASCULAR ASSESSMENT IN SYSTEMIC SCLEROSIS

Despite broad patient-to-patient variability in SSc presentation and disease severity, Raynaud's phenomenon (RP), a symptom complex related to digital vascular compromise in response to cold temperature or stress, is almost universally present in patients with this diagnosis [12]. Examination of the face and hands for telangiectases, as well as finger pulp assessment for pits and digital ulcerations (DU) is important for each patient, not only for diagnosis but also, for serial clinical management. The clinical disease progression of RP to DU in SSc represents micro vessel leak with hemorrhages, progressive capillary loss, and overt tissue ischemia [1]. There are several investigative tools that can be used to specifically examine vascular involvement of the hand and mouth that may reflect vascular pathogenesis in other organs.

NAILFOLD CAPILLAROSCOPY

Nailfold capillaroscopy is a safe, noninvasive tool to morphologically study the microcirculation in a patient with RP [13]. The importance of capillaroscopy is underscored by the fact that abnormal capillaries score two points of the nine required

for classification of SSc [3]. As such, all clinicians diagnosing SSc must have access to capillaroscopy and a familiarity with the technique [14]. Nailfold videocapillaroscopy (NVC) is the gold standard for assessment of peripheral microvascular morphology and thus allows classification and scoring of capillary abnormalities with respect to different microangiopathy patterns (early, active, and late) at the nailfold level [15]. Early phase microangiopathy is characterized by well preserved capillary architecture with a few dilated capillaries and microhemorrhages. The active pattern typically demonstrates mildly disorganized capillary architecture with many dilated capillaries and microhemorrhages along with avascular areas. The late pattern shows severely disorganized capillary architecture with dilated capillaries and microhemorrhages, but more significantly, a marked reduction in the number of capillary loops with large avascular areas. Even with limited training and experience, agreement for the identification of active and late patterns is achievable [16]. The late pattern on NVC is an independent predictor of DU in SSc [17]. Unfortunately, access to and training in NVC is not readily available in some countries, where this procedure is not reimbursable and due to the time it takes, is not feasible for serial use in clinical care [18].

Capillary assessment by dermatoscopy (used synonymously with the term dermoscopy) due to its low cost, quick acquisition of images and more frequent use amongst non-SSc specialists, is a valid clinical tool for nailfold assessment in a patient with RP [19]. There are a few important aspects to documentation when using a dermatoscope, including documentation of the magnification used, which typical ranges from $10\times$ to $30\times$, and the attachment of a device to allow photo documentation. While dermatoscopy does not provide the detailed assessment that is given by NVC, it can successfully identify the nailfold SSc-pattern as well as identify nonspecific abnormalities that can subsequently referred for NVC available in subspecialty centers that care for SSc-spectrum diseases [20].

The procedure for dermatoscopy is like NVC. Each subject should be acclimatized to the exam room for a minimum of 15 min before the nailfold is examined at room temperature of about 21–22°C. Like NVC, a thin layer of oil is applied to the nailfold of the second to fifth digit on both hands to enhance sharpness of images. However, unlike NVC the dermatoscopy is not placed directly on the nailfold. The distance of the dermatoscope from the nailfold is determined by image sharpness that is influenced by either the steady hand of the operator, or a platform that can fix the device, since clear images require no movement. The automated focusing system of a

dermatoscope results in the possibility of slight variation in magnification, and in general, provides a single, wide view image.

Nailfold capillaroscopy plays a significant role in the diagnosis of systemic sclerosis, as microvascular damage is an early marker of disease. It is also useful to assess the severity of disease. Structural abnormalities, such as devascularization areas and distortion of the capillary bed architecture, characteristic of the late microvascular damage, are strong predictors of the occurrence of DU in this population of patients. Abnormal nailfold capillaroscopy findings are associated with the presence of pulmonary arterial hypertension (PAH) in patients with SSc and correlated with PAH severity [21]. However, there is no consensus on its role in the follow-up of SSc patients [20]. Training of healthcare providers assessing RP, especially fellows and rheumatologists, in this technique is an important unmet need in SSc [18].

ORAL CAPILLAROSCOPY

Oral regions of the mouth can be examined by microscopy in a noninvasive method that assesses microcirculation. Oral capillaroscopy is performed with a sterile probe cap and can be applied to incisor, buccal and sublingual regions. One study of 20 SSc patients, and 20 age- and sex-matched controls using a portable videocapillary CapiScope (KK Technology) with a Sidestream Dark Field (SDF) camera demonstrated decreased oral vasculature in SSc patients [22]. Green light emitting diodes (from the SDF camera) is absorbed by hemoglobin in RBC, which allows RBC visualization in contrast to the vascular background that allows indirect measurement of the glycocalyx layer in sublingual capillaries. A study of 26 subjects (16 SSc patients and 10 healthy controls) reported that sublingual microcirculation and glycocalyx are impaired and that SDF imaging findings correlate with those of NVC [8]. Another study of sublingual capillaroscopy in 39 SSc patients, found a significant correlation between intravital microscopy of the sublingual microcirculation and NVC in terms of sublingual total microvascular density and microangiopathy evolution score, which includes the sum of three scores for loss of capillaries, disorganization of the microvascular array, and capillary ramifications [23"]. Serial use of a noninvasive and automated sublingual microvascular function testing and glycocalyx measurement in the clinical setting is needed to best understand the implication of these findings.

LASER TECHNIQUES

Capillaroscopy and laser Doppler techniques can be used together to complement each other in morphologic and functional evaluation of microcirculation. Laser Doppler techniques assess the skin capillary perfusion by measuring the Doppler shift induced by laser light scattering of moving red blood cells whereas laser speckle contrast imaging (LSCI) measures the fluctuating granular pattern produced by laser light reflected on moving red blood cells. Laser Doppler Flowmetry (LDF) has excellent speed, but poor reproducibility, requires skin contact, and due to single point measurement, has high spatial variability. Laser Doppler imaging (LDI) has good reproducibility but is slow at capturing changes in cutaneous perfusion and thus, not good at recording rapid changes in perfusion. LSCI is faster at capturing changes in cutaneous perfusion but is not good for assessing areas of low perfusion. Studies comparing laser Doppler techniques and conventional NVC showed that cutaneous perfusion measured by LDF correlated well with NVC findings [24""]. More studies are needed for validation of LSCI in SSc.

Although the validated method to study the morphological vascular alteration in SSc patients is NVC, laser speckle contrast analysis (LASCA) is helpful in the evaluation of functional damage of microvascular system [24""]. LASCA is a safe, noncontact, noninvasive microvascular imaging modality that is a less time-consuming technique compared to NVC and can be used to quantify peripheral blood perfusion in the cutaneous microcirculation over large skin areas. LASCA used alone or together with reactivity tests, is useful for the monitoring of disease progression, response to treatment and DU outcome [24""]. LASCA is a credible instrument in patients of Black ethnicity with SSc [25]. LDF at the single fingertip level correlates with LASCA, but LASCA has a lower intra-operator variability than LDF, can evaluate larger skin areas, is significantly less time consuming and more readily accepted by patients [24""]. Although LSCI is like LASCA, the contrast is calculated on a single pixel over several time frames. LSCI has a spatial resolution which is five-times larger than that of LASCA, but it has a poor temporal resolution. LSCI of the hand demonstrates lower perfusion in SSc patients than healthy controls and directly correlated with the NVC findings [26,27]. There is a good correlation between peri-oral and lip LSCI to mouth-opening in SSc patients, but no significant difference was observed between SSc and healthy subjects at the peri-oral area [28].

INFRARED THERMOGRAPHY

Infrared thermography (IRT) indirectly measures the cutaneous thermoregulation process to produce an image according to the temperatures emitted by the human body and can be obtained using portable digital thermal cameras attached to a mobile phone,

known as mobile thermography [29]. There is good correlation between LSCI and IRT for the assessment of digital perfusion [30]. IRT is an effective tool for assessing patients with rheumatic disease, but protocols require recording acclimatization time, distance between the camera and the individual, temperature, and ambient humidity [31▪▪]. IRT assessment of SSc hands is often combined with a local cold challenge to allow dynamic vascular assessment under conditions thought to simulate those responsible for an attack of RP. A local cold challenge does not account for the influence of convective and conductive heat exchange on surface skin temperature, thus does not truly recapitulate RP [32]. Although IRT measurements correlate only moderately with density of capillaries, abnormal initial thermography associates with nailfold capillaroscopy patterns and identifies SSc that are more likely to develop digital ulcers and require more frequent surgical debridement [33,34]. Of interest, baseline thermographic temperature is influenced by gender but, not race and trends show decreased perfusion in tobacco users relative to non-smokers, which highlights the importance of subject characterization [35]. Additionally, while not specifically studied in SSc, lower facial skin and submental triangle region temperatures, measured by IRT, can help identify patients with obstructive sleep apnea [36]. Though IRT devices are valuable for assessing skin circulation, they require prospective clinical studies to determine the validity, reliability, sensitivity, and specificity of these measurements for routine use in patients who are at risk for vascular disease and wound development [37].

IMAGING OF CALCINOSIS

Radiography, high-frequency ultrasound (HUS), computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) can be used to quantify the vascular complication of calcinosis [38,39▪]. Radiographs and HUS are the least expensive options for following calcinosis lesions. Radiographs allow rating of calcinosis and a description of the morphological pattern, such as nodular, sheet-like, reticular, amorphous, and linear [40]. By HUS, which is a low-cost, point of care, nonionizing imaging modality, calcinosis is described as hyperechoic foci with or without acoustic shadowing, which may increase detection, but may be more time intensive and is dependent on sonographer experience with less reproducibility [39▪]. The addition of Doppler imaging modes can result in artifact [41]. Nonetheless, HUS is helpful for following cutaneous ulcers in SSc [42].

Whole-body fluorine-18 fluorodeoxyglucose PET/CT can identify widespread soft-tissue calcinosis characterized by elevated glucose uptake in SSc [43]. Without the PET component, traditional CT can provide information regarding adjacent anatomic structures, which can guide a surgical approach to management [44]. Novel CT approaches, such as 3D visualization and dual-energy, provide better visualization, but are limited for serial use by cost and radiation exposure [39▪]. MRI with high contrast resolution and multiplanar imaging effectively evaluates soft tissue pathology without associated radiation exposure, but cost limits feasibility. Furthermore, conventional MRI sequences may not be able to identify small foci of calcinosis, but the addition of gradient-echo imaging can improve detection [45].

ANGIOGRAPHY AND OPTICAL COHERENCE TOMOGRAPHY

Vascular lesions of the hand are unique and more difficult to image because of the terminal vascular network, thus, to guarantee a high-quality exam the hand should be evaluated independently and not as part of an upper limb protocol [46▪▪]. Conventional angiography is the gold standard for vascular abnormalities. CT angiography (CTA) is performed with two successive acquisitions after the injection of iodinated contrast media. There are advanced CT imaging techniques such as dynamic CTA and super high-resolution (SHR)-CTA, which allow a clear visualization of the most distal arteries [46▪▪]. Dynamic contrast-enhanced magnetic resonance angiography (MRA) yields comparable information to conventional angiography about vascular anatomy, stenosis, obstruction, and vessel inflammation. Tissue characteristics influence MRI signal intensity, which can be manipulated pharmacologically for the purpose of contrast enhancement through altering the relaxation time of the tissue [47]. MRA is usually based on the administration of a gadolinium-based contrast agent and time-resolved sequences. Image contrast is the difference in brightness between an area of interest and the surroundings such that the larger the difference in brightness between different tissue types, the easier it usually is to differentiate them from each other. Contrast-enhanced T1-weighted fat-suppressed sequences provide a means to evaluate thickening and enhancement of the arterial wall. MRA of the hand can help rule out vasculitis mimics but is usually not indicated if drug or chemical related, frostbite, or vaso-occlusive disease is suspected [46▪▪].

Optical coherence tomography angiography (OCT-A) is method to directly visualize capillary-level vascular and structural features within skin *in*

vivo, which has the potential to provide new insights into the pathophysiology, as well as dynamic changes of SSc skin [48]. OCT-A visualizes vasculatures from two separate layers of skin, the small capillaries of the superficial papillary dermis and the larger vessels of the deeper reticular dermis [49]. OCT-A imaging of the nailfold correlates with microvascular injury classically described by NVC [49]. The development of dynamic OCT is proposed a standardized imaging technique that could potentially provide a quantitative outcome measure in clinical trials and practice [50▪▪].

VASCULAR FUNCTIONAL STUDIES WITH PERIPHERAL ARTERIAL TONOMETRY, DIGITAL THERMAL MONITORING, FLOW MEDIATED DILATION, AND AUTONOMIC NERVOUS SYSTEM INVESTIGATIVE TOOLS

The peripheral arterial tonometry (PAT) technique that measures arterial pulse volume changes in the finger as a result of vasomotion (vasoconstriction and vasodilatation) identified early endothelial changes in smaller arterioles and microvascular beds in early diffuse SSc [51]. The PAT technique compares pulse amplitude at the fingertips before and after a 5-min arm-cuff-induced reactive hyperemia. However, the PAT probe includes a fingertip cuff that obstructs microvasculature at the point of measurement; therefore, may not be able to accurately evaluate microvascular reactivity at the fingertip. Like the PAT technique, digital thermal monitoring (DTM) is performed during a 5-min arm-cuff-induced occlusion to induce reactive hyperemia and indirectly measures endothelial function, perfusion, and vasodilator ability. Both DTM and PAT are automated, but DTM of vascular reactivity assesses Doppler ultrasound hyperemic, low frequency, blood velocity of radial artery and a fingertip vascular function without fingertip occlusion. The DTM method measures both cutaneous microvascular and vascular reactivity that result in increased blood flow to the fingers because of reactive hyperemia. A single center study of 34 SSc subjects identified that DTM correlated to flow mediated dilatation (FMD), which is a test of endothelial function in the brachial artery [52].

FMD in SSc has identified that endothelial dysfunction seems to be primarily present in microvasculature [53]. When FMD is combined with endothelium-independent, nitroglycerin-mediated dilatation (NMD), FMD is impaired prior to NMD in SSc, suggesting assessment of FMD in the preatherosclerotic stage may have a beneficial diagnostic, prognostic, and therapeutic relevance [54]. FMD is reported as an independent predictor of DU [17].

FMD can be combined with carotid ultrasound to measure carotid intima-media thickness (CIMT) and carotid atheroma plaques (AP) in order to detect accelerated atherosclerosis or macrovascular disease. CIMT is reported at older ages and after longer disease duration in SSc [54]. Macrovascular disease is more common among SSc with diastolic dysfunction of the left ventricle on echocardiogram [55▪]. A study of 70 SSc patients identified that glucocorticoids may be associated with an early vascular damage in these patients detected by FMD and carotid ultrasound [56]. This highlights the value of serial vascular assessment in SSc.

Autonomic nervous system (ANS) involvement, consisting of parasympathetic under activity and sympathetic overdrive, is regularly described in SSc [57]. Increased heart rate, diminished heart rate, and blood pressure variability are the most reported alteration [57]. Gastrointestinal involvement is reported to correlate to ANS involvement [58]. Quantitative sudomotor axon reflex test (QSART) is designed to stimulate the autonomic nervous system and evaluate how nerves that regulate sweat glands responds to stimulation. A SSc QSART protocol for skin symptoms (digital ulcers, pernio-like eruptions, subcutaneous calcifications, telangiectasia, nailfold capillary dilatation/bleeding and degree of skin sclerosis) and skin surface temperature is under investigation in Japan for a observational clinical study [59]. This study adopts two evaluation points, summer and winter, to observe effects of temperature on sweating. The interaction of vascular and neurological symptoms are captured in this functional study.

Irrespective of PAT, DTM, FMD, or QSART studies, it is important to highlight that mean blood pressure (BP) is an important determinant of arterial stiffness in SSc [60]. Tobacco cessation and BP monitoring is mandatory for SSc patients for both detection of scleroderma renal crisis (SRC) and cardiovascular risk reduction [61]. Thus, while exciting advances in the field will help inform SSc vasculopathy progression, perhaps the most important routine, cost-effective, and noninvasive imaging technique for vascular involvement in SSc is home BP monitoring.

CONCLUSION

Vascular assessment of the skin and hands in SSc ideally captures the natural history of vasculopathy and fibrotic transition in a cost-effective methodology that captures quantifiable parameters of disease activity and damage with minimal training requirements (Table 1) [62]. Although nailfold capillaroscopy has a critical role in diagnosis, practical

Table 1. Noninvasive vascular methods

Noninvasive vascular method	Vascular data provided	Principle strength	Main weakness
Nailfold capillaroscopy	Morphological	Standardized protocol	Trained operator
Sublingual capillaroscopy	Functional	Automated	Availability
Laser speckle contrast imaging (LSCI)	Functional	Noncontact	Inadequate in low perfusion
Laser speckle contrast analysis (LASCA)	Functional	Intra-operator variability	Spatial resolution
Infrared thermography	Functional	Specific for skin circulation	Detailed protocol
Calcinosis assessment • Ultrasound (US) • CT • MRI • PET	Morphological	• US: cost • CT/MRI: preoperative anatomic information • PET: widespread evaluation	• US: trained operator • CT: radiation • MRI: misses small foci • PET: cost
Optical coherence tomography angiography (OCT-A)	Morphological	Quantitative in two layers of skin	Validation in SSc
Peripheral arterial tonometry (PAT)	Functional	Automated	Fingertip occlusion
Digital thermal monitoring (DTM)	Functional	Automated	Requires adequate baseline temperature
Flow mediated dilatation (FMD)	Functional	Standardized protocol	Trained operator
Quantitative sudomotor axon reflex test (QSART)	Functional	Captures neurologic skin symptoms	Trained operator

applications for serial monitoring are limited by standardization, training, and time-constraints. Costs of imaging are an important consideration for advances in CT, MRI, and PET as applied to longitudinal characterization of vasculopathy. Techniques including automated oral capillaroscopy, PAT, DTM, FMD, IRT, and OCT require prospective clinical studies to determine the validity, reliability, sensitivity, and specificity of these measurements for routine use in SSc patients who are at risk for vasculopathy progression. Nonetheless, the importance of collaborative efforts to standardize assessment of SSc disease progression is critical for longitudinal vascular assessment of the skin and hands to inform discovery [63]. Serial vascular assessment remains at the forefront of critical unmet needs for SSc.

Conflicts of interest

There are no conflicts of interest.

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Calcinosis in systemic sclerosis

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Purpose of review
The aim of this study was to provide updated information on the prevalence, pathogenesis, diagnostics and therapeutics of calcinosis cutis associated with systemic sclerosis (SSc).

Recent findings
Observational studies show ethnic and geographical differences in the prevalence of calcinosis. In addition to clinical and serological associations, biochemical studies and in-vivo models have attempted to explain theories behind its pathogenesis, including prolonged state of inflammation, mechanical stress, hypoxia and dysregulation in bone and phosphate metabolism. Long-term use of proton pump inhibitors may increase the risk for calcinosis in SSc. Few single-centre observational studies have shown mild benefit with minocycline and topical sodium thiosulfate.

Summary
Calcinosis cutis is the deposition of insoluble calcium in the skin and subcutaneous tissues. It affects up to 40% of SSc patients and causes significant morbidity. Long disease duration, features of vascular dysfunction and osteoporosis have been associated with calcinosis. Altered levels of inorganic pyrophosphate and fibroblast growth factor-23 have been implicated in dysregulated phosphate metabolism that may lead to calcinosis in SSc. Plain radiography can help with diagnosis and quantifying the calcinosis burden. Surgical treatment remains the most effective therapy when feasible. At present, no medical therapies have proven efficacy in large randomized controlled trials.

Keywords
calcinosis cutis, systemic sclerosis, therapeutics

INTRODUCTION

Calcinosis cutis is defined as the accumulation of insoluble calcium salts in the skin and subcutaneous tissues. Systemic sclerosis (SSc) is a heterogeneous disorder primarily characterized by fibrosis of skin and internal organs as well as vascular dysfunction. SSc is prone to dystrophic calcification, which occurs in areas of damage associated with normal serum calcium and phosphorus levels. X-ray diffraction studies of draining calcinotic material in SSc patients confirm that the main inorganic component is hydroxyapatite, similar to that of bone [1]. Calcinosis can affect up to 20–40% of SSc patients [2]. The prevalence may vary depending on ethnicity, geographic location and diagnostic approach. On the basis of physical examination or clinically indicated x-rays, it is more common in whites (up to 38%) compared with Asian ethnicity (9%) [2,3]. It has a prevalence of 10% in North Africa [4], 29% in the Middle East [5] and 24% in Japan [6]. However, subclinical calcinosis detected on imaging studies was found to be present in up to 50% of patients within 4 years of disease onset in Japan [7■]. Calcinosis decreases quality of life for SSc patients due to pain,

decreased hand function, ulceration, infection, and occasionally nerve compression. There are currently no effective medical therapies for calcinosis.

LOCATION AND ITS IMPACT

Calcinosis most commonly affects the hands, particularly the distal phalanges of the thumb and index fingers, which are prone to repetitive trauma [8] (See Figs. 1 and 2). In addition to reduced hand mobility [9■■], calcinosis in the hands and wrists can also lead to peripheral neuropathy [10■,11,12]. An ultrasound study showed that peritendinous

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KEY POINTS

- Biochemical studies support the association of calcinosis with prolonged vascular dysfunction, inflammation and dysregulated phosphate metabolism in systemic sclerosis.

- Low level of evidence supports medical therapies such as topical sodium thiosulfate and oral minocycline for SSc-related calcinosis.

- When lesions are accessible, surgical removal is the most effective treatment option for SSc-related calcinosis.

calcifications are frequently associated with hand contractures in SSc patients [13]. Other common locations include the forearms, elbows and extensor surface of the knees [14]. Unusual locations like the spine or face are reported in some studies [15–19]. Spinal calcinosis, which was found to occur commonly in association with acral calcinosis [20], can lead to radiculopathy, spinal stenosis or cord compression [15–18,21].

ASSOCIATED DISEASE CHARACTERSTICS AND PATHOGENESIS

The exact pathogenesis of calcinosis is largely unknown, but there are some clinical and serological associations. Distinct antibody profiles, including anticentromere [22] and anti-PM/Scl (Polymyositis/Scleroderma) [23], have been reported to be associated with calcinosis in some, but not all, studies [24]. Long disease duration is clearly associated with calcinosis development [23], and this may be representative of prolonged exposure to an inflammatory milieu. Inflammation has broad actions on several processes involved in calcinosis, including vascular dysfunction, bone metabolism and phosphate metabolism, as discussed below.

Vascular dysfunction and hypoxia

Many studies have established that calcinosis in SSc is associated with features of vascular dysfunction including loss of digital pulp, acro-osteolysis and presence of digital pitting scars [2,6,8,20,22,25▪▪, 26–29]. A Japanese cross-sectional study showed that calcinosis in the facial region is commonly associated with multiple external root resorption, which is akin to the loss of digital pulp in the hands [30]. Hypoxia is likely the link between vascular dysfunction and calcinosis in SSc. Vascular dysfunction combined with defective angiogenesis causes hypoxia in tissues [23], with markers of hypoxia, including keratinocyte GLUT-1 (glucose transporter), as well as advanced glycation/lipoperoxidation end products (AGEs) and their receptors (RAGE), present within the endothelium and the papillary dermis in SSc patients with calcinosis [31].

FIGURE 1. Calcinotic material extruding from the index finger of a 45-year-old woman with systemic sclerosis.

FIGURE 2. Calcinotic lesion with surrounding erythema in the thumb of 50-year-old woman with systemic sclerosis.

A pilot study with 20 SSc patients showed reduced perfusion in the superficial skin layers involving calcinotic areas compared with noncalcinotic areas [32]. Ulnar artery occlusion was found to be highly associated with radiograph-confirmed calcinosis in 43 SSc patients [33▪]. Repetitive episodes of ischemia-reperfusion injury due to vascular dysfunction can lead to hypoxia and increase products from oxidative stress [31,34]. Furthermore, areas prone to repetitive trauma are commonly involved sites for calcinosis. Injury to collagen, elastin or subcutaneous fat combined with hypoxia can lead to tissue necrosis, releasing in denatured proteins, which promotes calcification [35]. Cellular organelles such as mitochondria can accumulate high amounts of calcium and phosphorus under prolonged inflammatory conditions and be released during muscle damage as demonstrated in in-vivo murine models, thereby acting as nucleation sites for calcinosis [36,37].

Medications may also contribute to promotion of a hypoxic environment and subsequent development of calcinosis. Proton pump inhibitors (PPIs), medications frequently used to treat gastro-esophageal reflux disease (GERD) in patients with SSc, may promote atherogenic pathways and calcification through inhibition of dimethylarginine dimethylaminohydrolase [38▪▪]. A retrospective cohort study of 199 SSc patients established a dose–response relationship between PPI use and calcinosis. This was further validated in a prospective cohort of 200 SSc patients who demonstrated an increase in odds of calcinosis of 4% with every 1 additional year on PPI therapy.

Mechanical stress or repetitive trauma

Calcinosis occurs most frequently in areas prone to repetitive trauma [8]. Mesenchymal stem cells (MSCs) showed osteogenic transformation when treated *in vitro* with blister fluid (rich in IL-31 or Th2 pathway) from the skin of SSc patients on stiff matrices, supporting the hypothesis that mechanical stress plays an important role in calcinosis [39].

Dysregulation in bone metabolism

The composition of calcinotic material in SSc is similar to that of bone, and dysregulation of bone metabolism has been implicated in calcinosis development [1]. A retrospective analysis of 5218 SSc patients from a multicentre international cohort showed that osteoporosis was associated with calcinosis [40]. It is likely that bone resorption, as it occurs in acro-osteolysis and osteoporosis, releases inorganic phosphate, which promotes osteoblastic differentiation of dermal fibroblasts [1]. Consistent with this interpretation, MSCs underwent osteogenic transformation upon in-vitro exposure to appropriate osteogenic media consisting of beta-glycerophosphate [41].

Dysregulation in phosphate metabolism

Inorganic pyrophosphate (PPi), which is derived largely from ATP, is an inhibitor of mineralization. Alterations in the PPi pathway, leading to reduced levels, can promote calcification. Several genes involved in maintaining PPi levels, including ABCC6

(ATP binding cassette), ENPP1 (ectonucleotide pyrophosphatase phosphodiesterase 1) and NT5E (ecto- 5' nucleotidase), have been implicated in other ectopic mineralization disorders [14,42]. The ENPP1 pathway may also be inhibited by inflammatory cytokines, in particular IL-1β [41]. PPi levels are reduced in ectopic mineralization disorders such as pseudoxanthoma elasticum. A recent study found that plasma PPi levels were also reduced in SSc patients as compared to healthy controls, but there was no significant difference between those with and without calcinosis [43▪▪]. Additional studies are necessary to further investigate the role of PPi and dysregulated phosphate metabolism in the pathogenesis of calcinosis.

FGF-23 (fibroblast growth factor 23) signals through its co-receptor KLOTHO in the proximal tubule of the kidney to downregulate sodium-phosphate cotransporters, leading to phosphaturia [44]. This pathway is disrupted in familial pseudotumoral calcinosis leading to hyperphosphatemic calcinosis. Although dystrophic calcification in SSc is associated with normal serum phosphate levels, it is nevertheless noteworthy that high levels of serum FGF-23 levels [45] have been associated with calcinosis in SSc. Thus, this pathway may be involved in SSc-related calcinosis.

GENETIC ASSOCIATIONS

The HLA-DRB1▪04 allele is associated with subcutaneous calcinosis in SSc [46]. Polymorphisms of matrix metalloproteinase-encoding genes are also associated with calcinosis in SSc [47]. Genetic studies in 1142 SSc patients showed some association of TNFSF4 polymorphisms (tumour necrosis factor ligand superfamily member 4) with calcinosis, thus implicating the importance of inflammation [48].

Genetic studies exploring genes involved in phosphate metabolism in SSc patients with a severe calcinosis phenotype are currently underway.

CLINICAL PRESENTATION

Calcinosis cutis lesions can present as subcutaneous nodules (circumscripta), which is the most common form in SSc patients. Other presentations include 'sheet-like' along the myofascial planes (calcinosis universalis) or extensive deposits covering larger surface areas (exoskeleton) [49–51]. Although pseudo-tumoral calcinosis has been reported in up to 3% of SSc patients [52], giant calcinosis can occur in patients with dermatomyositis and SSc overlap syndrome [53]. On the basis of the shape and consistency on palpation, the lesions can be further classified into mousse (soft with chalky-like liquid under the skin), net (diffuse thin network), plate (large uniform agglomerate) or stone (palpable as a single or multiple stones of hard consistency) [14,54].

DIAGNOSTIC MODALITIES

Plain radiography of affected areas is the most common imaging modality used to detect calcinosis (Fig. 3). A validated radiographic scoring system for longitudinal assessment of calcinosis, with excellent inter-rater and intra-rater reliability, was developed by Chung *et al.* [25▪▪]. Ultrasound can be used at the point of care (Fig. 4) and has specificity close to 95–100% for detecting calcinosis compared with X-ray [55].

Although computed tomography (CT) is more sensitive than X-rays and provides information on surrounding structures such as neurovascular bundles [56▪], radiographic estimation of calcinosis volume seems to correlate well with that of CT [57]. Artificial

FIGURE 3. Calcinosis deposits in the fingers as detected on a plain radiograph.
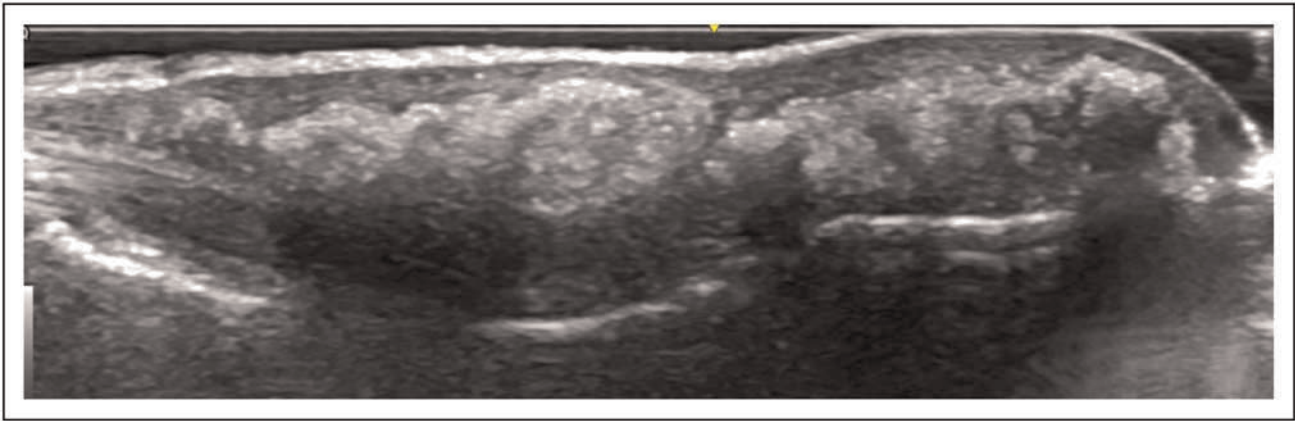
FIGURE 4. An aggregate of calcinosis detected by ultrasound on the volar aspect of the finger near the proximal interphalangeal joint.

intelligence is being applied to dual energy CT scans in SSc patients with calcinosis of the fingers to more accurately estimate calcinotic burden, but further validation studies are necessary [58,59]. Novel 3D-cinematic picturization through CT scan is also being evaluated in detection of calcinosis universalis [60].

MANAGEMENT

As the pathogenesis behind calcinosis is still being unraveled, and outcome measures to use in clinical trials are still being validated, thus far, we have no effective treatment options.

General measures

As longstanding hypoxia and trauma play a crucial role in the development of calcinosis, avoidance of trauma can help prevent new lesions. Appropriate wound care and antimicrobial treatment are needed for nonhealing and infected ulcers, respectively. Pain management is critical in the treatment of calcinosis, particularly for lesions involving pressure points or causing nerve compression.

Local and noninvasive therapies

Herbal therapies such as Holoil (neem oil and Hypericum perforatum) employed in wound care facilitated complete healing in 45% of calcinotic lesions (involving the hands) in 21 SSc patients over a mean duration of 40 days. It softens the lesions and facilitates easy excision of the calcinotic material. Although it needs to be validated in a larger cohort, it proved to be well tolerated in this pilot study [54].

Topical sodium thiosulfate (STS) helps in clearing calcinotic lesions by acting as a calcium chelating agent. In addition to individual case reports, a retrospective study showed improvement in 68% of those treated with 25% sodium thiosulfate in zinc oxide [61,62]. Topical STS is generally dispensed through compounding pharmacies. A recent systematic review found improvement in 81% (39 of 48 patients) of patients over a mean duration of 4.9 months [63■]. Side effects included skin irritation, allergy to zinc and occasionally pain with application.

Local and minimally invasive therapies

Intralesional STS (1 ml/cm^2) injected monthly into calcinotic plaques of a patient with diffuse cutaneous SSc resulted in nearly complete resolution in 3 months [64]. A case series of six patients (including five SSc patients) with calcinosis treated with intralesional injection every week (12.5–150 mg each dose) for 4 weeks showed significant improvement in 100% of the SSc patients [65]. Intradermal or local anesthesia is often employed with this procedure, despite which burning pain at the site of injection is reported by many patients.

Extracorporeal shockwave therapy is a minimally invasive procedure that is proving to help with pain relief (through nerve decompression) associated with recalcitrant calcinotic lesions that are not amenable for surgery. A prospective study reported analgesic improvement in two out of three patients with three sessions done at 3-week intervals [66]. A 12-week study involving four SSc patients resulted in significant pain relief in two of them [67]. CO_2 laser also showed significant pain improvement in six out of nine patients. Common side effects include scarring, hyperkeratosis and ulcer recurrence [63■].

Medical therapies

Table 1 outlines the various systemic medications that have been in use for calcinosis along with their typical dosing and common adverse effects.

Calcium channel blockers

Calcium channel blockers are frequently prescribed for calcinosis and thought to inhibit calcium efflux into tissues, in addition to vasodilation. A retrospective analysis done in a cohort of 78 patients with calcinosis showed that 10 out of 18 treated patients had partial response [68]. However, a case series of 12 SSc patients treated with diltiazem 60 mg three times a day showed only a slight radiographic improvement in calcinosis in three patients over a mean duration of 8 years [69].

Bisphosphonates

Bisphosphonates have shown some benefit in calcinosis associated with juvenile dermatomyositis [70], but large-scale studies in SSc are lacking. They combat calcinosis by decreasing macrophage activation and reducing bone turnover. A retrospective analysis of seven patients treated with intravenous (i.v.) pamidronate (including two patients with SSc) showed improvement in pain and nonprogression of severe calcinosis cutis in five out of seven patients (71%) including one SSc patient. It was given at a dose of 70–75 mg every 12 weeks for a mean duration of 24 months. Osteonecrosis of the jaw occurred in one patient [71].

Minocycline

Minocycline, with its combined antimicrobial and anti-inflammatory properties, is commonly used for calcinosis. Robertson *et al.* [72] described clinical improvement in eight out of nine treated patients in an open-label study with a dose of 50–100 mg daily over a mean duration of 4.8 months.

A large observational study showed clinical improvement in 34 out of 78 patients (43.6%) with multiple courses of 50–200 mg minocycline for 6–12 weeks [73].

Treprostinil

A pilot study of oral treprostinil, a prostacyclin analogue and powerful vasodilator, showed nonprogression of SSc-associated calcinosis radiographically in four out of five patients who completed the trial [74▪▪].

Rituximab

Rituximab is a mAb directed against the CD20 antigen of B lymphocytes, thereby causing B-cell depletion. A patient with extensive calcinosis showed improvement with two courses of rituximab given at a dose of 375 mg/m^2 4 weeks apart [75]. An observational study consisting of eight SSc patients with refractory calcinosis showed that a mean number of 3.12 rituximab cycles (1 g every 2 weeks) showed significant improvement in four patients (50%) with regards to pain, and two of these had near complete radiographic resolution in calcinosis [76]. No serious infections were reported in this study.

Other immunosuppressants

There are a few independent case reports showing some beneficial effect from leflunomide [77], infliximab [78], apremilast [79] and i.v. immunoglobulins [80]. The JAK inhibitor tofacitinib has proven beneficial in calcinosis associated with dermatomyositis but has not yet been evaluated in SSc-associated calcinosis [81].

Intravenous sodium thiosulfate

Intravenous sodium thiosulfate has been a popular treatment for calciphylaxis associated with end-stage renal disease [82,83] and similarly has been employed for recalcitrant calcinotic lesions associated with dermatomyositis. Several case reports showed improvement in pain and reduction in size of the calcinotic lesion [84–86]. This treatment is being explored for calcinosis in SSc patients as well.

Surgical treatments

Surgical excision of calcinotic lesions is an effective approach, particularly for periarticular lesions in the hands causing nerve compression [87▪] and/or severe physical discomfort. Spinal canal stenosis causing radiculopathy treated with decompressive laminectomy results in symptom alleviation [18]. A retrospective analysis done at a single centre showed significant improvement in analgesia in six out of 39 patients who underwent elective surgical debulking of calcinosis. The most common complications included recurrence of calcinosis (15%), delayed wound healing (13%) and wound infection (10%).

CONCLUSION

Although a significant contributor to disease morbidity, very little is known about the origins of calcinosis in SSc. Several studies have established a strong association of calcinosis with long disease duration and features of vascular dysfunction. Further studies are necessary to better understand the role of bone metabolism and inflammation in the pathogenesis of calcinosis. Surgical excision when feasible is the most effective treatment. Topical therapies can be employed for uncomplicated small

Table 1. Systemic medical therapies for calcinosis in systemic sclerosis

Drug class	Mechanism of action	Drug (dosage)	Efficacy	References	Common adverse effects
Calcium channel blockers	Vasodilation by blocking voltage sensitive Ca^{2+} channels on vascular smooth muscle and decrease calcium efflux	Diltiazem (oral 60 mg t.i.d.) X 6.5 years	3/12- PR	Vayssairat et al. [69]	Hypotension, peripheral oedema, heart blocks
		Diltiazem (oral up to 480mg/day)	9/17- PR	Balin et al. [68]	
		Amlodipine (oral 10mg o.d.)	1/1- PR	Balin et al. [68]	
Bisphosphonates	Reduce macrophage activity and bone turnover	Pamidronate (i.v. 70–75 mg every 12 weeks) X 8.6 cycles	5/7- nonprogression	Rauch et al. [71]	Osteonecrosis of jaw, Flu like illness
Tetracyclines	Antimicrobial and inhibit MMPs	Minocycline 50–100 mg (oral o.d.) X 4.8 months	8/9- PR	Robertson et al. [73]	Dizziness, bluish discoloration of calcinotic lesions
		Minocycline 50–200 mg (o.d.)X 6–12 weeks	34/78- PR	Carmen Fonseca et al. [72]	
Prostacyclin agonist	Vasodilation	Treprostinil (oral 0.125 mg TID and increase as tolerated) X 1 year	4/12- Non progression	Chung et al. [74▪▪]	Gastrointestinal intolerance, headache
B-cell depletion	mAb against CD20 antigen of B lymphocytes	Rituximab (i.v. 375 mg/m² every 4 weeks) X 2 courses	1/1- PR	Daoussis et al. [75]	Secondary infections
		Rituximab (i.v. 1 g every 2 weeks) X3.1 cycles	4/8- pain improvement 2/8- PR	Narvaez et al. [76]	
Leflunomide	Inhibit pyrimidine synthesis, anti-inflammatory	Leflunomide (20 mg oral o.d.) X 12 months	1/1- PR	Lee et al. [77]	Liver enzyme elevation
TNF-alpha inhibitors	Suppress TNF-alpha	Infliximab (i.v. 3 mg/kg at 0, 2, 6 weeks and then every 8 weeks) X 41 months	1/1- CR	Tosounidou et al. [78]	Secondary infection, allergic reactions
PDE4 inhibitor	Inhibits IL-17, IL-23 and TNF-alpha	Apremilast (oral 30mg b.i.d.) X3 months	1/1- PR	Qiblawi et al. [79]	Secondary infections
Intravenous immunoglobulin (IVIG)	Suppress macrophage and complement pathway	IVIG (2 g/kg/month) X 3 months	1/1- PR	Schanz et al. [80]	Headache, nausea, dizziness, small increase in risk of thrombosis
JAK inhibitors	Inhibit macrophage and complement pathway	Tofacitinib (5 mg oral b.i.d.) X 3 months	3/3- PR	Shneyderman et al. [81]	Increased risk of thrombosis, malignancy, secondary infections
Intravenous Sodium thiosulfate	Increase solubility of the calcium by compounding and help clearing	12.5–25 g sodium thiosulfate three times a week		Badawi et al. [83] Mageau et al. [84] Song et al. [85]	Fatigue, nausea, vomiting, metabolic acidosis not requiring treatment

b.i.d., twice a day; CR, complete response; IL, interleukin; IV, intravenous; JAK, Janus Kinase; MMPs, matrixmetalloproteinases; o.d., once a day; PR, partial response only; t.i.d., thrice a day; TNF, tumour necrosis factor.

calcinotic lesions but will require long-term adherence to show mild benefit. Systemic medical therapies such as minocycline can be useful in treating the inflammation associated with calcinosis, but immunosuppressants should be used cautiously given the sparsity of evidence supporting their efficacy. Validated outcome measures and larger clinical trials are much needed to establish strong treatment recommendations for this debilitating condition.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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Gastrointestinal involvement in systemic sclerosis: pathogenesis, assessment and treatment

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Purpose of review
The majority of patients with systemic sclerosis (SSc) will experience involvement of their gastrointestinal over the course of their disease. Despite the high prevalence of gastrointestinal involvement in SSc, the strategies pertaining to the assessment and treatment for this clinical dimension of SSc have historically been limited. However, the present review highlights recent research contributions that enhance our understanding of SSc-GI patient subsets and provides updates on pathogenic mechanisms of disease, assessment and symptom-directed management.

Recent findings
In the past few years, several studies have identified risk factors for more severe gastrointestinal disease in SSc and have provided insight to optimize diagnosis and management of SSc-GI symptoms. This article also provides a review of currently available investigations and therapies for individual SSc-GI disease manifestations and reflects on actively evolving areas of research, including our understanding the role of the gut microbiome in SSc.

Summary
Here, we provide important updates pertaining to the risk stratification, assessment, diagnosis and management of SSc patients with gastrointestinal symptoms. These findings provide opportunities to enhance patient care and highlight exciting opportunities for future research.

Keywords
gastrointestinal tract, microbiome, motility, scleroderma, systemic sclerosis

INTRODUCTION

Gastrointestinal tract involvement occurs in nearly all patients with systemic sclerosis (SSc) [1–3]. Gastrointestinal manifestations of SSc often arise early in the disease course, may be progressive in nature and represent a leading cause of morbidity and mortality [2]. Any region of the gastrointestinal tract may be involved, and it is not uncommon for patients to experience simultaneous involvement of different regions at one time. Numerous studies have demonstrated that SSc-GI involvement adversely affects psychosocial functioning, contributing to diminished quality of life, disability, depression and anxiety, and in severe cases, death [4–6].

Although the burden of gastrointestinal disease in SSc is high, treatment options are limited. No approved therapies for SSc-GI manifestations exist. Current treatments largely target symptoms, and there is no evidence that therapies approved for other manifestations of SSc [e.g. interstitial lung disease (ILD)] prevent progression of SSc-GI involvement [7■■]. The lack of objective disease activity measures and trial endpoints for gastrointestinal manifestations of SSc, combined with the heterogenous nature of the natural history of SSc-GI involvement, has hindered our ability to study potential disease-modifying therapies in this clinical area of SSc.

The purpose of the present scoping review is to summarize the clinical features and management of SSc-GI involvement. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for scoping reviews [8], we searched PubMed (January 1990 to May 2022) using

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KEY POINTS

- Diverse clinical manifestations of SSc-GI involvement exist, and careful history taking and diagnostic testing can identify the underlying cause of symptoms.

- Treatment of gastrointestinal manifestations involves a holistic approach combining pharmacotherapy with lifestyle modification, including dietary adaptations.

- Disease-modifying therapies are greatly needed to prevent SSc-GI progression and promising therapeutic targets include the autonomic nervous system, immune system and gut microbiome.

the search terms 'systemic sclerosis' or 'scleroderma' in combination with the terms 'gastrointestinal' and 'gut'. We largely selected publications from the past 18 months; however, high-quality older publications were included. No PubMed filters or limits were applied to maintain a broad search strategy. We also conducted a manual search of references cited in original research studies and review articles on gastrointestinal involvement in SSc to identify additional relevant articles. Although primary research publications were prioritized, review articles are also cited to provide opportunities for further reading on specific topics.

PATHOPHYSIOLOGY

As significant clinical heterogeneity exists in SSc-GI disease [6,9▪,10,11], the underlying pathophysiology is complex and may vary across patient subsets. The classic hypothesis proposed by Sjogren nearly 3 decades ago suggested that SSc-GI disease arises from a three-step sequential process, which includes neural dysfunction, smooth muscle atrophy and fibrosis [12]. However, autopsy studies have revealed that smooth muscle atrophy, rather than significant fibrosis, is prominent in the SSc-GI tract and that areas of atrophy are not associated with significant inflammation or vasculopathy [13,14]. As such, some have proposed that a primary neural or smooth muscle insult drives gastrointestinal dysfunction and that atrophy, not fibrosis, is the primary outcome of this process [13].

A reduction in neuromuscular communication may contribute to smooth muscle atrophy. Growing evidence suggests that enteric neurons and smooth muscle are targeted by the autoimmune response in SSc [15], and that functional autoantibodies to muscarinic 3 receptors (M3R) play an important role in SSc-GI dysmotility in a small subset of patients with rapidly progressive lower bowel disease [16–19].

These antibodies bind to and block the M3R, preventing acetylcholine from binding to and stimulating gastrointestinal smooth muscle. Antibodies to vinculin, RNPC3 and nicotinic acetylcholine receptors in autonomic ganglia (AChR) are also associated with gastrointestinal symptoms in SSc, though these antibodies have not been shown to be pathogenic [20▪▪,21,22]. However, in patients without SSc, anti-AChR antibodies targeting ganglia are known to interfere with cholinergic synaptic transmission and are associated with slow gastrointestinal transit [23].

Dysfunction of the autonomic nervous system likely also contributes to SSc-GI disease [16,24]. The vagus nerve, a key mediator of autonomic function, plays a dominant role in regulating oesophageal motility and lower oesophageal sphincter function, both of which may be disrupted in SSc [25,26]. Autonomic dysfunction is reported among patients with SSc-GI disease, and a higher overall burden of autonomic symptoms correlates with increased overall gastrointestinal severity [24,26], and specifically with anorectal motility disorders [27,28], gastric compliance [29,30] and oesophageal dysmotility and dysfunction [26,30,31]. Furthermore, the frequent overlap between abnormal gastric emptying and oesophageal dysmotility in SSc suggests that common pathogenic mechanisms may exist in difference regions of the gastrointestinal tract [32]. Interestingly, a loss of the Interstitial Cells of Cajal (ICC) is also reported on SSc oesophageal disease. The ICCs are part of the sensory units of vagal afferents, which provide pacemaker activity to the smooth muscles and can generate peristalsis in the absence of innervation. These cells are key mediators of communication between the enteric nerves and smooth muscles, suggesting again that disrupted communications between the gastrointestinal nerves and muscles likely contribute to SSc gastrointestinal dysfunction [13]. In summary, many distinct abnormalities in the neuromuscular communications exist in the gut in patients with SSc. Each of these mechanisms may play a role in driving the clinical gastrointestinal manifestations, although it remains unclear which patients are affected by each type of dysfunction and how much upstream vascular dysfunction disrupts neural control and contributes to disease pathogenesis.

UPPER GASTROINTESTINAL TRACT INVOLVEMENT

Up to 90% of patients with SSc have symptoms of upper gastrointestinal disease [33,34] Patients may present with symptoms of laryngo-esophageal [35–38] or gastroesophageal reflux disease (GERD)

(e.g. hoarseness. oropharyngeal dysphagia, reflux, heartburn), lower oesophageal sphincter (LES) dysfunction [39], oesophageal dysmotility (distal dysphagia) and gastroparesis (e.g. early satiety, bloating, nausea, vomiting and unintentional weight loss) [1,40,41]. Symptoms may occur in isolation, or in combination, which can complicate both diagnosis and management. Gastrointestinal bleeding may also complicate SSc, and arise from esophagitis, oesophageal ulcers, gastritis, gastric ulcers or gastric antral vascular ectasia (GAVE).

Diagnostic testing for upper gastrointestinal tract involvement

As gastrointestinal symptoms in SSc may be attributable to dysfunction in different regions of the gut, diagnostic testing may be helpful in identifying affected areas, particularly because certain gastrointestinal therapies preferentially target specific gastrointestinal regions.

In patients who have mild upper gastrointestinal symptoms, lifestyle management with or without over-the-counter gastrointestinal medications are the recommended first-line interventions prior to testing. If oropharyngeal dysphagia is present, blood work to screen for elevations in muscle enzymes and/or relevant antibodies may be appropriate [36]. If symptoms persist despite negative testing, a modified barium swallow study can be helpful in evaluating the swallow function (Table 1). If nondiagnostic, further evaluation with laryngoscopy through ENT may be warranted [38].

The diagnostic testing for GERD refractory to first and second-line therapies usually begins with an upper endoscopy (EGD) to screen for abnormalities in the oesophageal and gastric mucosa (Table 1).

Such abnormalities may include findings such as oesophageal strictures, esophagitis or gastritis, opportunistic infections (e.g. *Candida* esophagitis), Barrett's oesophagus (reported in up to 12% of women with SSc) or even a malignancy [42]. Importantly, several distinct mechanisms may contribute to symptoms of GERD in SSc. In patients with symptoms of distal dysphagia and/or persistent symptoms of GERD despite high-dose acid blocking agents, a high-resolution oesophageal manometry study (HREM) with pH testing and impedance may be warranted. HREM may be useful in differentiating between patients with normal vs. abnormal oesophageal motility (i.e. ineffective oesophageal motility or absent peristalsis). HREM can also identify abnormal lower oesophageal sphincter pressures and hiatal hernia, which may impact therapeutic decisions. Furthermore, these findings can be helpful for patient risk stratification. For example, absent contractility and a hypotensive lower oesophageal sphincter on HREM are risk factors for Barrett's [42]. In addition, a multiple rapid swallow study, which may be performed during manometry [43], can identify patients with peristaltic reserve, which is a good prognostic indicator of long-term oesophageal function [44].

Gastric emptying and/or whole gut transit testing (i.e. scintigraphy or smart pill) also play an important role in the assessment of refractory upper gastrointestinal symptoms in SSc (Table 1). A recent study found that SSc patients with gastroparesis by scintigraphy were likely to have other areas of abnormal transit in the gut. Combined liquid and solid gastric emptying studies were found to be more sensitive in detecting delayed gastric transit compared with solid gastric emptying studies (74 vs. 55%, respectively). Moreover, percentage liquid

Table 1. Common diagnostic tests for upper gastrointestinal tract involvement in systemic sclerosis

Test	Purpose
Modified barium swallow study (i.e. Video fluoroscopic swallowing study)	Impaired swallowing and/or clearance of food and liquids
Laryngoscopy	Abnormal laryngeal structure or function (e.g. impacts swallowing, breathing, cough)
Barium swallow	Evaluate for stricture, obstruction, GERD
Upper endoscopy	Evaluate oesophageal and gastric mucosa
High resolution oesophageal manometry	Evaluate for upper or lower oesophageal sphincter dysfunction, oesophageal dysmotility and hiatal hernia
pH impedance testing	Determine the amount of reflux that occurs in a typical 24-h period, whether symptoms are attributable to reflux episodes and whether acid suppressive therapy is adequate
H. pylori breath test	Diagnose active *H. pylori* infection, and determine whether treatment cured an *H. pylori* infection
Gastric emptying study	Screen for gastroparesis

emptying correlated best with Reflux ($\rho= -0.33$, $P = 0.01$) and Distension ($\rho = -0.30$, $P = 0.03$) scores on the UCLA GIT 2.0 survey [10].

Treatment of upper gastrointestinal tract involvement

The treatment of oropharyngeal dysphagia involves diagnosing and treating the underlying cause of symptoms and reducing the risk of aspiration [41] (Table 2). Although H2 blockers and proton pump inhibitors remain the standard of care for GERD, a new class of acid blocking agents is emerging. These potassium-competitive acid blockers inhibit proton pump potassium–exchange and do not depending on gastric acid for activation. One of these medications, known as vonoprazan, is available in Japan and can facilitate the healing of erosive esophagitis and improve reflux symptoms in patients with refractory GERD [45■,46,47]. If the diagnostic work-up suggests that oesophageal dysmotility and/or a hypotensive LES is driving the symptoms of refractory reflux, prokinetics may be considered for symptom control [48,49]. The addition of

prokinetics to PPI therapy in a large cohort of non-SSc patients with refractory GERD recently demonstrated that combination therapy resulted in improved QoL and fewer reflux episodes [50]. Buspirone was also found to alleviate upper gastrointestinal symptoms in SSc. In an open-label trial, buspirone significantly increased LES pressures and decreased symptoms of heartburn and regurgitation in patients with SSc patients who were already taking PPI [39]. Although data in favour of prokinetic use for the management of oesophageal dysmotility in SSc is limited, a trial of prokinetics may be considered if dysmotility is present.

Surgery in SSc is usually reserved for refractory cases; however, a recent systematic review was conducted to determine whether surgical treatment is feasible and well tolerated in SSc patients with refractory GERD. A total of seven studies, including 101 patients were included, and 63 patients (62.4%) underwent open fundoplication, 17 (16.8%) laparoscopic fundoplication, 15 (14.9%) Roux en-Y gastric bypass (RYGB) and six (5.9%) esophagectomy. Recurrent symptoms were identified in up to 70% undergoing fundoplication and 30% of patients

Table 2. Treatment for the most common upper gastrointestinal tract symptoms in systemic sclerosis

Symptoms and causes	Intervention and effect on the GI tract
Oropharyngeal dysphagia	
From laryngeal oesophageal reflux: Treat GERD (see below)	Reduces laryngeal irritation
From myositis: immunosuppression	Improves laryngeal muscle function
Distal dysphagia	
From stricture:	EGD with dilation will allow bolus to pass more easily
From dysmotility:	Promotility agent (e.g. metoclopramide, bethanechol, pyridostigmine) to enhance smooth muscle contraction
From infection or esophagitis:	Treat infection or inflammation to alleviate tissue irritation
GERD	
Aggravated by suboptimal habits: Lifestyle modification	Avoid large meals and eating within 3-4 h of laying down. Minimize intake of aggravating foods. Sleep with head of the bed elevation
Suspicion of too much acid exposure of unclear cause	Antiacid therapy to reduce acidity of reflux and/or reduces reflux episodes
From oesophageal dysmotility and food not passing efficiently	Promotility agent (as above) to enhance oesophageal transit
From a weak lower oesophageal sphincter	Tighten LES to reduce reflux (e.g. buspirone, metoclopramide)
From gastroparesis:	Treat gastroparesis with dietary modification and promotility agents if needed (see below) to improve transit and accommodation
Early satiety and nausea	
From gastroparesis:	Consider gastroparesis diet (smaller more frequent meals, reduce fibre and fat, eat solids first); Consider supplemental medication if needed (e.g. promotility agent, appetite stimulant, medications that help with gastric accommodation) to improve transit, accommodation and nausea

undergoing RYGB, although minimally invasive RYGB was thought to be feasible and well tolerated based on short-term results [51].

Lower gastrointestinal tract involvement

Dysmotility is the defining feature of lower gastrointestinal tract involvement in SSc and may be present in the small intestine, large intestine and/ or the anorectum [52]. In contrast to the upper gastrointestinal tract, lower gastrointestinal tract involvement typically arises in patients with established SSc and less commonly presents in patients with very early or early SSc [53]. However, the symptoms of lower gastrointestinal tract involvement are among the most troubling symptoms SSc patients experience, as they can profoundly affect a patient's social functioning and emotional well being. For instance, many patients will avoid eating outside of the home or traveling if it involves a long journey. This can lead to social isolation and may contribute to feelings of helplessness and anxiety.

Small intestine involvement

Dysmotility of the small intestine occurs in 40–88% of patients with SSc based on manometry studies [54]. Symptoms often include distension and bloating; prolonged episodes of either constipation or diarrhoea are also common. Weight loss can occur, and although the differential diagnosis for weight loss in SSc is broad (Table 3), malabsorption should be considered in any patients with unintentional weight loss. Diagnostic tests include abdominal x-rays, small intestinal manometry, scintigraphy, wireless motility capsules and computed tomography (CT)/MRI enterography [40] (Table 4).

Small intestinal bacterial overgrowth (SIBO) [55] is estimated to occur in 30–62.5% of patients with SSc [56]. The cause of SIBO in SSc is likely multifactorial and may be due to use of agents that

Table 4. Diagnostic tests for lower gastrointestinal tract involvement in systemic sclerosis

Test	Pathologic findings
Abdominal x-ray	Dilated bowel loops; Tightly packed valvulae conniventes
Intestinal manometry	Low-amplitude contractions; Absent or prolonged migrator motor complexes
Scintigraphy	Slow colonic transit
Wireless motility capsules[a]	Slow colonic transit
CT/MRI enterography	Small intestine involvement; Extraluminal pathology
Hydrogen or methane breath tests	SIBO
Colonoscopy	Obstructing lesions; Mucosal inflammation; Telangiectasias
Barium swallow	Obstruction; Pseudo-obstruction
Defecography	Rectal outlet obstruction
Video capsule endoscopy	Intra-luminal small intestine pathology
Faecal fat, pH tests; Measurement of fat soluble vitamin levels	Malabsorption
Endoanal ultrasound or MR pelvis	Soft tissue masses; Atrophy of internal anal sphincter
Surface electromyography	Sphincter faecal incontinence

[a]Please note, wireless motility capsules are contraindicated in patients with known, severe gastroparesis or GI strictures.

suppress gastric acids, dysmotility of the small and/or large intestine, as well as a weakened ileocecal valve. Regardless of cause, symptoms of SIBO are highly disruptive and may include nausea, vomiting, early satiety, bloating, diarrhoea, excessive flatulence and weight loss [57]. Hydrogen and methane breath tests after an oral glucose or lactulose bolus are the most commonly used diagnostic tests for SIBO [58], although the sensitivity for these tests is suboptimal, with some studies reporting a sensitivity of only 62% [59]. Interestingly, prospective

Table 3. Differential diagnosis for weight loss in systemic sclerosis

Increased caloric output	Decrease caloric intake
Increased work of breathing	Decreased appetite
Increased effort to move due to physical challenges (e.g. arthropathy, myopathy, joint contractures, diffuse skin disease)	Difficulty with mechanical digestion (e.g. decreased oral aperture, poor dentition, dry mouth)
Increased inflammation due to underlying SSc	Difficulty swallowing
Increased inflammation due to infection[a]	SIBO, Malabsorption
Increase psychosocial stress related to SSc or external life stressors	Medication side effect (e.g. diarrhoea, nausea, vomiting)

[a]Patients with SSc are at an increased risk for infection due to various factors, including malnutrition, immunosuppressant medications, abnormal organ architecture (i.e. parenchymal lung disease causing interstitial abnormalities) and aberrant immune function due to underlying SSc.

studies have demonstrated that a sizable percentage of patients with SSc have evidence of SIBO based on breath testing, even in the absence of gastrointestinal symptoms [56]. These findings are consistent with recent research demonstrating that alterations in the lower gastrointestinal tract microbiota are a feature of patients with early SSc [60■]. Therefore, it is plausible that dysbiosis is not necessarily a consequence of dysmotility in SSc; instead, dysbiosis may be a driver of dysmotility, similar to irritable bowel syndrome [61].

Pneumatosis (cystoides) intestinalis is a rare complication of SSc that is associated with multiple gas-filled cysts arising within the wall of the intestine [62,63]. Several hypotheses for pathogenesis have been proposed, including: (1) a disruption in muscosal integrity during immunosuppression; (2) the production and absorption of gaseous products from bacterial carbohydrate fermentation; (3) increased wall permeability from SSc-related smooth muscle atrophy and fibrosis [64–68]. A population-level review, not specific to scleroderma reported that the colon was more commonly impacted than the small bowel. Pneumoperitoneum may complicate the clinical picture, though oftentimes surgical intervention is not required.

Large intestine involvement

Constipation is the most common clinical feature of large intestine involvement in SSc [53]. Dysfunction of neuropathic and myopathic processes contributes to delayed colonic transit leading to symptoms such as distension or fullness after meals, abdominal pain

Table 5. Treatment for the most common lower gastrointestinal tract symptoms in systemic sclerosis

Symptoms and intervention(s)	Predominant effect of intervention on GI tract
Constipation	
Docusate sodium	Softens stool through increasing osmotic pressure
Senna	Stimulates peristalsis and increases fecal water content
Bisacodyl	Stimulates peristalsis in the colon; increases fluid and salt secretion
Milk of Magnesia	Softens stool through increasing osmotic pressure
Lactulose	Softens stool through increasing osmotic pressure
Linaclotide, Plecanatide, Lubiprostone	Actively stimulate secretion of electrolytes and water into the intestinal lumen and accelerate colonic transit
Prucalopride	Accelerates GI motility, FDA approved for chronic constipation
Pyridostigmine	Accelerates GI motility
Small frequent meals	Stimulate natural peristalsis
Diarrhoea	
Loperamide	Inhibit peristalsis; use with caution
Limit foods high in FODMAPs, especially raw fruits and vegetables	May lessen symptoms
Fluid resuscitation	Treat dehydration
Increased foods naturally high in probiotics and prebiotics	Potentially improve bacterial balance in GI tract
SIBO	
Antibiotics	Potentially improve bacterial balance in GI tract
Limit consumption of simple carbs (white flour, white sugar	May lessen symptoms
Anorectal dysfunction	
Physiotherapy	Improve pelvic floor strength
Biofeedback	Re-enforce connection between central nervous system and
Sacral nerve stimulation	Improve incontinence
Percutaneous tibial nerve stimulation	

and straining during bowel movements [69]. Risk factors for delayed colonic transit include female sex, presence of telangiectasia, presence of anticentromere antibodies, past history of smoking and a Medsger gastrointestinal severity score of at least 3 [9■]. After constipation, diarrhoea is the second most common clinical feature of large intestine involvement in SSc. The diarrhoea may be due to various causes, including paradoxical (i.e. overflow) diarrhoea and/or overzealous treatment of constipation with stimulant laxatives.

Intestinal pseudo-obstruction affects approximately 10% of patients [70]. Associated with delayed colonic transit [71], pseudo-obstruction results from the inability to move intestinal luminal contents forward in the absence of a mechanical obstructive process. Patients complain initially of nausea and abdominal pain and eventually the inability to pass flatus and increasing abdominal girth [72]. Although intestinal pseudo-obstruction does not represent a true obstructive process, this condition is often recurrent, necessitates hospitalization in many cases and can be fatal [73].

Anorectum involvement

Involvement of the anorectal dysfunction occurs in 50–70% of patients with SSc [74]. Faecal incontinence is the most common symptom and is largely due to neuronal dysfunction [75]. Rectal prolapse can also occur. In this scenario, patients will perceive a bulging sensation in their anus and complain of chronic stool leakage [76]. One small study reported a high recurrence rate of rectal prolapse occurs after restorative surgery [77], rendering the treatment of this complication challenging. Other anorectal clinical manifestations include haemorrhoids, which can develop secondary to chronic constipation in SSc.

Treatment of lower gastrointestinal tract involvement

As symptoms of lower gastrointestinal tract involvement may arise due to dysfunction in different regions of the lower gastrointestinal tract [e.g. diarrhoea may be due to faecal incontinence (anorectum), malabsorption (small intestine) and/or colonic dysmotility (large intestine)], the first step in the management is to identify the underlying cause of symptoms [1,3,41]. Careful history taking, combined with diagnostic testing (Table 4), can often reveal the driving factor for symptoms [41]. Treatment is then tailored according to the suspected underlying cause. It can take time to establish an effective treatment regimen for lower gastrointestinal tract symptoms. The optimal approach combines

pharmacological interventions with lifestyle modifications, including dietary adaptations (Table 5).

CONCLUSION

Of all the organ systems affected in SSc, the gastrointestinal tract has the most diverse clinical manifestations related to SSc and the least number of evidence-based treatment options available. This paradox represents a significant challenge for patients and their healthcare providers. Emerging research on novel motility measurement modalities, and the roles of the autoimmune response and gut microbiome in SSc have the potential to propel this field forward and improve how we care for patients who suffer from gastrointestinal complications of SSc.

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Conflicts of interest

There are no conflicts of interest.

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Contribution of keratinocytes to dermal fibrosis

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Purpose of review

The cellular pathogenesis of fibrotic disorders including systemic sclerosis (SSc) remains largely speculative. Currently, the altered function of endothelial cells and fibroblasts under the influence of an inappropriate immune response are considered central pathogenic events in SSc. Adding to this complexity, novel evidence here reviewed suggests that keratinocytes may concur in the development of skin fibrosis.

Recent findings

Epidermal equivalents (EE) generated from primary SSc keratinocytes display a distinct gene expression program when compared to healthy donor (HD) EE. SSc-EE, among others, exhibited enhanced oxidative and metabolic response pathways. Immunohistochemical studies demonstrated similarities between SSc-EE and SSc epidermis including altered keratinocyte differentiation, enhanced expression of activation markers, and reduced rate of basal keratinocytes proliferation. SSc-EE supernatants more than HD-EE modified the inflammatory and extracellular matrix deposition/resorption program of dermal fibroblasts. Further evidence indicated that the relative lack rather than the excess of interleukin-25 in keratinocytes may contribute to enhanced dermal fibrotic changes. Overall, these data support keratinocyte-intrinsic SSc-related modifications.

Summary

Improved methods for engineering epidermal and skin equivalents are helping to address the question whether keratinocyte alterations in SSc are primary and capable to dysregulate dermal homeostasis or secondary following dermal fibrotic changes.

Keywords

fibroblast, fibrosis, inflammation, keratinocyte, scleroderma

INTRODUCTION

The potential contribution of the epidermis and in particular of keratinocytes to the pathogenesis of dermal fibrosis has been neglected for long time. Recent work, however, has shown that the homeostatic relationships normally regulating the crosstalk between epidermal and dermal cell constituents is altered in scleroderma, thus reinforcing the concept that keratinocytes may take part to scleroderma pathogenesis. Previous work, exploring physiological conditions, demonstrated that keratinocytes stimulate fibroblasts, mainly through the production of interleukin (IL)-1, inducing in fibroblasts the production of keratinocyte growth factor (KGF) also known as fibroblast growth factor 7. In turn, fibroblasts affect keratinocyte viability, proliferation, and differentiation mainly by producing KGF. This interrelationship is modulated by a variety of other factors which participate to homeostatic crosstalk between keratinocytes and fibroblasts reviewed in [1]. Importantly, healthy keratinocytes were known to affect extracellular matrix (ECM) deposition and resorption by influencing fibroblasts, favoring resorption over deposition. Under this perspective, previous work addressing SSc-specific epidermal alterations showed that compared to healthy donor (HD), SSc keratinocytes conditioned media increase type-I collagen (Col-I) production by enhanced oncostatin M (OSM) production [2], an effect independent from transforming growth factor (TGF)-β [3], favoring enhanced IL-1-dependent gel contraction in which both TGF-β and endothelin-1 (ET-1) were needed [4]. By enhanced connective tissue growth factor (CTGF) and S100A9 production, SSc keratinocyte could also favor fibroblast production of Col-1 as well as fibroblast migration and

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proliferation [5]. Interestingly, SSc fibroblasts were reported to respond to keratinocyte conditioned medium with higher production of Col-I and a similar production of matrix metalloproteinase-1 (MMP-1), resulting in an increased ratio of col-I over MMP-1, suggestive of decreased ECM turnover [6].

PROGRESS IN RECONSTITUTED SKIN MODELS

When addressing the role of the epidermis and dermis in SSc pathogenesis, it has to be taken into consideration that these anatomical structures are not faithfully reproduced in *in vitro* systems in which both keratinocytes and fibroblasts are cultured in 2D systems and when the respective conditioned media are used to stimulate the cell counterpart. Although a method for isolating and culturing skin cells, including endothelial cells, fibroblasts, keratinocytes, and melanocytes from small SSc skin biopsies has been published [7], increased efforts in the field of tissue engineering have recently led to the development of 3D human models with improved intercellular interactions and tissue microenvironment observed in skin fibrosis [8]. Self-assembled or scaffold-based models exploit the ability of fibroblasts to generate 3D dermal-like constructs, producing their own ECM. The addition of TGF-β or other pro-fibrotic regulators is a simple mean of inducing fibrotic shift [9]. Incorporation of SSc fibroblasts results in a model with altered collagen structure, characterized by a more mature and aligned fibrillar structure, leading to increased stromal rigidity and upregulation of innate immune signaling genes [10]. The layering of keratinocytes on overlapping sheets of self-assembled fibroblasts, followed by exposure to air–liquid interphase, leads to the formation of a fully stratified epidermis (i.e. containing basal, spinous, granular and corneal layers), making these models appropriate for mimicking the interaction between the dermal and epidermal compartments [11]. Of interest, immune cells may be included within self-assembled skin (saS) models. Thus, peripheral blood monocytes incorporated in skin equivalents – generated from SSc fibroblasts and SSc keratinocytes – differentiated into M2-like cells and resulted in increased thickness and stromal rigidity. Of interest, greater numbers of M2-like macrophages populated SSc-saS compared with HD-saS, thus suggesting a reciprocal activation between macrophages and fibroblasts resulting in increased tissue thickness and stiffness [12▪▪]. Decellularization of living tissue is also a promising approach and a porcine, decellularized, intestine scaffold has been exploited to reconstruct human skin equivalent by the sequential seeding of endothelial cells, fibroblasts and keratinocytes resulting in a fully polarized epidermis, dermis and a functional vasculature. This model recapitulated key features of SSc skin upon TGF-β stimulation, including the transdifferentiation of fibroblasts into myofibroblasts and excess ECM deposition and was sensitive to nintedanib treatment [13]. Additional models have been established in the recent past. One consisted on human skin equivalents fully generated from healthy or SSc donor skin cells *in vitro* then grafted onto SCID mice. This model allowed the replication of the fibrotic phenotype when grafted tissues were of SSc origin. Interestingly, in this *in vivo* model the generated skin from healthy donors acquired *bona fide* SSc characteristics when humanized monoclonal antibodies specific for the platelet derived growth factor (PDGF) receptor were injected into the graft [14]. An additional model was based on the organotypic culture of healthy, full, human skin obtained by esthetic surgery which showed enhanced collagen and reduced MMP-1 production under the influence of TGF-β. In this model, the exposure to IL-17 resulted in enhanced production of MMPs counteracting the profibrotic activity of TGF-β [6]. Future expected developments in tissue engineering potentially useful to address pathophysiological aspects of skin fibrosis including the influence of keratinocytes on the development or resolution of dermal fibrosis could be based on the use of induced pluripotent stem cells (iPSCs) [15] or organ-in-chip technologies [16]. Thus, future efforts will help in investigating the subtle modification governing and altering the crosstalk between epidermis and dermis in fibrotic conditions.

KERATINOCYTES IN DERMAL FIBROSIS

A powerful method to test whether *ex vivo* SSc may differ from HD keratinocytes would be the study of differentially expressed genes detected by gene

arrays or RNA sequencing in bioptic tissues or single cell preparations. However, such studies have principally focused on fibroblast, endothelial cells, and inflammatory infiltrating cells rather than on keratinocytes. This notwithstanding by comparing genes differentially expressed in full skin biopsies from 61 SSc from the Genetics vs. Environment in Scleroderma Outcome Study cohort to 36 HD, it was found among others that enriched pathways included 'keratinocyte differentiation' in the subgroup of SSc individuals positive for anticentromere antibodies [17]. In another study, by comparing the signature scores in early vs. late diffuse disease, the keratinocyte signature was higher in late disease [18]. When performing consensus clustering and meta-analysis on three genome-wide datasets, a community containing modules enriched for keratinocyte-specific processes was found to make contact with the inflammatory module [19]. Thus, while sparse, unbiased analytical approaches based on gene expression are hinting to keratinocyte alterations in SSc. Future keratinocyte-centered studies should provide stronger evidence of their potentially dysregulated genes in SSc epidermis.

Systemic sclerosis epidermal equivalents

Engineered epidermal equivalents (EE) were used by us to investigate whether keratinocyte activation and differentiation as well as gene expression could distinguish EE generated by using primary SSc keratinocytes from their HD counterpart [20■■]. Interestingly, SSc-EE exhibited aberrant differentiation, enhanced expression of activation markers, and a lower rate of basal keratinocyte mitosis, reproducing most of the abnormalities histologically detected in SSc epidermis. RNA sequencing analysis revealed that, compared to HD-EE, SSc-EE were characterized by lower expression of homeobox gene family members. Using the Hallmark gene set from the Molecular Signatures Database both metabolic and oxidative stress molecular pathways resulted to be enhanced in SSc-EE. This is in agreement with previous abundant experimental data supporting a role of oxidative stress in the pathogenesis of SSc [21]. We went further and could demonstrate that conditioned medium generated from EE enhanced the production of IL-6, IL-8, MMP-1, Col-I, and fibronectin by fibroblasts. Notably, this effect was twofold higher in the presence of conditioned medium generated form SSc EEs (with the exception for Col-I and fibronectin). These data support the notion that SSc keratinocytes have an intrinsically altered differentiation program, which is maintained when keratinocytes adapt to in vitro conditions, while proliferating and generating a stratified epidermis

in the absence of cues derived from other cell types or from structured dermis [20■■]. Further work should test whether epigenetic mechanisms may be involved.

Less is more

The interesting, understudied possibility that the relative lack rather than the excess of a particular soluble mediator of inflammation could be involved in the pathogenesis of scleroderma received recent attention. Starting from the hypothesis that IL-25 (member of the IL-17 family, known also as IL-17E), acknowledged for being produced by epithelial cells, could be involved in fibrotic processes, we assessed its presence in SSc skin and found that its expression was reduced in SSc compared to HD epidermis. In EE generated using primary HD keratinocytes IL-25 regulated several molecular pathways related to wound healing and ECM remodeling and the conditioned medium from IL-25-primed keratinocytes enhanced the fibroblast production of MMP-1, IL-6, IL-8, but not of Col-I nor fibronectin. However, IL-25 significantly reduced the production of Col-I when applied directly to fibroblasts. This evidence was supportive for the role of IL-25 in participating to skin homeostasis and its decreased expression in SSc could contribute to skin fibrosis by favoring ECM deposition over degradation [22■]. Similarly, from the functional point of view, the EBI3 (constituting IL-35 in conjunction with p35) expression was found by others to be decreased in keratinocytes of the SSc epidermis compared to HD. Of interest, the injection of EBI3 in the skin of mice enhanced fibrosis. Indeed, EBI3 was able to downregulate the protein and mRNA expression of type I or type III collagen, in the presence or absence of TGF-β, by reducing collagen mRNA stability [23]. Thus, the keratinocyte-specific decrease of two distinct cytokines characterizes SSc epidermis. This decreased expression appears to have functional consequences in homeostatic processes regulating dermal ECM turnover favoring fibrosis. Future studies should address the therapeutic potential of targeted therapies aiming at increasing the keratinocyte production of such cytokines.

Keratinocytes in additional models of skin fibrosis

There are some similarities between SSc and the sclerodermatous chronic form of the graft versus host disease (cGVHD) in terms of skin fibrosis. A major difference may be due to the mechanism of damage of the epidermis which may be mediated by allogenic CD8+ T cells in cGVHD. In this respect, a recent publication reports an increased production

of TGF-β by epidermal cells, especially of the basal cell layer, in samples from patients with cGVHD compared to acute GVHD and HD. By establishing a model of cGVHD-like sclerodermatous changes in genetically modified mice transferred with cytoxic CD8 T cells specifically targeting keratinocytes, these authors observed reduced fibrosis when CD8 T cells were interferon-gamma (IFN-γ) deficient. The reduced fibrotic reaction correlated to a lower expression of TGF-β by keratinocytes undergoing apoptosis. These results indicate that keratinocytes produce higher amounts of TGF-β1 in the presence of IFN-γ when undergoing apoptosis, thus participating to sclerodermatous changes [24].

Taking advantage from the integration of single-cell and bulk transcriptome data among the dysfunctional cell types expressed in hypertrophic scars, a fibroproliferative skin disorder characterized by excessive ECM deposition, the proportion of a particular subtype of keratinocytes named by the authors KC-2 was reduced when compared to healthy skin. Cell-cell communication analysis revealed intercellular contacts between some fibroblast types and KC-2. Interestingly, syndecan 4 (SDC4), a receptor expressed also in KC-2 which could bind multiple ligands, was downregulated in hypertrophic scars, suggesting that the reduced proportion of KC-2 and apoptotic phenotype of KC-2 might be associated with the downregulation of SDC4 [25]. In summary, evidence gathered in experimental models of fibrotic skin diseases highlight the role of keratinocytes and their involvement in processes associated to dermal fibrosis.

ARE ABNORMALITIES IN SYSTEMIC SCLEROSIS KERATINOCYTES PRIMARY OR SECONDARY?

The question arises whether the morphological and functional alterations described in SSc keratinocytes and skin are secondary to pathological events arising elsewhere, in particular in the dermis or whether they are not the consequence but rather the cause of other pathologic features, including increased ECM deposition (Fig. 1). Or as a further possibility whether these abnormalities arise in parallel under the influence of factors driving keratinocyte, fibroblast, and other cell types altered activities. No firm answer is available to this question at the time being. Nonetheless, we would like to stress evidence generated in animal models which may support the contention of a primary role of keratinocytes in the cascade of pathogenic events leading to SSc.

The first report addressing this issue took advantage from gene silencing of the transcription

factor Friend leukemia virus integration 1 (Fli1) in keratinocytes [26]. Fli1 was known to be constitutively suppressed in dermal fibroblasts, dermal microvascular endothelial cells, and perivascular inflammatory cells in lesional and nonlesional SSc skin, therefore implicated in SSc pathogenesis. The authors generated keratin 14 (K14) – expressing epithelial cell-specific Fli1 knockout mice, which spontaneously developed dermal and esophageal fibrosis with epithelial activation. Furthermore, these mice developed autoimmunity and interstitial lung disease, thus demonstrating the potential instructive role of ''altered'' keratinocytes in SSc pathogenesis. As a note of caution in interpretating these findings, the authors were able to demonstrate that Fli1 directly regulated AIRE expression in epithelial cells including in the thymus and its absence resulted in down-regulation of autoimmune regulator (AIRE), a transcription factor involved in central tolerance. This leaves open the door on whether thymic abnormalities were also mechanistically responsible of the scleroderma-like phenotype developing in keratinocyte Fli1 KO mice [26].

Second, a manuscript recently deposited in bioRxiv shows that the transcription factor Snail is overexpressed in the epidermis of SSc patients and a transgenic mouse in which Snail is specifically hyper-expressed in keratinocytes under the K14 promoter is sufficient to induce several features of human SSc. Mechanistically, the authors show that the matricellular protein Mindin is produced by Snail transgenic skin keratinocytes and favor fibrogenesis by inducing inflammatory cytokines and collagen production in resident dermal fibroblasts [27].

Additional work should clearly provide further data in favor of an instructive role of keratinocytes in directing dermal fibrosis. Nonetheless, the evidence here summarized provides ground to address this interesting question.

CONCLUDING REMARKS

The observations that SSc epidermis has an abnormally increased number of stratified keratinocytes, that the expression of several keratinocyte products distinguish SSc from HD, that epidermal equivalents generated from primary SSc keratinocytes display a distinct gene expression program compared to the HD counterpart, that primary SSc keratinocytes cultured *in vitro* respond differently to exogenous stimuli compared to HD are all pieces of evidence indicating that SSc-specific molecular cues characterize keratinocytes and epidermis. These peculiarities may be primarily or secondarily part of wider SSc dysfunctions resulting, among others, in abnormal ECM deposition and fibrosis. Future work should address
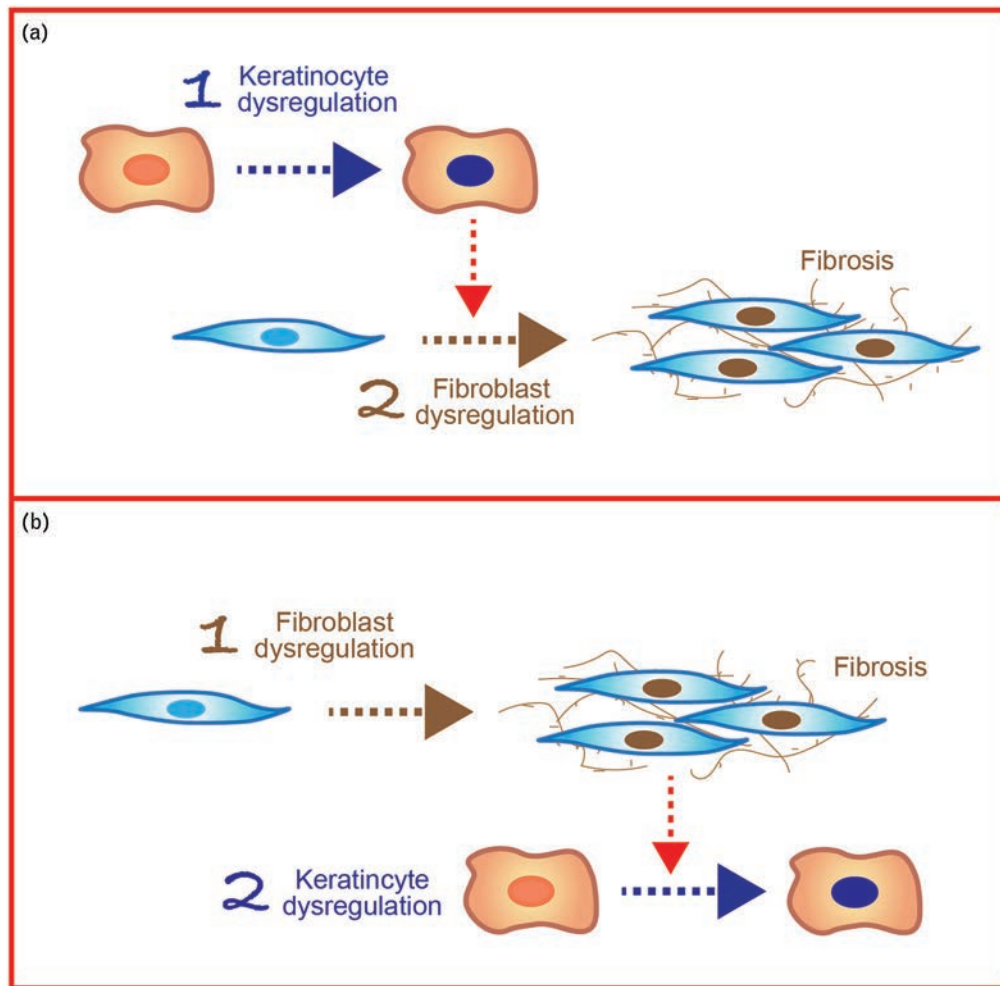
FIGURE 1. Potential interactions relevant to keratinocyte and fibroblast dysregulation in SSc. (a) Keratinocyte precedes and modulates fibroblast dysregulation. (b) Fibroblast precedes and modulates keratinocyte dysregulation. Dashed lines indicate processes. The changes in the color of the nuclei indicate differential gene expression programs.

the question whether cell-autonomous keratinocyte abnormalities may drive secondary abnormalities in cells populating the dermis or whether they respond to cues generated in the SSc-dermis whether due to cell signals or structural constrains associated with fibrosis and increased dermal stiffness. Single cell gene expression studies investigating SSc in comparison to HD epidermis as well as improved methods of epidermis and skin *in vitro* engineering using primary cell lines generated from individuals affected by various clinical forms of early or late SSc should bring solid answers to these questions. In any case, it remains an attractive perspective the possibility of targeting keratinocyte abnormalities to harness clinical scleroderma, which continues to be an incurable disease.

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Conflicts of interest

C.C. has received honoraria from Boehringer Ingelheim and CSL Behring. Other authors declare no conflicts of interest in relationship to this manuscript.

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Role of cellular senescence in the pathogenesis of systemic sclerosis

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Purpose of review
Systemic sclerosis (SSc) is a chronic rheumatic disease that is characterized by immune activation, vasculopathy and fibrosis of the skin and internal organs. It has been proposed that premature onset of ageing pathways and associated senescent changes in cells contribute to the clinical and pathological features of SSc. The aim of this review is to critically review recent insights into the involvement of cellular senescence in SSc.

Recent findings
Cellular senescence plays a critical role in SSc pathogenesis, particularly involving endothelial cells and fibroblasts. Immunosenescence could also contribute to SSc pathogenesis by direct alteration of cellular functions or indirect promotion of defective immune surveillance. Molecular studies have shed some light on how cellular senescence contributes to fibrosis. Recent and planned proof-of-concept trials using senotherapeutics showed promising results in fibrotic diseases, including SSc.

Summary
There is increasing evidence implicating cellular senescence in SSc. The mechanisms underlying premature cellular senescence in SSc, and its potential role in pathogenesis, merit further investigation. Emerging drugs targeting senescence-related pathways might be potential therapeutic options for SSc.

Keywords
cellular senescence, scleroderma, senotherapeutics

INTRODUCTION

Systemic sclerosis (SSc) is a systemic autoimmune disease of unknown cause and incompletely understood pathogenesis. SSc is characterized by progressive fibrosis, vascular involvement and inflammation in the skin and multiple internal organs. The disease shows substantial patient-to-patient heterogeneity, follows a chronic and often progressive course, and is associated with substantial disability and high mortality. There is currently no cure or disease-modifying therapy for SSc, and management is suboptimal and largely symptom-based.

The peak incidence of SSc is reported to occur between 45 and 64 years of age [1]. Notably, age at disease onset was one of the characteristics that best predicted patient survival; advanced age was associated with worse survival [2]. Similarly, in the Scleroderma Lung Study I and II, older age was also associated with increased mortality [3]. In addition, pulmonary, renal and cardiac complications are more prevalent in older SSc patients [4,5]. Could the generally greater age at disease onset, higher frequency of SSc diagnosis in patients older than 45 years and higher mortality in older patients imply that SSc represents a disease of accelerated ageing? Here, we will review recent experimental data supporting the involvement of cellular senescence, a hallmark of ageing, in SSc. The potential of using drugs targeting cellular senescence, so-called senolytic therapies, in treating SSc will also be discussed.

HALLMARKS OF CELLULAR SENESCENCE

Originally dismissed as a cell culture artifact, cellular senescence is now viewed as a fundamental cellular mechanism of normal tissue homeostasis, disease

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KEY POINTS

- Cellular senescence, an irreversible exit from the cell cycle with both beneficial and harmful biological functions, is a hallmark of ageing, and contributes to age-related health decline through direct and indirect mechanisms.

- Accumulating evidence support the presence and potential pathogenic roles of cellular senescence in fibrosis in a variety of conditions, including SSc.

- Cellular senescence might be directly responsible for the emergence of pathogenic myofibroblasts in SSc, and their apparent resistance to apoptosis.

- Emerging evidence suggests that senolytic therapy is feasible for fibrotic conditions, and might be associated with clinical improvement and reduction of tissue senescent cell burden.

and ageing. Cellular senescence is involved not only in embryonic development but also in tissue remodelling such as wound healing [6,7]. In certain circumstances, cellular senescence acts as a protective mechanism. For instance, cellular senescence suppresses malignant transformation by preventing cancer cell proliferation [8]. However, cellular senescence can also promote cancer development by altering the cellular microenvironment, thus acting as a double-edge sword. Indeed, persistent tissue accumulation of senescent cells could negatively impact homeostasis, leading to inflammation and age-related diseases.

Depending on how it is triggered, cellular senescence can be classified as replicative senescence, physiological senescence, drug-induced senescence and stress-induced senescence (Fig. 1). In addition to irreversible growth arrest and inability to replicate DNA, senescent cells undergo a series of morphological and molecular alterations that distinguish them from multiplying cells. Cells undergoing senescence have enlarged, flattened and irregular cell shapes. This is typically coupled with increased numbers of lysosomes, which express β-galactosidase and contain lipofuscin granules. The mitochondria in senescent cells produce reactive oxygen species (ROS) and express high levels of antiapoptotic proteins. Senescent cells are also characterized by nuclear changes, including enlarged nuclei, impaired nuclear integrity, global epigenetic changes of the chromatin landscape, formation of senescence-associated heterochromatin foci (SAHF) and DNA segments with chromatin alterations reinforcing senescence (DNA SCARS). Senescence is associated with overexpression of cell cycle inhibitors, particularly p21 and p16, which block

cyclin-dependent kinases (CDKs)-cyclin complexes from phosphorylating retinoblastoma protein (Rb), leading to cell cycle arrest. Another striking feature of cellular senescence is the expression of a suite of proteins termed the senescence-associated secretory phenotype (SASP). The SASP comprises a variety of biologically active factors including growth factors, cytokines, chemokines, extracellular matrix (ECM) proteins and enzymes. These secreted factors signal in both autocrine and paracrine fashions, and can themselves induce senescence in neighbouring cells, allowing senescence to spread. This cellular senescence cascade is depicted in Fig. 1.

IMPLICATIONS OF CELLULAR SENESCENCE FOR SYSTEMIC SCLEROSIS PATHOGENESIS

Do senescent changes in various cell types implicated in the pathogenesis of SSc contribute to disease development and progression? Here, we summarize the phenotypic and functional changes of senescent cells and discuss the implication of these changes in the context of SSc-associated vasculopathy, inflammation and fibrosis.

Vasculopathy-endothelial cells

Senescent endothelial cells show characteristic changes in gene expression and function including lower nitric oxide production due to downregulation of eNOS, and increase in expression of PAI-1, which is one of the hallmark factors of SASP (Fig. 2). These endothelial alterations are associated with functional changes, including lower angiogenic and proliferative capacities, as well as inflammation, atherogenesis and increased thrombosis risks [9]. Notably, senescent endothelial cells also undergo endothelial-to-mesenchymal transition (Endo-MT), a process that contributes to organ fibrosis [10▪]. Recent studies reveal that these changes phenocopy the hallmarks of SSc endothelial cells [11–13], suggesting that cellular senescence is associated with, and might play a role in, endothelial dysfunction in SSc.

Vasculopathy-vascular smooth muscle cells

Alterations in vascular smooth muscle cells (VSMCs) contribute to the proliferative vasculopathy noted in SSc. VSMCs isolated from SSc skin biopsies showed increased proliferation and viability, and resistance to apoptosis, compared with VSMCs isolated from healthy skin [14]. In contrast, senescent VSMCs showed lower proliferative capabilities but display a pro-inflammatory SASP characterized by
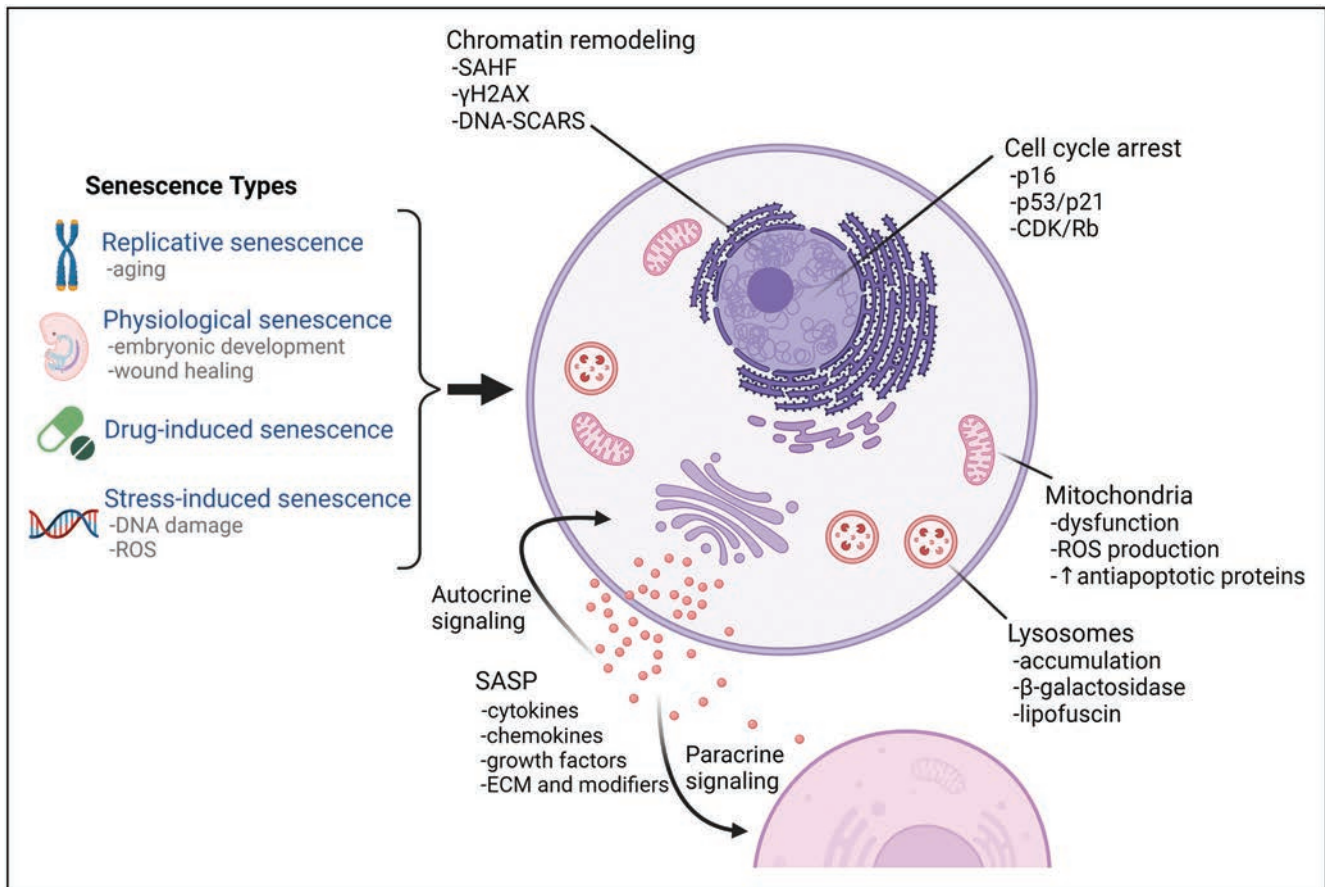
FIGURE 1. Hallmarks of cellular senescence. The senescent phenotypes are classified by how they are triggered: replicative senescence, physiological senescence, drug-induced senescence and stress-induced senescence. The cellular mechanisms and alterations of senescence include chromatin remodelling, cell cycle arrest, mitochondrial and lysosomal changes, and expression of senescence-associated secretory phenotype (SASP). These secreted factors signal not only in an autocrine fashion to reenforce the senescent phenotype, but also paracrinally to affect neighbouring cells.

secretion of MMP-9 and IL-1 [15]. Production of ECM components such as collagen is downregulated in these cells. In addition, it has been shown that VSMC senescence promotes atherosclerosis and necrotic core formation [16]. Taken together, the data suggest that VSMC senescence does not directly contribute to proliferative vasculopathy in SSc, as they are unable to replicate. However, it is possible that senescent VSMCs promote expansion of a subset of VSMCs into synthetically active cells indirectly through SASP, contributing to the proliferative vasculopathy phenotype in SSc.
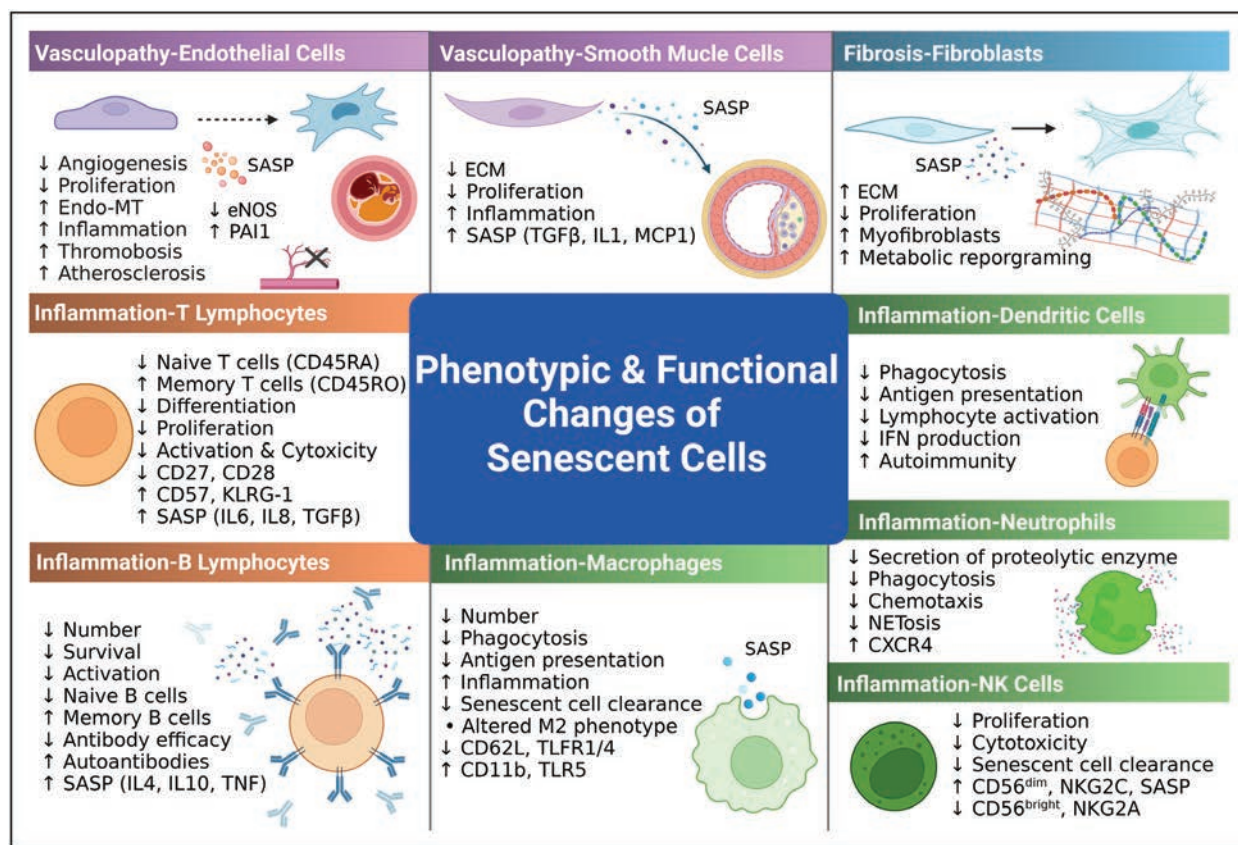
Fibrosis-fibroblasts

In addition to growth arrest, senescent fibroblasts are characterized by increased ECM production, increased glycolysis and other metabolic changes, and various features of activation, including myofibroblast transformation [17]. The secretome of senescent fibroblasts comprises proinflammatory cytokines (TNF-α, TGF-β, IL-6), chemokines (MCP-1), growth factors (FGF, CTGF, PDGF), MMPs and other bioactive factors [18■]. These secreted proteins can act in a self-amplified network to affect the local microenvironment and spread the senescent signal to neighbouring cells. All of these properties of senescent fibroblasts have the potential to contribute to SSc fibrosis.

Immune cell senescence

When senescence occurs in cells of the immune system, it alters long-term immune responses, and consequently affects the capacity of the host to fight infections and other inflammatory processes. Indeed, immunosenescence disturbs both innate and adaptive immunity, both of play significant roles in SSc pathogenesis [19]. In general, age-related immune alterations include increase in monocytes, decreased lymphocytes and naive cells, and increased memory cells (Fig. 2).

FIGURE 2. Summary of phenotypic and functional changes of senescent cells. Cells that play critical roles in SSc pathogenesis are listed. Senescence in stromal cells (endothelial cells, vascular smooth muscle cells and fibroblasts) is associated with promoting SSc vasculopathy and fibrosis. Immunosenescence, which is associated with alteration of immune functions in both innate and adaptive immune subsets, could also promote autoimmunity and inflammation pertinent to SSc.

Inflammation-innate immunity

Dendritic cells are central in coordinating immune responses and play key roles in maintaining tolerance and immunity. In the circulation and in the skin, these cells produce inflammatory mediators to activate other immune cells as well as fibroblasts to promote fibrosis [20,21]. When senescent, dendritic cells show impaired antigen presentation, interferon production and phagocytosis, leading to an increased risk of autoimmunity (Fig. 2). The ability of neutrophils to chemotax, migrate, form NETs and phagocytize is impaired with age. Senescent neutrophils express high levels of CXCR4 and become responsive to SDF-1α [22]. Although neutrophils from SSc patients have been shown to harbour functional defects [23], other studies suggest that NET formation is augmented in these SSc [24,25]. It is thus difficult to assess the contribution of neutrophil senescence in SSc pathogenesis. In SSc, natural killer (NK) cells show decreased expression of chemokine and activation receptors [26]. Moreover, CD56^{bright} NK cells, which produce cytokines but are weakly cytotoxic, are reduced in SSc patients

[27,28]. These resemble some of the properties of senescent NK cells (Fig. 2). Macrophage senescence is associated with defective phagocytosis and reduced potency, as shown by decreased IL-10 production by M2 macrophages [29,30]. Macrophages from SSc patients show an activated and profibrotic transcriptome and phenotype [31,32]. It is still unclear whether senescent macrophages contribute to SSc pathogenesis, although senescent macrophages and their SASP promote inflammation and fibrosis in a mouse model of radiation-induced pulmonary fibrosis [33].

Inflammation-adaptive immunity

SSc is associated with significant changes in adaptive immunity. Abnormal activation of B cells is prominent in SSc [34,35], and B cells are expanded with increased naive population and reduced activated memory B cells [36–38]. In addition, regulatory B cells are decreased and functionally impaired in SSc, as shown by reduced IL-10 production [39]. Interestingly, activated B cells in SSc produce higher

levels of IL-6 and TGF-β compared with ones from healthy controls [40]. The numbers and percentages of B cells are significantly decreased with age [41]. There is also a shift in the proportion of different B cell subsets. Late memory B cells, which are defined as CD19+IgG+IgD-CD27- B cells, are increased in elderly compared with younger individuals [42]. In contrast, IgD-CD27+ B cells are significantly decreased. Senescent B cells show intrinsic defects such as decreased somatic hypermutation, defective class switch recombination and reduced antibody affinity and neutralization capacity. It also results in increased SASP production and autoantibody production [43]. The senescent phenotype of B cells is summarized in Fig. 2. Senescent B cells, or so-called age-associated B cells (ABCs), have been implicated in autoimmune and autoinflammatory disorders, including systemic lupus erythematosus [44,45], however, their role in SSc has not been fully elucidated. An early study suggested that ABCs, defined as CD11c+CD21- B cells, are present in higher frequency in the blood of SSc patients [46]. Comprehensive characterization of these cells and determination of their mechanistic implications in SSc warrant further research.

A declining CD4+/CD8+ ratio in T cells is associated with immunosenescence [47]. Similar to B cells, ageing causes shrinking of the population of naive T cells and an increase in memory T cells [48]. These T cells lose the expression of CD27 and CD28, but express NK markers including CD57 and KLRG-1 [49]. Senescent T cells include a fraction of cells that are CD45RO+, termed T effector memory cells [50]. Functionally senescent T cells acquire a proinflammatory phenotype, harbouring features of Th1, Th17, Tfh and Treg cells [51]. They produce SASPs that are characterized by pro-inflammatory cytokines. In SSc, there is an increased proportion of CD27-CD28- in CD8 T cells in the circulation [52]. CD4+CD28- T cells are also reported to be expanded [38]. In addition, CD8+CD28- T cells, which produce high levels of IL-13, have been shown in SSc skin [53]. Th17 cells are expanded in SSc [54]. CD4+/CD8+ ratio is increased in SSc patients compared with controls. CD4+ T cells display an activated phenotype in SSc with elevated activation markers CD69 and GITR [54]. The apparent impact of senescent T cells in SSc is not known. It has been shown that adoptive transfer of senescent T cells into young mice accelerated angiotensin-induced cardiovascular damage and kidney fibrosis in an IFN-γ-dependent manner [55]. The expansion of CD8+CD28- T cells in SSc implies that at least a subset of T cells undergo senescence in this disease. Indeed, these cells have been shown to undergo rapid replication compared with CD8+CD28+ T cells, coupled with

significantly shortened telomeres, suggestive of a replicative senescent phenotype [56].

Together, these studies indicate that cellular senescence might play a critical role in SSc pathogenesis, particularly involving endothelial cells and fibroblasts. Immunosenescence could contribute to SSc pathogenesis in two distinct ways: senescent immune cells can directly induce inflammation and autoimmunity through their altered cellular functions; and senescent immune cells are defective in immune surveillance, including for senescent cells. Impaired ability of senescent macrophages, NK cells and CD8+ T cells to eliminate senescent cells might indirectly promote SSc pathogenesis. Indeed, it has been shown that senescent cells are predominantly cleared by the immune system [57].

CELLULAR SENESCENCE HAS BEEN IMPLICATED IN FIBROTIC DISEASES INCLUDING SYSTEMIC SCLEROSIS

Cellular senescence and fibrosis have been linked. Although cellular senescence plays a beneficial role in physiologic repair and wound healing, persistence of senescent cell accumulation and their release of SASP factors promote fibrosis [58]. As mentioned earlier, cellular senescence can result from various types of stress, including telomere attrition, DNA damage, oxidative stress and even mitochondrial dysfunction [59,60■■,61,62]. There is accumulating evidence that cellular senescence is elevated in fibrotic diseases. For instance, in idiopathic pulmonary fibrosis (IPF) and SSc-associated interstitial lung disease, epithelial cells and fibroblasts display a senescent transcriptome signature and significantly increased levels of the senescence marker p16INK4A [63■■,64,65].

By secreting SASP, including ECM proteins (fibronectin, various collagens and laminins), the matrix remodelling proteases (MMP-1, 3, 10, 12, 13 and 14) and growth factors implicated directly in fibrosis (TGF-β, PDGF, IL-6) [66,67], senescent cells promote chronic inflammation [68], epithelial-to-mesenchymal transition (EMT) [69] and profibrotic phenotype changes of fibroblasts and macrophages [70] in a paracrine fashion. Fibroblasts explanted from skin biopsies of SSc patients with early diffuse disease displayed significantly elevated levels of the senescence effectors *CDKN2A* (encoding p16), *TP53*, *PAPPA*, *IGFBP*s, *PDGFB*, *TNF*, chemokine ligands and multiple ECM remodelling genes (unpublished data). The association of senescence in SSc is also shown at the genomics level. Whole exome sequencing of microdissected areas of dermal fibrosis in skin biopsies from patients with early disease with severe skin/lung involvement revealed the

presence of large number of somatic mutations [71▪▪]. The mutation pattern exhibited a clock-like 'senescence' signature that resemble what is seen in cancer. The authors speculate that genomic instability and somatic hypermutation is critical in SSc pathogenesis that drives fibrosis and inflammation.

As shown in numerous studies, senescent IPF fibroblasts are less sensitive to cytotoxic and pro-apoptotic signals, resulting in their accumulation in tissues [64,72]. Overexpression of α-smooth muscle actin and ECM components by senescent fibroblasts promotes the development of fibrosis [73]. The apoptosis-resistant phenotype of senescent lung fibroblasts and myofibroblasts has been attributed to multiple mechanisms. In IPF, senescent fibroblasts exhibit decreased levels of the pro-apoptotic proteins Bak and Bax, and increased levels of the anti-apoptotic proteins Bcl-2 family proteins Bcl-2, Bcl-W and Bcl-XL [17,74,75]. This imbalance of pro- and anti-apoptotic proteins contributes to failure of senescent fibroblasts to be properly eliminated. Senescent IPF lung fibroblasts are also highly resistant to apoptosis induced by Fas ligand and TNF-associated apoptotic ligand. In these cells, levels of FasL, TRAIL and caveolin-1 (Cav-1) are reduced, while AKT activity is elevated [76].

SENOTHERAPEUTICS: TARGETING CELLULAR SENESCENCE IN SYSTEMIC SCLEROSIS

As summarized above, increasing evidence links cellular senescence to the pathogenesis and progression of SSc. Senescent cells accumulate in fibrotic skin and lung. Assuming that their persistent accumulation in these tissues is detrimental, could targeting cellular senescence represent a viable therapeutic strategy?

Senotherapeutics describes a field focused on therapeutically eliminating or disabling senescent cells in a variety of human conditions [77,78]. Examples of senotherapeutics include senolytics, which are drugs that remove senescent cells, or senomorphics, which are compounds that modulate SASP expression and function. It is perhaps not surprising that there is also substantial interest in targeting cellular senescence in fibrotic diseases. Recent pilot studies provide some evidence for the efficacy of 'senolytic' therapy in the treatment of fibrotic diseases, including SSc. In a single-arm, open-label clinical trial, 12 SSc patients with interstitial lung disease were treated with the putative senolytic drug dasatinib (a tyrosine kinase inhibitor) for 9 months. Three patients (25%) showed clinical improvement, which correlated with a decrease in the level of a senescence-related gene set in skin biopsies [79]. In

contrast, patients who failed to show clinical improvement with dasatanib therapy showed no change in the senescence signature. These intriguing pilot observations suggest that dasatanib might have targeted senescent cells in the skin, which possibly contributed to the clinical improvement in these patients. It is unclear why only a subset of treated patients demonstrated clinical improvement and a decline in senescence gene signature levels; of note, 'improvers' in this trial tended to have higher senescence signatures prior to therapy compared with 'nonimprovers'. Another pilot study sought to evaluate senolytic treatment in IPF, using a 'senolytic cocktail'. In this open-label clinical trial, intermittent combination therapy with dasatinib as well as quercetin (a flavanol) for 3 weeks resulted in significant improvements in physical performance, although pulmonary functions and circulating levels of senescence and fibrosis markers remained unchanged [80]. In another open-label short-term phase 1 pilot study, patients with diabetic kidney disease were treated with the senolytic cocktail. In this study, once-daily oral administration of 100 mg dasatinib along with 1000 mg quercetin for 3 days was associated with a reduction in the epidermis and adipose tissue of p16INK4A-positive cells (38%) and p21CIP1-positive cells (30%) [81].

To date, there is a scarcity of clinical evidence on the effectiveness and safety of senotherapeutics, including systemic effects and side effects of this treatment strategy. Most senotherapeutics currently being investigated are repurposed drugs or dietary interventions. However, the next generation of senotherapeutics, including navitoclax, HSP90 inhibitors, as well as senomorphic drugs including JAK inhibitors and rapamycin, are on the horizon. It is likely that effective senolytic therapy can be administered episodically rather than chronically, potentially limiting adverse effects, as opposed to senomorphics that need continuous administration.

CONCLUSION

Experimental evidence supports the concept that cellular senescence is present in SSc and plays a critical role in disease establishment and progression. Potential use of senotherapeutics in SSc would be expected to be an effective treatment approach. Clearly, many substantial hurdles remain before larger clinical trials of senolytic therapy for fibrosis can be considered. It will be necessary to further assess the specificity of such therapies in killing senescent cells, and characterize their cytotoxic effects, to demonstrate their long-term safety, and to identify reliable markers for response [82▪]. Furthermore, adding senotherapeutics as a combination therapy in conjunction with

other drugs modulating various pathways in SSc pathogenesis may have added benefits and result in improved outcome for this complex and potentially fatal disease.

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Conflicts of interest

There are no conflicts of interest.

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Insights into molecular and clinical characteristics of very early systemic sclerosis

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Purpose of review
The early heterogenous presentation of systemic sclerosis (SSc), in particular without skin involvement, has been a confounding factor delaying early diagnosis. In fact, early signs of SSc as Raynaud's phenomenon and puffy fingers, are also typical of other connective tissue diseases (CTDs) such as mixed CTD and undifferentiated CTD. In the last decade, a significant effort has been dedicated in defining molecular characteristics that could be used as early SSc biomarkers. In this narrative review, we address the present situation where several clinical scenarios are in search of a correct positioning into the prescleroderma (pre-SSc) phase as well as in the very early phase of SSc.

Recent findings
Literature data showed that a part of patients classified as sine scleroderma SSc (ssSSc), mixed CTD and undifferentiated CTD may already belong to the very early phase of SSc, thus having a different pattern of progression to SSc. Recently, the very early diagnosis of systemic sclerosis (VEDOSS) criteria has been validated.

Summary
while the area of pre-SSc still remains fuzzy, the VEDOSS study has shown that a 'window of opportunity' does exist also for SSc. In the very next future, this may allow to start the treatment to prevent the disease progression to a more advanced fibrotic stage.

Keywords
prescleroderma, systemic sclerosis, very early diagnosis of systemic sclerosis

INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease (CTD) characterized by a wide clinical heterogeneity, ranging from a milder subset to a more severe one, with a rapid widespread involvement of internal organs and skin fibrosis [1].

According to the 1980 American College of Rheumatology (ACR) classification criteria, it was necessary to detect skin fibrosis to classify a patient as SSc. It should be noticed that usually skin fibrosis is the main pathognomonic characteristic of SSc even if it is typical of the advanced phase. In the last 4 decades, these criteria have been widely used also as diagnostic criteria, despite their poor sensitivity for detecting early SSc patients. In fact, the use of these criteria has delayed either the disease diagnosis or the therapy [2]. Over the years, several attempts have been made to overcome the limits of the ACR 1980 criteria [3,4] The early heterogenous presentation of SSc, in particular without skin involvement, has been a confounder which does not allow a clear orientation and reach the diagnosis. Moreover, early signs of SSc as Raynaud's phenomenon and puffy fingers, are also typical of other CTDs such as mixed connective tissue disease (MCTD) and undifferentiated connective tissue

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KEY POINTS

- Early signs of SSc as Raynaud's phenomenon and puffy fingers are aspecific and typical of other CTDs such as mixed mixed CTD, undifferentiated CTD as well as of systemic sclerosis sine scleroderma: this can delay diagnosis in particular in absence of skin involvement.

- The very early diagnosis of SSc and the identification of progressive patients is of paramount importance because half of all incident organ manifestations occur simultaneously within 2 years from the onset of Raynaud's phenomenon.

- Most of the molecular SSc markers are present already in VEDOSS patients characterized by very few disease signs.

- Patients with the same molecular activation may share a common biological process and similar specific signalling pathways.

- VEDOSS represents a 'window of opportunity' for SSc, because a targeted therapeutic strategy, proportional to the disease features, may be chosen to stop disease progression.

disease (UCTD), and this element has further created confusion in SSc diagnosis [5,6].

It is well known that Raynaud's phenomenon is the most typical presenting sign of UCTD (80%) [5]. The definition of UCTD includes a wide spectrum of clinical pictures as patients with recent onset of symptoms and unclassifiable clinical pictures, patients with 'stable UCTD', a distinct clinical entity characterized by at least one clinical manifestation of connective tissue diseases, positive antinuclear antibodies (ANA) and disease duration of at least 3 years and patients with 'organ-dominant' conditions and ANA or other serological features or mild clinical symptoms [6]. Literature data report that from 4 to 15% of UCTD patients evolved to a definite SSc over the years [7]. In MCTD patients, Raynaud's phenomenon and puffy fingers are the most common presenting symptoms also in MCTD patients [8]. In the early stages, patients often present with one of the following features: Raynaud's phenomenon, puffy fingers, sclerodactyly, arthralgias, arthritis, myalgias, myositis and malaise [9]. Furthermore, Cappelli *et al.* showed that out of 161 patients, initially diagnosed as MCTD, after a mean follow-up of 7.9 years, 93 (57.9%) still had the diagnosis of MCTD while 17.3% were diagnosed with SSc [8]. For the abovementioned reasons, a part of patients classified as MCTD and UCTD [8,10] are already cases that may belong to the very early phase of SSc, thus having a different pattern of progression to SSc.

In this narrative review, we address the present situation where several clinical scenarios are in search of a correct positioning into the early phase of SSc, ranging from the pre-SSc to the very early diagnosis of systemic sclerosis (VEDOSS), to sine scleroderma SSc (ssSSc), also passing through the UCTD/MCTD confounders.

THE MYTH OF SINE SCLERODERMA SYSTEMIC SCLEROSIS

To date, several studies showed that sine scleroderma SSc (ssSSc) is a distinct clinical entity. In 1989, ten SSc-interstital lung disease (ILD) men without skin involvement, later defined as ssSSc, were characterized by positive ANA, oesophagal dysmotility, ILD, Raynaud's phenomenon and abnormal nailfold capillaries. About 6/10 developed skin sclerosis from 4 months to 7 years after ssSSc diagnosis [11].

In 1996, Fine *et al.* [3] provided a revised definition of SSc subsets, including also ssSSc defined as patients without skin involvement but with ANA and specific antibodies, as well as ILD, scleroderma renal crisis, cardiac or gastrointestinal involvement.

In 2000, Poomorghim *et al.* evaluated patients with ssSSc, comparing them with patients with SSc and limited cutaneous involvement (lcSSc). Out of 555 patients without diffuse SSc they found 49 (9%) ssSSc patients. They reported that other than the absence of skin thickening, the ssSSc group had no significant differences in internal organ involvements, laboratory features, serum autoantibody type or survival rate compared with patients with lcSSc. This leads them to suggest to include ssSSc in the disease classification [12].

Some years later, in another study six SSc-ILD (usual interstitial pneumonia and nonspecific interstitial pneumonia) patients did not present skin thickening, sclerodactyly and digital oedema: some had telangiectasia, four had Raynaud's phenomenon with abnormal nailfold capillaroscopy (NVC) modifications, all gastroesophageal reflux, and three oesophagal dysmotility. All were nucleolar ANA positive and only one was topo I positive, whereas the others were anti-Th/To positive. These authors suggested that the presence of the clinical features, despite the absence of skin involvement were already defining SSc in particular in presence of ILD [13].

In 2012, Tolosa *et al.* studied patients from the Spanish register and investigated either preSSc (defined by Raynaud's phenomenon, SSc NVC changes, specific autoantibodies without skin thickening) and ssSSc (defined by Raynaud's phenomenon, scleroderma clinical features and SSc-specific autoantibodies without skin sclerosis) proposing a

Table 1. Criteria defining presystemic sclerosis and very early diagnosis of systemic sclerosis

	Raynaud's phenomenon	Specific ATB	Capillaroscopy	Puffy fingers	Digital ischemic changes
1995, pre-SSc, Fine [3]	X	X	X		X
2001, pre-SSc, LeRoy and Medsger [4]	X	X		X	
2021, VEDOSS, Bellando Randone *et al.* [37▪▪]	X	X	X	X	

ATB, auto-antibodies; SSc, systemic sclerosis; VEDOSS, very early diagnosis of systemic sclerosis.

modification of the Leroy-Medsger criteria [4,14]. In 2013, 79/947 Brasilian SSc patients (8.3%) were identified as ssSSc with a significant esophageal involvement (83.1%) and ILD (63.2%) [15].

In 2014, Simeón-Aznar *et al.* found that there were no significant differences in disease features between ssSSc and lcSSc and that ssSSc Spanish patients fulfilled ACR criteria much less than lcSSc (13%/77%, $P < 0.0001$). They concluded that ssSSc and lSSc were practically similar but suggested to consider ssSSc a different subset to avoid misdiagnosis and to deepen internal organ investigation [16].

In 2021, 33 ssSSc patients were studied by De Almeida Chaves *et al.* [17] The majority of patients were anticentromere positive (26, 33%), whereas one was topo I positive and one PmScl positive.

In 2022, in 1054 SSc Portuguese patients, Freitas *et al.* identified preclinical SSc in 13% and ssSSc in 3.3%, as well as puffy fingers in 62.1% dSSc and 47.3% lSSc ($P < 0.01$). It is important to highlight that the authors did not provide the definition of preclinical SSc neither addressed the VEDOSS criteria in their work to identify very early patients [18].

Finally, ssSSc has also been described in children: seven out of 52 Juvenile SSc patients were characterized by classic SSc clinical features but without skin involvement: six patients presented primary myocardial involvement and three secondary to pulmonary arterial hypertension; two patients died, whereas one had heart transplantation. These data suggest that ssJSSc has a high morbidity as well as a significant mortality [19].

In practice, ssSSc might be either a distinct disease subset or just a very early phase of SSc in which the skin is not yet involved. For this reason, some ssSSc cases may belong to the very early phase of SSc (VEDOSS) where the skin involvement is practically represented by puffy fingers only.
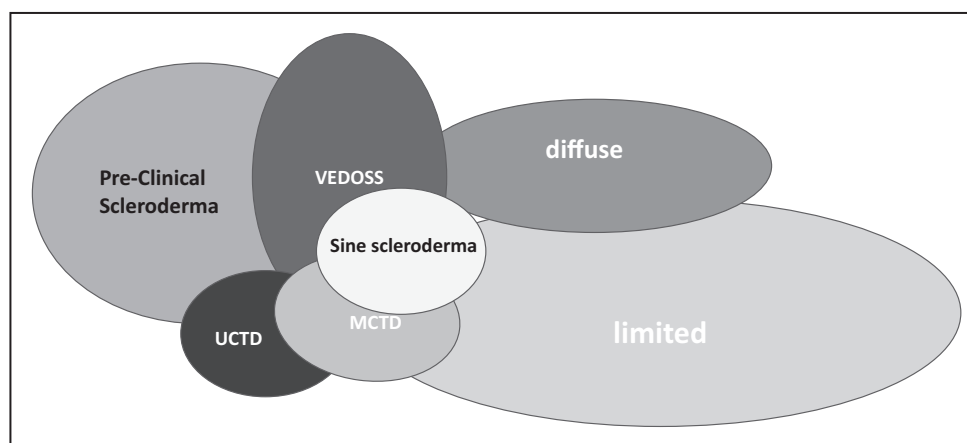
THE VERY EARLY DISEASE

The terminology identifying patients in the early phase of SSc has been heterogeneous over the last decades. In 1995, the pre-SSc was coined for patients with Raynaud's phenomenon, nailfold capillary

modifications, specific autoantibodies (topo I, centromere or nucleolar) and digital ischaemic changes [3] (Table 1). In 2001, LeRoy and Medsger [4] provided criteria for the limited subset of SSc (lSSc) defining a group of pre-SSc having Raynaud's phenomenon and either an SSc-type nailfold capillary pattern or SSc-selective autoantibodies, without puffy fingers and any other sign of definite SSc (Table 1). In 2013, the ACR–European League Against Rheumatism (EULAR) classification criteria significantly increased the sensitivity to classify SSc patients with a minimal skin involvement. Today, even though the early detection of SSc is possible, it is a challenge to identify patients at a high risk of progression into definite SSc [20]. The very early diagnosis of SSc (VEDOSS) and the identification of progressive patients is therefore of paramount importance because half of all incident organ manifestations occur simultaneously within 2 years from the onset of Raynaud's phenomenon [21]. However, at disease onset, the high variability of disease presentation (Fig. 1) and severity may make difficult the identification of predictors of morbidity and mortality. Therefore, patient stratification is important to guide treatment decisions.

Furthermore, it has been suggested that the intrinsic gene expression could help to in a more homogeneous classification of SSc patients [22,23]. Patients with the same molecular activation may share a common biological process and similar specific signalling pathways. This may link the molecular subset with the clinical response to a specific treatment. In SSc, immunophenotypic abnormalities have been observed. In fact, higher proportions of activated Th1 and Th17 cells and an abnormal B-cell differentiation may suggest that a stratification of SSc patients may be possible based on the immune cell phenotype, thus allowing a targeted treatment.

MOLECULAR CHARACTERISTICS OF THE VERY EARLY DISEASE

The detection of autoantibodies against specific nuclear antigens such as centromere proteins A

FIGURE 1. The scleroderma cluster of disease is presented, starting from prescleroderma to very early diagnosis of systemic sclerosis and definite systemic sclerosis (diffuse and limited disease). Undifferentiated connective tissue disease and mixed connective tissue disease are also attached to the area of prescelroderma and very early diagnosis of systemic sclerosis.

and B, Topoisomerase or specific subunits of RNA polymerase 3, among others, carries a strong diagnostic value for SSc because of both their sensitivity and specificity for the disease. Beyond their diagnostic role, specific autoantibodies are also associated with differential risk for type and severity of organ involvement, including skin fibrosis, pulmonary artery hypertension or ILD, suggesting that they are somehow implicated in specific molecular events leading to the different organ manifestations [24]. Significantly, the same antibodies are detectable quite sometime before the onset of clinical manifestations, with time span ranging from 1 to 4 years for anti-Topoisomerase antibodies and up to 10 years or more for antibodies against centromere proteins [25]. This observation could lead to speculate that the molecular events leading to tissue fibrosis (for which anti-Topoisomerase antibodies have a positive predictive value) are quicker than the ones associated with vascular fibrosis (which is more frequent in patients with anticentromere antibodies). Despite these speculations, the observation of these antibodies before the clinical onset of disease has been the main driver for the research into early detection and the definition of early and very early disease [26,27].

In fact, despite there has been so far no evidence supporting a direct pathogenetic role for ANA antibodies in the molecular mechanisms responsible of disease, their presence and their specificity is clearly supporting the notion that the autoimmunity associated with or leading to the clinical manifestations of Scleroderma is already present for quite sometime before the onset of the symptoms.

The only exceptions to this observation are the Raynaud's phenomenon and also the Gastroesophageal Reflux, which are often present in the very early disease [21] together with autoantibodies. Therefore,

it could be hypothesized that the tissue modifications associated with these SSc clinical manifestations could be associated with autoimmunity.

Beyond autoantibodies, in the last 10 years, since the definition of early SSc and VEDOSS, there has been a multitude of studies detecting SSc biomarkers before the onset of clinical symptoms. These include mainly biomarkers of immune activation, including CXCL4 plasma concentration, the expression of interferon-inducible genes or the concentration of their protein products [28–30]. More recently, it has also been shown that skin biopsies from patients without clinically detectable skin fibrosis already show pathognomonic SSc signs [31▪▪]. These included increased extracellular matrix accumulation as assessed by Masson Trichrome, increased perivascular infiltration by CD45 positive cells, and initial derangement of CD31 staining, all histopathological markers of SSc. These latter observations suggest that the clinical detection of skin thickening may happen quite late in the pathogenesis of tissue damage and that we may start to postulate the concept of a biological diagnosis of SSc based on the detection of molecular SSc signatures.

In conclusion, most of the molecular SSc markers are present already in VEDOSS patients characterized by very few disease signs. This observation, may support an early intervention as well as define the evidence of an SSc disease continuum.

CLINICAL CHARACTERISTICS OF VERY EARLY DISEASE

The VEDOSS is today a reality. The possibility to reach a very early diagnosis is supported by criteria of suspicion represented by the three red flags – Raynaud's phenomenon [26,32], puffy fingers,

ANA – that are then supported for a final diagnosis by the presence of abnormal NVC and SSc-specific autoantibodies. The very early diagnosis opens the possibility to exploit the window of opportunity also for a very early treatment [26,27,33]. In fact, VEDOSS represent, at the moment, the earliest SSc stage, where the organ damage is not yet established because the disease has not yet fully progressed to fibrosis remaining still in a phase of subclinical internal organ involvement [21,34].

In the last years, several studies analysed the VEDOSS characteristics in a different group of patients with Raynaud's phenomenon and at least one manifestation of SSc, in search of predictors for the progression to SSc.

Recently, Blaja *et al.* highlighted the heterogeneity of VEDOSS patients: 102 patients did fulfil neither the 2013 ACR/EULAR nor the 1980 ACR classification criteria but had a clinical expert diagnosis of SSc with Raynaud's phenomenon and additional features as puffy fingers, SSc-specific antibodies, SSc pattern on NVC, or any organ involvement characteristic for SSc. Authors reported that these patients could be divided in an early and in a very mild long-standing SSc, at risk of progression. This study showed that these two subgroups of early/mild SSc cannot be differentiated based on clinical features at first presentation and that they need to be differently followed up and different considerations for therapeutic interventions than patients with very early disease at risk of progression [35■■].

Valdirene *et al.* [36■■] in a cross-sectional single-centre study evaluated a cohort of 217 patients showing that among patients with Raynaud's phenomenon the combination of VEDOSS characteristic were the strongest predictors of progression to SSc at a median follow-up of 4 years.

Moreover, the data from the EUSTAR multicentre study showed that Raynaud's phenomenon patients stratification could be achieved according to their risk of developing definite SSc at 24, 36 or 60 months. Specifically, Raynaud's phenomenon patients with SSc-specific autoantibodies and either puffy fingers or nailfold capillary changes have more than 80% risk of progressing to definite SSc within 5 years, and around 50% risk at 30 months [37■■].

THE UNMET NEED AND THE THERAPY

Today, when patients are diagnosed as VEDOSS, UCTD or MCTD, they may be exposed to the risk of progression of internal organ involvement and complications because of a 'wait and see' strategy. However, in case an immunosuppressive treatment is decided, the risk of an overtreatment is real in those cases that will not progress. Therefore, the treatment in VEDOSS patients, and also in other conditions like pre-SSc and UCTD/MCTD with SSc features, is today an unmet need. In fact, this is an important decision to be taken as an opportunity to prevent organ damage, thus overcoming the fear of an overtreatment. Indeed, the area of pre-SSc still remains fuzzy but is now, after the validation of the VEDOSS criteria, a new area to be more clearly defined after a thorough clinical and experimental investigation. This strategy is corroborated by the very recent evidence that in pre-SSc patients, the increase of endostatin and the decrease of basic fibroblast growth factor and platelet-activating factor were both associated with a rapid progression to definite SSc [38■■].

CONCLUSION

The VEDOSS study has shown that, like in rheumatoid arthritis, a 'window of opportunity' does exist also for SSc, where a targeted therapeutic strategy, proportional to the disease features, might be chosen. In the very next future, this may allow to start the treatment to prevent the disease progression to a more advanced fibrotic stage [37■■].

In the last 40 years, the classification of SSc has gone through several revisions leading to a more clear vision of the possible clinical pictures of the disease. In Fig. 1, the evolution of classification and diagnosis criteria are presented together with pre-SSc as a new entity which deserves now the greatest attention of the clinical and experimental researchers to clarify and define what is preceding the VEDOSS phase of the disease.

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Type 1 interferon activation in systemic sclerosis: a biomarker, a target or the culprit

CURRENT OPINION

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Purpose of review
Activation of the type 1 interferon (T1 IFN) pathway has been implicated in the pathogenesis of systemic sclerosis (SSc) by an increasing number of studies, most of which share key findings with similar studies in systemic lupus erythematosus (SLE). Here we will focus on the evidence for T1 IFN activation and dysregulation in SSc, and the rationale behind targeting the pathway going forward.

Recent findings
An increased expression and activation of T1 IFN-regulated genes has been shown to be present in a significant proportion of SSc patients. TI IFN activation markers have been found to predict and correlate with response to immunosuppressive treatment as well as severity of organ involvement. As inhibition of the IFN-α receptor has been proven to be effective in active SLE, benefit may be seen in targeting the IFN pathway in SSc.

Summary
The role played by T1 IFN and its regulatory genes in SSc is becoming increasingly evident and strikingly similar to the role observed in SLE. This observation, together with the benefit of type 1 IFN targeting in SLE, supports the notion of a potential therapeutic benefit in targeting T1 IFN in SSc.

Keywords
interferon regulatory factors, plasmacytoid dendritic cells, systemic sclerosis, Toll-like receptors, type 1 interferon

INTRODUCTION

Systemic sclerosis (SSc) is a progressive, heterogenous multisystem autoimmune disease, which is characterized by autoimmune activation as well as a pathognomonic tissue and vascular fibrosis [1,2]. It has the greatest mortality amongst the major rheumatic diseases [1,3,4▪▪]. Genetic predisposition combined with triggers activating a persistent immune response at the level of the tissue is thought to drive the pathogenetic process in SSc. Type 1 interferons (T1 IFNs) are a family of cytokines playing a key role in response to viruses and a variety of danger and damage signals, triggering innate immune activation. The dysregulation in T1 IFN signalling has now been implicated in the pathogenesis of certain autoimmune diseases, including SSc and systemic lupus erythematosus (SLE) [4▪▪,5,6]. Clinical evidence of the harmful effects of T1 IFN in SSc, is provided by a randomized, placebo-controlled trial of IFN-α, in patients with early diffuse SSc, where the trial had to be stopped early because of a deleterious effect seen in the lung function of the treatment group. The withdrawal and serious adverse event rates were also greater in the treatment group than in the placebo group [7].

Here we will focus on the evidence for T1 IFN activation in SSc, the potential mechanisms leading to its dysregulation, the predictive role on disease progression and the rationale to target the pathway going forward.

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KEY POINTS

- The activation of T1 IFN has been implicated in the pathogenesis of SSc and SLE, with inhibition of the IFNα receptor being recently shown to be effective in active SLE.

- An increased serum concentration of ISGs is detectable in a variable proportion of patients with SSc, even at the very early stages of the disease, before onset of clinically detectable damage.

- The origin and triggers of T1 IFN, and how the interactions between genetic and environmental factors, leads to dysfunction in the T1 IFN response remains unclear.

- IFN activation markers have been found to predict and correlate with response to immunosuppressive treatment as well as in the severity of organ involvement.

- Clinical trials of T1 IFN antagonists in carefully selected SSc patients would lead to a better understanding of the role T1 IFN plays in SSc pathogenesis, potentially improving outcomes in certain SSc patients.

FROM DANGER SENSORS TO INTERFERON-STIMULATED GENES: A MULTIFACETED DEFENSE MACHINERY THAT CAN LEAD TO IMMUNE-MEDIATED TISSUE DAMAGE

T1 IFNs are a heterogenous family of cytokines, which provide a robust first line of antiviral defence. Type 2 and 3 interferons have partially different roles, which are outside the scope of this review and have been summarized elsewhere [8,9].

T1 IFN can be divided into five classes in humans: α, β, ω, ε and κ. All five, T1 IFN classes signal through the same type 1 IFN heterodimeric receptor complex constituting IFN-α receptor 1 (IFNAR1) and IFNAR2 subunits.

Secretion of T1 IFN in the extracellular space is the terminal event of an 'innate' response mechanism to a variety of danger and damage stimuli. The detection of repetitive molecular patterns displayed by a pathogen (pathogen-associated molecular patterns or PAMPs) is one of the stimuli, which is 'sensed' by the pattern recognition receptors (PRRs) [10]. There are four classes of PRRs – the Toll-like receptors (TLRs), the nucleotide-binding oligomerization domain-like receptors (NLR), the retinoic acid inducible gene I (RIG-I) and the C-type lectin receptors [11]. They all differ in ligand recognition, signal transduction and cell localization.

TLRs are the most extensively studied class of PRRs and consist of 10 types (TLR 1–10) [10,12,13]. TLRs are expressed on most nucleated cells, and

once they are engaged with their ligand, they lead to T1 IFN pathway activation. While this is true in most cells, plasmacytoid dendritic cells (pDCs) are the cells that are 'professionally' differentiated to secrete vast amounts of T1 IFN in response to TLR engagement. For this reason, they are believed to play a central part in the T1 IFN-mediated immune response and their role has been implicated both in the pathogenesis of SLE [14] and SSc [15■,16].

The first indirect evidence of a putative involvement of pDC in the aberrant T1 IFN activation in SSc was suggested by a proteome-wide analysis showing that CXCL4 in the plasma of SSc patients was substantially higher than healthy controls, and it predicted the presence and worsening of lung fibrosis and pulmonary hypertension. In the same study, the authors implicated pDC as one of the potential sources of CXCL4 [17]. More recently, CXCL4 has been found to function as a Damage Associated Molecular Pattern (DAMP) sensor. Lande *et al.* observed that CXCL4 organiszd microbial and self-DNA into liquid crystalline complexes that amplified TLR9-mediated IFN-α production in pDCs. Importantly, CXCL4-DNA complexes were present *in vivo*, and correlated with T1 IFN in SSc blood and skin, revealing a direct link between CXCL4 overexpression and T1 IFN production in patients with SSc [18]. Another study also indicated the infiltration of SSc skin by pDCs, where they were chronically activated, producing high levels of IFN-α and CXCL4. CXCL4 was under the control of phosphatidylinositol 3-kinase δ, which was linked to the aberrant presence of TLR8 on pDCs in SSc patients. CXCL4 was also found to potentiate the activities of TLR8-induced and TLR9-induced IFN production in SSc pDCs [16].

Importantly, Ross *et al.* [15■] have shown that functional inhibition of pDCs was effective in preventing skin activation and fibrosis in preclinical models of SSc, similar to what has been observed in SLE [14].

In another study, anti-CXCL4 antibodies were shown to be present in at least half of SSc patients and correlated with serum/plasma IFN-α levels. Recently, CXCL4 itself was found to behave as a self-antigen, maintaining a vicious cycle by promoting T1 IFN activation via pDCs and anti-CXCL4 antibodies by B cells, sustaining the SSc IFN signature [19]. Further work with CXCL4 has interestingly shown that the anti-CXCL4 antibodies were present in patients with VEDOSS (very early diagnosis of systemic sclerosis), suggesting that this mechanism can intervene very early in the pathogenesis of disease, before clinically apparent tissue damage [20].

Activation of TLRs have also been found to play a role in interstitial lung disease (ILD). TLR3 activation by poly I:C has been reported to increase lung

inflammatory proteins including the cytokines CCL3, CCL5 and CXCL10, in airway epithelial cells. Importantly, TLR3 knockout mice showed protection against the inflammatory response [21]. TLR4 has also been implicated in pulmonary and skin fibrosis, with the ability to activate IRF5 [22].

Pathogens have long been proposed as a trigger for autoimmune illnesses, and one mechanism for this is 'molecular mimicry' between self-derived and pathogen-derived molecules. Another mechanism, occurs through the inability to clear the pathogen, resulting in infection persistence, and repeated stimulation of the innate immune cells via TLRs [23,24]. Farina and colleagues have shown evidence of infectious Epstein–Barr virus (EBV) in monocytes triggering SSc. Induction of EBV viral lytic genes resulted in the induction of TLR8 expression in both healthy control and SSc monocytes infected with EBV [25]. Further, Farina *et al.* [26] have shown that EBV can infect endothelial cells and fibroblasts in SSc skin, leading to an aberrant TLR activation. A novel mechanism has also now been demonstrated by which human monocytes bound to EBV recombinant virus are capable to transfer EBV to the endothelial cells. In the same study, EBV lytic antigens in scleroderma dermal vessels were detected, suggesting EBV could target endothelial cells in SSc skin, activating TLR 9 in the process and possibly contributing to the vascular injury seen in SSc [27].

Beyond classic pathogens, there is increasing evidence for an important role played by mitochondria, in the events driving T1 IFN activation and subsequent autoimmunity. It is widely accepted that fragmentation in mitochondrial DNA (mtDNA), can lead to the activation of T1 IFN pathway, through cGAS (cytosolic cyclic GMP-AMP synthase), a specific cytosolic receptor for free DNA, which, in turn, activates the endoplasmic reticulum membrane protein, stimulator of interferon genes (STING). cGAS-STING activation by mtDNA was shown to be positively associated with T1 IFN and IL-6 expression in SSc as well as in SLE [28,29]. Consistent with these findings, mtDNA has been found to be at increased concentration in SSc plasma, with the ability to function as DAMPs and interact with PRRs [30]. This is one of the putative mechanisms by which necrotic cells or those under stress have been found to activate TLR 9 and the double-stranded DNA sensor, cGAS.

Interestingly, it has been also proposed that mtDNA could be damaged as a consequence of oxidative stress because of high exposure to reactive oxidative species (ROS) produced by the mitochondria itself [28].

Regardless of the source of its secretion, T1IFN signal through IFNAR1 and IFNAR2, which in turn activate Janus kinase (JAK)-signalling pathway downstream [4▪▪,8]. This consists initially with phosphorylation of pre-associated JAK1 and tyrosine kinase 2 (TYK2), which triggers kinase activity of signal transducers and activators of transcriptions 1 and 2 (STAT1 and 2) via cross-phosphorylation. This leads, in turn, to the recruitment of IFN-regulatory factor 9 (IRF9), a member of the family of transcription factors called IFN Regulatory Factors (IRFs), for their ability to regulate the expression of T1 IFN and its effects on target gene expression. IRF9 together with STAT1 and 2 form a complex known as the IFN-stimulated gene factor 3 (ISGF3). This complex translocates to the nucleus to bind to IFN-stimulated response elements (ISRE) in order to induce a family of genes that for this reason are called interferon-stimulated genes – ISGs [4▪▪,8]. A summary of the different pathways and key factors mentioned above leading to T1 IFN activation is shown in Fig. 1.

GENETICS AND EPIGENETICS OF TYPE 1 INTERFERON DYSREGULATION IN SYSTEMIC SCLEROSIS

Familial association studies have previously shown that family history appears to be the strongest known risk factor for SSc. It was found that amongst first-degree relatives of SSc patients, the prevalence of the disease was 0.33%, with a relative risk factor of 13 when compared with the general United States population, which had a prevalence of 0.026% [31]. A twin study including 42 twin pairs (24 monozygotic and 18 dizygotic), found that the overall concordance of SSc was only 4.2% (1 out of 24) in monozygotic twins and 5.6% in dizygotic twins. The concordance, however, of antinuclear antibodies (ANAs) was significantly higher in monozygotic twins vs. dizygotic twins (90% vs 40%), suggesting that concordance for autoimmunity was much higher than the one for clinical disease phenotype. Consistent with these findings, a study in 4612 first-degree relatives of 1071 probands revealed an increased risk for familial autoimmunity among subtypes of SSc, with thyroid diseases and SLE showing the most significant increased prevalence when compared with control families, together with Raynaud's phenomenon and ILD [32].

The most frequent form of genetic variation in humans is the single-nucleotide polymorphism (SNP), which influences protein function and is key to personalized medicine [33]. In a recent meta-analysis of Genome-Wide Association Studies (Meta-GWAS), which included 26 679 individuals, 27 independent genome-wide associated signals were identified, which included 13 new-risk loci, and nearly doubled the number of genome-wide hits
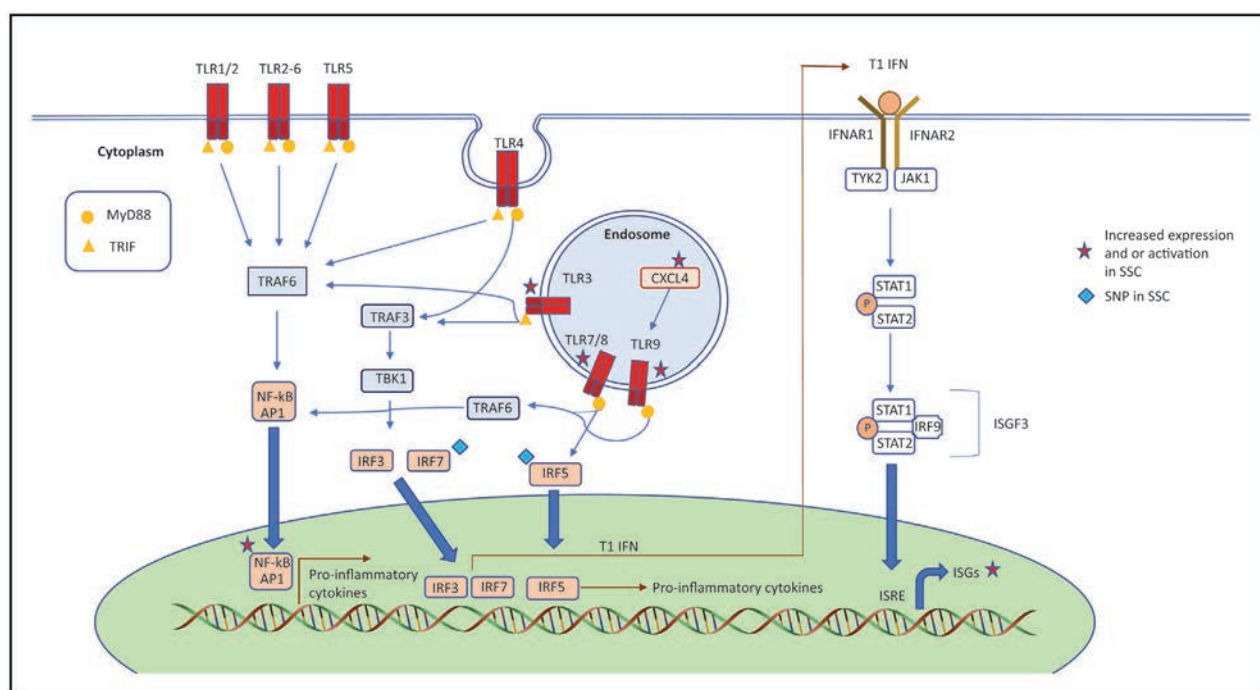
FIGURE 1. Different pathways and key factors leading to T1 IFN activation and ISG release.

previously reported in SSc [34]. This meta-analysis has suggested a variety of IFN-signalling loci, including T1 IFN regulatory factors IRF4 [35], IRF5 [36,37], IRF7 [34,38] and IRF8 [34,39,40]. (Fig. 1) Interestingly, apart from SSc, the genes have also shown an association with SLE [41–44]. Tyrosine kinase 2 (TYK2) [45], and STAT4 [34,46] are genes that have also been linked to SSc genetic susceptibility.

A shared genetic background of autoimmune diseases is clearly seen in GWAS, but additionally a vital role played by environmental factors (air pollution, infection and chemical substances, such as silicon) [47], and epigenetic influences in the pathogenesis of SSc has been suggested. Links to the pathogenesis of SSc have been previously reported for all the major epigenetic alterations, including DNA methylation [48–50], histone modifications [51,52], noncoding small (miRNA) and long (lncRNA) RNA transcript expression [53–56]. For instance, MiR-618 was found to be significantly overexpressed in SSc pDCs, causing an IRF8-dependent inhibition of pDC differentiation and activation, as well as increased production in IFN-α upon TLR9 stimulation [57]. LncRNAs are a larger class of transcribed RNA molecules, that are not translated but regulate gene expression [58]. It has recently been shown that a group of lncRNAs were modulated in a T1 IFN-dependent manner in human monocytes in response to TLR4 activation [59]. Among the lncRNAs, the negative regulator of the IFN response (NRIR) was found significantly upregulated in-vivo in SSc monocytes, and affected the expression of the ISGs, CXCL10 and CXCL11. Therefore, dysregulation of NRIR in SSc monocytes may play a part in contributing to the aberrant IFN response present in SSc patients [59].

EVIDENCE OF INCREASED TYPE 1 INTERFERON ACTIVATION IN SYSTEMIC SCLEROSIS

Due to the difficulty of directly measuring T1 IFN levels from human samples, an 'interferon signature' including the levels of expression of the transcript levels of multiple known ISGs has been widely used for this purpose. This method established the presence of increased T1 IFN in SLE, and more recently in other rheumatic diseases [60]. The first reported finding of an IFN signature in SSc dates back to 2006 [61]. Since then, it has been shown that an IFN signature in blood is found in a large proportion of SSc patients [5,62,63]. It has been also shown that activated monocytes and macrophages can be a potent source of T1 IFN and other profibrotic factors, stimulating the proliferation of fibroblasts and extracellular matrix accumulation [64]. An IFN signature in monocytes has even been found at the earliest phases of SSc, before overt fibrosis, suggesting of this being an early event in SSc pathogenesis [10].

A higher IFN signature in SSc whole blood or plasma has been found to correlate with the antibody profiling, where antitopoisomerase and anti-U1-RNP antibodies were associated with a higher IFN

signature [5,65]. Correlation of this higher IFN signature was also seen in more severe vascular manifestations and lung involvement [65–68]. Organs known to be targeted in SSc such as the skin and lung, have also demonstrated an overexpression of ISGs in SSc patients [69,70].

Upregulation of ISGs in the skin of SSc patients was also demonstrated in skin biopsy gene expression studies [70,71]. A study performing microarrays from lung tissue revealed upregulation of ISGs in addition to TGF-β-regulated genes in SSc patients with ILD, with an increased expression of ISGs, associated with a higher rate of progression in ILD [69]. Interestingly, a recent multiomic comparative analysis of the serum profile, peripheral blood cells and skin ISG expression in SSc patients showed that the serum protein profile correlated more closely with the transcriptome of the skin than that of the PBMCs. This may be because of a spill-over effect from diseased end organs and suggests that IFN-inducible chemokine concentration may be a better predictor of tissue IFN activity than PBMC ISG expression levels [72,73■].

Apart from the trial in IFN-α mentioned in the introduction of this review, case reports have been documented of the development of SSc in individuals treated with T1 IFN for other conditions. Interestingly, Anifrolumab (anti-IFNAR1 monoclonal antibody) in a phase 1 trial of SSc patients led to the suppression of the IFN signature and TGFβ signalling in SSc skin [74]. Additionally, in a graft-versus-host disease (GVHD) mouse model of SSc, neutralization of IFNAR1, and consequent normalization in the overexpression of T1 IFN-inducible genes, led to a marked reduction in the dermal fibrosis [75]. Consistent with these findings, in SSc patients treated with high-dose cyclophosphamide followed by rescue autologous hematopoietic stem cell transplantation, clinical response strongly correlated with normalization in T1 IFN module by RNAseq of peripheral blood cells [76].

The close mirroring of disease activity of T1 IFN activation has also been shown in the analysis of the SLS2 trial. Assassi *et al.* [77■■] have shown that higher serum IFN-inducible chemokine score predicted a better clinical response in both the cyclophosphamide and the mycophenalate mofetil arms. Importantly during the second year of the study, higher serum IFN score predicted worse clinical course in patients put on placebo, supporting the notion that IFN activation in SSc is deleterious, unless immunosuppressive treatment is initiated.

Vascular injury plays an important role in organ dysfunction in SSc, and it is the main driver of disease in patients with the limited cutaneous subset (LcSSc) of SSc. T1 IFN has been implicated in the dysregulation of the vascular remodelling process in SSc. Myxovirus-resistance protein A (MxA), which is induced by T1 IFN, was found to correlate with digital ulcerations and lower pulmonary forced vital capacity in SSc [78]. T1 IFN has also been shown to contribute to the increased vascular permeability in SSc through downregulation of Fli1 (friend leukemia integration 1 transcription factor) and vascular endothelial cadherin (VE-cadherin) in endothelial cells and fibroblasts [79]. Features of SSc vasculopathy were also seen in mice with conditional deletion of Fli1 in endothelial cells confirming that T1 IFN-mediated downregulation of Fli1 enhanced the development of SSc [80].

Consistent with these observations, IFN-inducible chemokines were found to predict progression of patients with LcSSc as far as a multi-morbidity score including skin, lung, vascular and gastrointestinal progression [81■].

Taken together, these observations suggest that T1 IFN is involved in both tissue and vascular fibrosis in SSc, strongly supporting the rationale for a direct therapeutic approach targeting the pathway.

CURRENT EXPERIENCE IN TYPE 1 INTERFERON TARGETING FOR DISEASE MODIFICATION

Dysregulation in the T1 IFN response has been shown to contribute to the development of autoimmunity. Although the clinical manifestations vary amongst the different types of autoimmune diseases, T1 IFN protein or transcript signatures have now been identified in many of them (SSc, SLE, dermatomyositis and Sjogren's disease) [5,10,82–85].

In SLE, up to 80% of patients were shown to have a T1 IFN signature, with around 50% having chronically elevated T1 IFN levels, detectable in blood [86,87]. SLE patients with high T1 IFN activity, also tend to have higher disease activity scores with a greater tendency to relapse whilst in remission and a lower response rate to placebo medication [88–90]. Similarly to what has been observed in SSc, deranged pDC activation also occurs in SLE, and monoclonal antibodies against pDC have recently shown benefit on cutaneous and musculoskeletal lupus [91–93].

The effectiveness of blocking IFNAR, which plays a critical role in T1 IFN signalling, has now been concretely demonstrated in SLE patients with the monoclonal antibody Anifrolumab. The phase III Tulip-2 trial met its primary end-point, with an improvement in overall disease activity vs. placebo [94], leading to Food and Drug Administration (FDA) and European Medicine Agency (EMA) approval for treatment in SLE.

The similarities of T1 IFN activation in SSc, therefore, informs the rationale to block IFNAR in SSc and determine its therapeutic effectiveness [4▪▪]. As mentioned above in this review, early phase 1 study of 34 SSc patients, showed that anifrolumab was well tolerated and showed peak inhibition of the T1 IFN signature in blood [95]. A follow-up mechanistic study showed that treatment with anifrolumab led to the reduction of the T1 IFN signature in whole blood and skin biopsy samples, demonstrating the suppressive effects of the anti-IFNAR1 antibody [74]. These findings provide further support for future larger double-blind, placebo-controlled trials of Anifrolumab in early SSc.

CONCLUSION

Over the past few years, substantial progress has been made in deconvoluting the immune complexity of SSc, which has led to identify key molecular and cellular components of T1 IFN signalling involved in disease pathogenesis. In spite of the progress made, many unanswered questions in the pathogenesis of SSc remain. The origin and triggers of T1 IFN, and the interactions played between genetic and environmental factors, leading to dysfunction in the T1 IFN response still remains a grey area. However, newly discovered function of molecules such as CXCL4, start to lead towards a better understanding of the connections between pDCs, the IFN continuum and the fibrotic process. Further studies are also needed to elucidate downstream processes linking the T1 IFN activation to the exaggerated fibrotic response in fibroblasts and other key effector cells implicated in SSc pathogenesis.

Specifically, the identification of specific ligands and signalling pathways driving T1 IFN signalling in SSc will need further investigation with in-vivo and in-vitro studies. This will improve our understanding of SSc pathogenesis, and will increase the armamentarium of the therapeutic targets that could be exploited to improve patient outcome.

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Anti–MDA5 dermatomyositis: an update from bench to bedside

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Purpose of review
This review summarizes the recent developments about anti-MDA5 antibody positive dermatomyositis with a focus on its pathogenesis, clinical features and treatment options of rapidly progressive interstitial lung disease, its most ominous complication.

Recent findings
Anti-MDA5+ dermatomyositis has a heterogeneous clinical spectrum with different patient subsets exhibiting widely different outcomes; severe acute interstitial lung disease is the main factor impacting prognosis. The pathogenetic role of anti-MDA5 antibodies is an active area of investigation.

Summary
Anti-MDA5+ dermatomyositis has a wider spectrum of manifestations than previously thought. A high index of suspicion is needed not to miss atypical presentations. In the setting of acute interstitial lung involvement, once a confident diagnosis is made, an aggressive approach with early combined immunosuppression affords the best chances of survival.

Keywords
anti-MDA5 antibodies, dermatomyositis, immunosuppressants, interstitial lung disease, rapidly progressive interstitial lung disease

INTRODUCTION

Immune-mediated inflammatory myopathies (IIM) are increasingly recognized as complex multisystem diseases with a wide spectrum of organ manifestations engendered in different proportions by inflammation, autoimmunity and vasculopathy [1,2,3]. The description and characterization of several myositis-specific and associated antibodies (MSAs and MAAs) has been a key contribution to defining different myositis clinical and pathophysiological subsets [4,5]. Among these, anti-melanoma differentiation antigen 5 (MDA5) antibodies have been associated with a definite subset of dermatomyositis patients showing prominent cutaneous and lung involvements with rapidly progressive interstitial lung disease (RP-ILD). The spectrum of anti-MDA5+DM is being explored further and subdivided into different clinical and prognostic subsets. Anti-MDA5 antibodies may also be found in the context of isolated lung involvement [6]; thus, the term 'anti-MDA5 syndrome' has been recently proposed [7▪▪].

Furthermore, a hyperinflammatory and hyperferritinemic state can be documented at the time of clinical worsening in some of these patients, bearing resemblance to severe cases of human SARS-CoV2 infection [8–11].

In contrast with classical forms of dermatomyositis, no strong association is consistently reported between MDA5+DM and malignancy. Recent research acquisitions have focused on describing the clinical spectrum associated with anti-MDA5 antibodies in Asian and non-Asian settings, in identifying predictors of RP-ILD and death, and on a deeper understanding of anti-MDA5 antibodies, whether as a directly pathogenic entity or as a marker of an underlying pathological process.

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THE BENCH: MDA5 AND ANTI-MDA5 ANTIBODIES

Originally described in melanoma cells and thence deriving its namesake, MDA5 is an antiviral pattern recognition receptor in humans. MDA5 is a cytosolic receptor that recognizes long strands of double-stranded RNA, a foreign molecular structure in eukaryotic cells. The origin of such molecules stems mainly from RNA viruses and DNA viruses, but dsRNA can also have an endogenous mitochondrial origin. Upon binding to dsRNA, through interaction with mitochondrial antiviral signalling protein (MAVS), MDA5 enhances the transcription of interferon-dependent (IFN) genes. In turn, MDA5 itself is encoded by a IFN-inducible gene (IFIH-1). Therefore, MDA5 sits at the origin of a positive proinflammatory and interferogenic feedback loop, occupying a critical regulatory position.

Hyperfunction of MDA5 due to gain-of-function mutations results in a spectrum of diseases sharing malformations, chronic inflammation and features of an interferonopathy with several rheumatological manifestations [12,13]. Furthermore, hyperstimulation of MDA5 by defective clearance mechanisms for mitochondrial dsRNA – for example in hypomorphic polynucleotide phosphorylase mutations – also results in an interferonopathy [14]. Importantly, the range of MDA5 subcellular localizations is not yet entirely clear: indeed, although MDA5 is classically described as a cytosolic receptor, it may relocate when abundant [15]. An overexpression of MDA5 in response to an index event may promote a shift in its subcellular localization, and it may encourage loss of tolerance to MDA5 and production of anti-MDA5 antibodies.

Anti-MDA5 could exert pathological effects on both ends of their functional spectrum: Anti-MDA5 antibodies that inactivate MDA5 may compromise antiviral responses, altering them to the point of indirectly producing an excessive, inefficient and damaging multisystemic inflammation to sustain viral clearance. On the opposite end, anti-MDA5 antibodies may stabilize MDA5 in an 'active' configuration, thus creating a constant danger signal at the origin of a pernicious positive feedback, producing the same hyperinflammatory state [16]. Several other mechanisms may be implicated in a direct anti-MDA5-mediated damage, such as formation of immune complexes together with MDA5, cell penetration with downstream pathway disruptions and antibody-dependent cytotoxicity. Anti-MDA5 could also simply be a marker of a dysfunctional antiviral response, with overexpression of MDA5 and loss of tolerance towards it as an epiphenomenon. However, it is increasingly clear that not all anti-MDA5 antibodies are made equal: in a recent study, Anti-MDA5 IgG-1 were found to be associated with RP-ILD and Anti-MDA5 IgA were found to be common, while the IgM isotype was more unusual [17]. In a different study, IgG1 and IgG3 anti-MDA5 antibodies were found to be independently associated with death and with RP-ILD, in contrast with IgG2 and IgG4 [18]. Titres of anti-MDA5 antibodies also seem to be higher in nonsurvivors and in RP-ILD patients, although this is not a universal finding [19,20]. Therefore, anti-MDA5 antibodies have potential roles both as *markers* and *makers* of a potentially devastating disease. In a general pathogenetic model (Fig. 1): an index event – presumably a viral infection – is met by a genetically susceptible host with an exuberant production of MDA5, loss of its subcellular localization, tissue damage and break of tolerance. A late immune response with delayed IFN production may promote this maladaptive process, whereas a rapid and orderly virus clearance through a timely initial burst of IFN production may avert further complications, in a similar manner to that described in COVID-19 [21,22]. Anti-MDA5 antibodies, once produced, may further exacerbate the process, leading to more inflammation and tissue damage, and engendering a cytokine storm in which high levels of IFN may mediate a vasculopathy through endothelial toxicity [23,24]. The healing response to the ongoing damage and ischemia would promote macrophage recruitment [25], fibrosis [26] and irreversible organ damage, especially in the lungs.

This conceptual framework bears several similarities with the human infection by SARS-CoV-2. Of note, anti-MDA5 antibodies have been found in COVID-19 patients, and their presence and titre showed an association to mortality [27]. On the
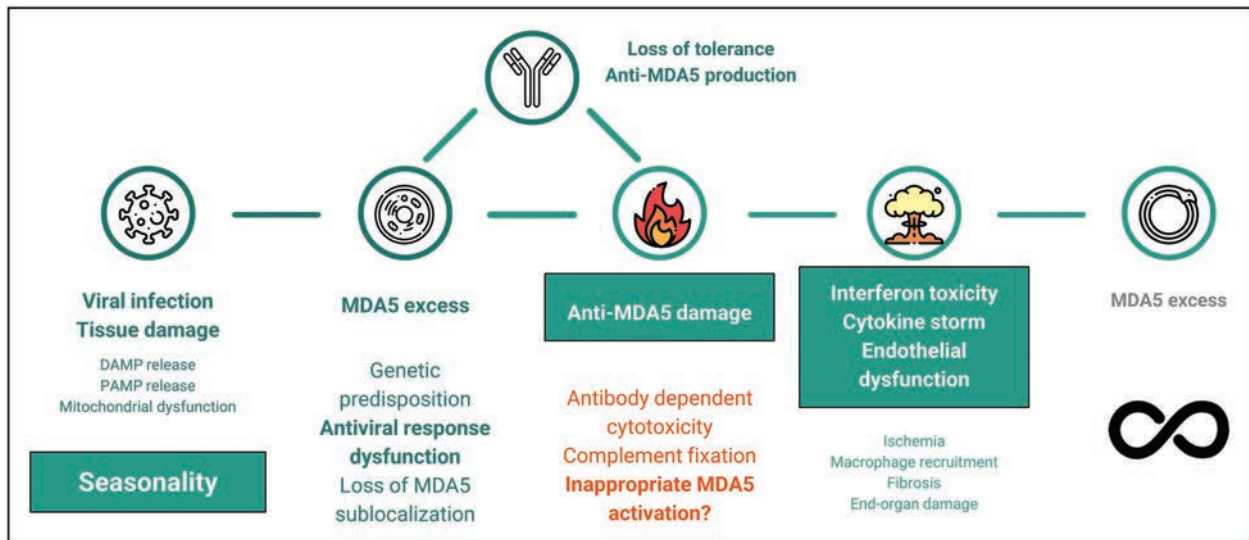
FIGURE 1. Proposed general pathogenetic model of the anti-MDA5 syndrome. DAMP, damage-associated molecular pattern; MDA5, melanoma differentiation antigen 5; PAMP, pathogen-associated molecular pattern. Icons made by Freepik from Flaticon.com.

contrary, nonspecific positive antibody tests are commonplace during viral infections, and anti-MDA5 titres were rather low compared with true anti-MDA5+DM patients.

THE BEDSIDE: CLINICAL CLUSTERS

The first descriptions of anti-MDA5+ dermatomyositis entailed a combination of clinically amyopathic dermatomyositis (CADM) with RP-ILD [28,29]. The cutaneous manifestations included hallmarks of dermatomyositis such as heliotrope rash, Gottron's papules and sign, and other typical dermatomyositis rashes such as V-neck and shawl signs. The presence of prominent cutaneous vasculopathy with skin ulcers was also an outstanding clinical feature.

Since then, the picture has evolved with the availability of retrospective data from both Asian and non-Asian cohorts [30–33]. In a recent unsupervised analysis on a French nationwide multicentre retrospective cohort [34], three clinical phenotypes were proposed: a 'rheumatoid cluster' exhibiting mostly arthritis and dermatologic involvement, with infrequent RP-ILD, a female predominance and a good overall prognosis; a male-predominant 'vasculopathic DM cluster' displaying severe vasculopathy in the form of Raynaud's phenomenon, skin ulcers and necrosis in addition to typical dermatomyositis rashes; in this group, rates of RP-ILD were intermediate (22.7%), as was the overall prognosis. Clinically relevant myositis (proximal weakness and high creatine kinase) was more prevalent in this subgroup. A 'RP-ILD cluster' with a grievous prognosis, high prevalence of ICU admission and very high rates of RP-ILD and death.

Some of these clusters are similar to other reports. In a recent single-centre retrospective Chinese cohort [35], three clusters emerged of which two were comparable to the French study: one mainly showing arthritis and mechanic's hands with low rates of RP-ILD and a good prognosis; one enriched in RP-ILD which was also exhibiting fever, hyperferritinemia and a far worse prognosis. In contrast, a different third cluster identified patients with high rates of typical cutaneous signs and enriched in clinically relevant myositis, with very low rates of RP-ILD (Table 1) [65].

In a retrospective analysis of the AENEAS group focusing on anti-MDA5+ patients as a whole [7■■], 89% of patients were diagnosed with myositis (dermatomyositis 43%, CADM 31%, polymiositis 5%, overlap myositis 11%); interestingly, the remainder 10% was diagnosed with interstitial pneumonia with autoimmune features (IPAF), not satisfying any other classification criterion. ILD was the main manifestation (72%); skin, joint and muscle involvement also showed a significant prevalence (74, 51 and 56%, respectively). Notably, rates of RP-ILD (21.5%) were lower than in Japanese reports, but in line with other European reports [32]. Onset of ILD was not confined to the first stages of the disease, but it could be diagnosed after a long course and, importantly, after prior treatment with potent immunosuppressants. Although the methodology differs, clinical clusters were not as clear-cut in this study, and arthralgia/arthritis and Raynaud phenomenon did not show a clear segregation in particular subgroups. Importantly, more than half of the patients did not show a positive antinuclear titre, stressing the need to

Table 1. Focus on recent descriptive cohorts and salient clinical characteristics of anti-MDA5+DM and non-DM patients

Reference	Salient clinical involvement	RP-ILD rate	Prognosis	Comment
Allenbach *et al.* n = 121 [39]	Cluster 1 ILD 100% Skin 100% • mechanic's hands 73.3%	RP-ILD 93.3%	3-month mortality 80%	
	Cluster 2 Skin 82.6% • Skin ulcers 37% ILD 82.6% Arthritis/arthralgia 82.6%	RP-ILD 17.4%	3-month mortality 0%	
	Cluster 3 Skin 95.4% • Skin ulcers 77.3% • Digital necrosis 31.8% Raynaud phenomenon 81.8% Proximal weakness 68.2% ILD 50%	RP-ILD 22.7%	3-month mortality 4.5%	
Yang *et al.* n = 96 [35]	Cluster 1 Arthritis 84.6% Mechanic's hands 51.3%	RP-ILD 7.7%	24-week mortality 2.6%	
	Cluster 2 V-neck sign 69.2% Muscle weakness 92.3%	RP-ILD 7.7%	24-week mortality = 0	
	Cluster 3 Fever 77.3% Elevated CRP 100% Hyperferritinemia > 1000 ug/L 75%	RP-ILD 77.3%	24-week mortality 54%	
Cavagna *et al.* n = 149 [7■■]	Overall Skin involvement 74% Symptomatic muscle involvement 49% Joint involvement 51% • symmetric polyarticular in 70% Skin ulcers 15% Raynaud phenomenon 30% Fever 29% At presentation Skin alone 14% Skin + ILD 13%	RP-ILD 21.5%	17% mortality at 36 months • 42% directly due to RP-ILD • 19% due to infection superimposed on RP-ILD	Focused on Anti-MDA5+ overall (10% diagnosed with IPAF)
Hensgens *et al.* n = 20 [65]	Overall ILD 95% Skin findings 87% Arthritis/arthralgia 60%	RP-ILD 45%	1-year mortality 45%	Higher Anti-MDA5 titres in RP-ILD although with shorter disease duration

The rates of RP-ILD, overall or in different clusters depending on the study, are reported. CRP, C-reactive protein; FU, follow-up; ILD, interstitial lung disease; IPAF, interstitial pneumonia with autoimmune features; RP-ILD, rapidly progressive interstitial lung disease.

actively look for Anti-MDA5 antibodies whenever clinical suspicion arises.

In severe cases, the disease may be complicated by signs of an hyperinflammatory, hyperferritinemic syndrome similar to severe COVID-19 [8]; this subset is often represented by acutely ill patients with RP-ILD, peripheral cytopaenias, high ferritin, elevated liver enzymes and haemostatic imbalances with both bleeding events and a proclivity towards disseminated intravascular coagulation. For example, spontaneous intramuscular haemorrhages have been described in acutely ill anti-MDA5+ patients,

often carrying a grave prognosis [36]. Some of these severe cases may satisfy criteria for macrophage activation syndrome [37], including the presence of haemophagocytosis at bone marrow examination [38]. Awareness of such haematologic manifestations as part of the clinical picture is of critical importance, because these may otherwise lead clinicians astray in what appears to be a time-sensitive and difficult-to-treat disease.

Taken together, recent evidence suggests that any patient presenting with a suspicion or a known positivity for anti-MDA5 antibodies should prompt

the treating physician to perform an assessment of a full patient history and a thorough examination of skin, muscle, joints and lungs; chest imaging with high-resolution computed tomography (HRCT) should be obtained expeditiously if any clinical signs of lung involvement are present; if not, at least pulmonary function tests (PFTs) and first-line chest imaging are advisable. Once any level of lung involvement is diagnosed, appropriate therapy and a tight multidisciplinary follow-up by Rheumatology, Pneumology and, if possible, Radiology should be arranged.

INTERSTITIAL LUNG DISEASE, RAPIDLY PROGRESSIVE- INTERSTITIAL LUNG DISEASE AND PREDICTORS OF POOR OUTCOME

RP-ILD is the main factor impacting prognosis in anti-MDA5+DM. Although ILD and RP-ILD can ensue at any point in the disease course, RP-ILD peaks in the first 6–12 months from diagnosis, and it drives mortality in this early period [39,40]. Predictors of both RP-ILD and mortality are therefore of great clinical interest.

The available data, derived from multivariate analyses of retrospective cohorts, point to the following factors as independently associated with ILD in the setting of anti-MDA5+DM: older age, a high neutrophil-to-lymphocyte ratio and/or lymphopenia, elevated LDH, elevated ferritin. The exact ferritin cut-off is variable among studies, with the majority reporting levels in excess of 1000 µg/l. Fever and elevated CRP have also been implicated in portending a worse prognosis (Table 2) [66–68]. These thought-provoking findings reinforce the notion of a dysfunctional antiviral response or a cytokine storm as the underlying substrate of the disease, at least in severe cases.

The co-presence of anti-Ro52 (SSA) antibodies has repeatedly been reported to be enriched in ILD

Table 2. Focus on recent studies reporting on associated factors to rapidly progressive interstitial lung disease and mortality in Anti-MDA5+DM

References	Outcome	Risk factors (except RP-ILD)
Zuo *et al.* [43]	RP-ILD	Fever OR 3.672 (1.794–7.516) Elevated ALT OR 2.355 (1.153–4.813) Elevated LDH OR 3.083 (1.517–6.266) Lymphopenia OR 2.141 (1.013–4.528) Elevated Ferritin OR 4.965 (1.973–12.498) Elevated CEA OR 2.276 (1.128–4.591) Elevated CA 15.3 OR 3.305 (1.502–7.272) Protective: Arthralgia OR 0.281 (0.138–0.570)
	Mortality	Ferritin > 2200 ng/ml AUC 0.66 (0.51–0.80)
So *et al.* [66]	RP-ILD	Age > 50 years HR 2.640 (1.277–5.455) LDH > 300 U/L HR 3.189 (1.469–6.918) Fever HR 1.903 (0.956–3.790) NLR > 7 HR 1.967 (0.942–4.107)
	Mortality	Age > 52 years HR 4.750 (1.692–13.333) LDH > 400 U/L HR 2.290 (1.009–5.198) Ferritin > 2800 pmol/l HR 3.042 (1.323–6.997)
Ouyang *et al.* [44■]	Mortality	Fever HR 24.6 (2.3–260.7) Ferritin > 1250 µg/l HR 51.1 (3.5–747.5) Elevated CEA HR 85 (1.1–6516.2)
Zhou *et al.* [67]	Mortality	Advanced age Lymphopenia Low serum albumin High LDH High ferritin
Lian *et al.* [68]	Mortality[a]	Ferritin > 636 ng/ml HR 2.62 (1.18–5.83) LDH > 355 U/l HR 3.59 (1.83–7.01) HRCT score HR 6.24 (1.47–12.56)

Where available, adjusted ORs, hazard ratios, AUCs and 95% confidence intervals are reported.
AUC, area under the curve; CA15.3, Cancer-Antigen 15.3; CEA, carcinoembryonic antigen; HR, hazard ratio; HRCT, high-resolution CT; LDH, lactic dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio.
[a]Analysis on a cohort of CADM-ILD patients, with Anti-MDA5+ as a subset.

and RP-ILD patients [41,42], confirming the not-so-benign profile of this antibody in the setting of autoimmune lung involvement. In recent reports, higher peripheral CD5-CD19+ B-cell counts and elevated carcinoembryonic antigen (CEA) and CA 15.3. were remarked on as independently associated with RP-ILD [43], in addition to the previously mentioned factors. Moreover, in a recent matrix prediction analysis [44▪], three factors (ferritin, CEA, fever) successfully predicted mortality at 6 months. The elevation of oncomarkers may raise suspicion of malignancy being implicated: conversely, CEA levels are heightened in many forms of lung injury such as in idiopathic pulmonary fibrosis and in active smokers [45]; moreover, no cases of adenocarcinoma were reported by the authors at extended follow-up in patients with elevated CEA who survived. Radiological patterns vary between reports but frequently show a combination of nonspecific interstitial pneumonia (NSIP) and organizing pneumonia findings with basal involvement and a rapidly progressive consolidative pattern [46,47]; a UIP-like pattern has also been reported [7▪▪]. Quantification of lung involvement at HRCT contributes to inform prognosis [48–50].

Importantly, although radiology may offer some crucial clues during the diagnostic stage, it remains challenging for any single radiological pattern to uniformly clinch the diagnosis of anti-MDA5 lung involvement *a priori* without supporting clinical and serological evidence; this reinforces the importance of actively looking for anti-MDA5 antibodies whenever clinically indicated.

THERAPEUTIC DEVELOPMENTS in RAPIDLY PROGRESSIVE-INTERSTITIAL LUNG DISEASE

No universal recommendations exist for treatment of anti-MDA5+DM. Outside of RP-ILD, current therapies are targeted towards the prevailing clinical manifestations whether it be arthritis, myositis, cutaneous rashes and vascular/vasomotor manifestations. In observational studies, employed drugs include glucocorticoids, antimalarials, methotrexate, mycophenolate mofetil, calcineurin inhibitors and azathioprine [7▪▪]. Intravenous immunoglobulins (IvIGs) and rituximab also have a role, especially as second-line interventions.

In the setting of RP-ILD, glucocorticoids in isolation do not seem to offer benefit and recent evidence supports early combined immunosuppression, with a low threshold for therapy escalation, and consideration to therapeutic plasma exchange (PEx) as salvage therapy in unresponsive cases (Table 3) [51,52▪]. The main strategy, supported by retrospective and prospective data, entails the combined use of high-dose glucocorticoids, for example intravenous methylprednisolone pulses 500 mg to 1 g/day for at least three consecutive days followed by 1 mg/kg/day, a calcineurin-inhibitor (CNI) and intravenous cyclophosphamide (CYC) $0.5–1.0 \, g/m^2$. In Japanese studies, early combination therapy yielded a better survival rate when compared with step-up therapy [53,54▪]. PEx could afford some incremental survival in cases not responding to combination therapy [55▪,56]. Of note, PEx outside of a combined immunosuppressive regimen appears to be of little value [54▪]. Combination therapy with glucocorticoids and a CNI, especially Tacrolimus, without CYC may yield similar results to triple therapy [57]. Among CNIs, Tacrolimus may perform better than Cyclosporin A [58]. Retrospective evidence suggests that the use of Rituximab as an add-on therapy to background immunosuppression could be a valid option [59]; an ultra-low dose regimen (100 mg single dose) also showed a nonstatistically significant trend towards response [60].

Apart from PEx, other salvage therapies include Polymyxin B Hemoperfusion, which unfortunately has not shown encouraging results [61]. Extracorporeal membrane oxygenation (ECMO), while not a disease-modifying therapy *per se*, can act as a bridge to recovery or bridge to transplantation through the most critical stages of lung dysfunction [62].

Obviously, an aggressive combined immunosuppression has the drawback of being at odds with the main other confounding factor at the diagnostic and follow-up stages: infection. In fact, infections remain an important cause of death in anti-MDA5+DM patients [7▪▪]. A swift microbiologic workup and close collaboration and shared decision-making between different specialist figures are therefore key to avert unfavourable outcomes in this difficult disease.

Lastly, JAK inhibitors have been reported to be effective, especially in early cases [63]. Isolated reports of a combined use of JAKis with RTX with good effect are also available [64]. Further controlled studies are needed to properly assess the treatment hierarchy.

CONCLUSION

The spectrum of disease manifestations associated with anti-MDA5 antibodies is complex and expanding. Anti-MDA5+DM encompasses different patient groups with different prognoses, with RP-ILD being the main prognostic watershed. Several challenges lie ahead, including obtaining a better understanding of the role of anti-MDA5 antibodies, and achieving clarity on which treatment is the most indicated within and outside the setting of

Table 3. Focus on selected key recent evidence on treatments of Anti-MDA5+-ILD. Studies employing control groups are reported

References	Design and intervention	Study population	Result
Shirakashi *et al.* [55■]	Retrospective case-control add-on PEx vs. no PEx	Anti-MDA5+ RP-ILD $n=38$ of which progressing under combined immunosuppression $n=13$	3-year survival of 62.5% in PEx group vs. 0% in no PEx group ($P=0.04$, significant)
Abe *et al.* [56]	Retrospective case-control add-on PEx vs. no PEx	Anti-MDA5+ RP-ILD under combined immunosuppression $n=10$	1-year survival 100% in PEx group vs. 25% in no PEx group ($P=0.033$, significant)
Mao *et al.* [60]	Retrospective case-control single 100 mg RTX infusion with or without CYC vs. CYC	Anti-MDA5+ ILD, RP-ILD in 92.5% $n=40$	180-day mortality 36.4% in RTX group vs. 63.2% in CYC alone group ($P=0.26$, nonsignificant)
Tsuji *et al.* [54■]	Prospective single-arm with historical control group Combined immunosuppression vs. traditional high-dose GCs with or without add-on PEx	Anti-MDA5+ ILD $n=44$	12-month survival 85% in combined immunosuppression group vs. 33% in traditional immunosuppression ($P<0.001$, significant) 12-month survival 85% in add-on PEx vs. 71% in no add-on PEx ($P=0.17$, nonsignificant)
Fujisawa *et al.* [58]	Prospective, randomized open-label 52 weeks trial Tacrolimus vs. Cyclosporine	Myositis-associated ILD, subgroup for Anti-MDA5+ patients $n=58$	Survival 88% in TAC group vs. 80% in CsA group ($P=0.63$, nonsignificant) Progression-free survival 63% in TAC group vs. 40% in CsA group ($P=0.32$, nonsignificant)
Chen *et al.* [63]	Prospective open-label with historical control group Tofacitinib vs. no Tofactinib	Anti-MDA5+ ILD, early (< 3 months) $n=50$	6-month survival of 100% in Tofacitinib group vs. 78% in control group (significant at $P=0.04$)

CYC, cyclophosphamide; PEx, plasma exchange; RTX, rituximab.

RP-ILD. Collaboration between the different medical specialties of Rheumatology, Pulmonology, Intensive Care and Radiology is paramount to achieve better outcomes.

Conflicts of interest

There are no conflicts of interest.

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Recent advances in the use of machine learning and artificial intelligence to improve diagnosis, predict flares, and enrich clinical trials in lupus

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Purpose of review
Machine learning is a computational tool that is increasingly used for the analysis of medical data and has provided the promise of more personalized care.

Recent findings
The frequency with which machine learning analytics are reported in lupus research is comparable with that of rheumatoid arthritis and cancer, yet the clinical application of these computational tools has yet to be translated into better care. Considerable work has been applied to the development of machine learning models for lupus diagnosis, flare prediction, and classification of disease using histology or other medical images, yet few models have been tested in external datasets and independent centers. Application of machine learning has yet to be reported for lupus clinical trial enrichment and automated identification of eligible patients. Integration of machine learning into lupus clinical care and clinical trials would benefit from collaborative development between clinicians and data scientists.

Summary
Although the application of machine learning to lupus data is at a nascent stage, initial results suggest a promising future.

Keywords
biomarker, clinical trials, diagnosis, gene expression, lupus, machine learning

INTRODUCTION

Artificial intelligence emerged as a computational field in the 1950s [1], but the promise of artificial intelligence to transform medicine has been increasingly recognized in the past 2 decades. Artificial intelligence and its subfield machine learning, a system of computer algorithms designed to identify patterns, make decisions and improve performance through experience, are undoubtedly powerful tools for discerning new relationships in clinical data. However, the transition from the use of machine learning analytics in basic science to the application of machine learning algorithms to biological data with proper validation and subsequent implementation in *a clinically meaningful way* has just begun. Considerable effort has been exerted to apply machine learning to further understand systemic lupus erythematosus (SLE, lupus), and indeed, the adoption of machine learning frameworks in lupus research has not lagged behind that of other disease areas (Fig. 1), but there still is only modest, if any, clinical implementation. Herein, we review recent advances in the use of machine learning for the diagnosis and management of lupus, with a focus on the future implications of this data analysis paradigm in supporting more precise and personalized clinical care and more effective clinical trials.

WHAT IS MACHINE LEARNING?

Machine learning is a powerful analytic approach in which computerized algorithms are trained to recognize patterns in existing data. From these patterns, algorithms build a mathematical model that can subsequently be applied to new data to predict a specified outcome. Machine learning is capable of

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KEY POINTS

- Machine learning can be applied to multiple types of lupus data, including electronic medical/health records, omics data, including gene expression, and histology images to classify or cluster samples, or predict outcomes.

- The application of machine learning to the diagnosis of lupus and prediction of flares may help standardize clinical practice across centers and identify previously unappreciated patterns that facilitate meaningful, personalized care, but validation and testing in external datasets are required.

- In the future, machine learning could be applied to enrich lupus clinical trials through the identification of eligible patients and those with upregulation of the targeted mechanisms.

determining the group or 'class' to which a sample belongs (classification), predicting the value of a specific parameter (regression), or grouping the sample into subsets based on similarity (clustering) [2]. Previous reviews have discussed the key features of machine learning and its application in rheumatic diseases [2–6] and this review will focus on the key goals of machine learning employment, rather than the technical parameters of model construction and interpretation. Critical to understanding the

potential of machine learning to provide meaningful information that might have clinical relevance, however, is an evaluation of the robustness of model performance. This is typically evaluated by constructing receiver operating characteristic (ROC) curves [7] for models and assessing the area under the ROC curve (AUC). In general, a model AUC greater than 0.9 is considered outstanding, greater than 0.8 is excellent, greater than 0.7 is modest, and greater than 0.6 is acceptable [7]. Implicit in the interpretation of model performance is the concept that it will be trained and validated in separate portions of a dataset and then tested in an unrelated dataset. In the absence of this rigorous approach, overfitting is frequently encountered.

HOW IS MACHINE LEARNING APPLICABLE TO LUPUS RESEARCH AND CLINICAL CARE?

Machine learning analysis and modeling *could* be applied to many aspects of lupus clinical care. However, a single machine learning model cannot be expected to solve all relevant problems (i.e., diagnose, predict flares, and predict appropriate treatment) within a single disease or entity [8]. To address disparate elements/concepts, multiple, different machine learning models are needed, each with proper training, validation, and testing in sufficiently large datasets [2].
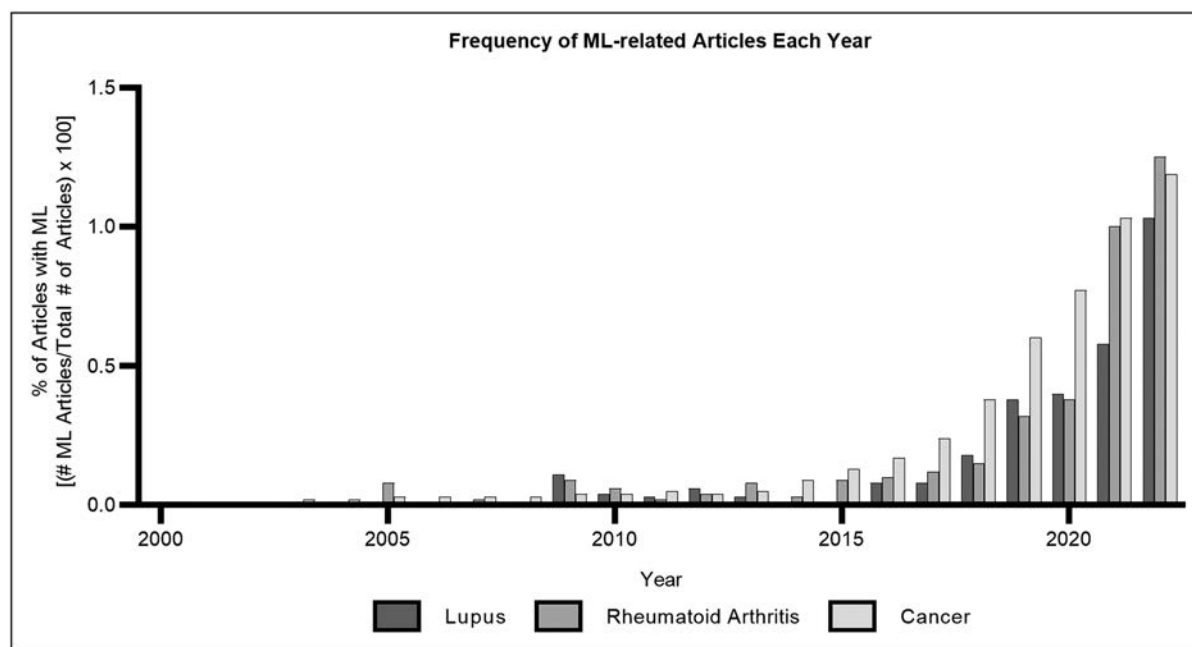
FIGURE 1. The frequency of machine learning articles in lupus each year is comparable with that in rheumatoid arthritis and cancer. Data for the number of machine learning articles each year were derived from a PubMed search of 'disease' AND 'machine learning' and then normalized by the number of 'disease' articles each year. 'disease' = 'lupus' or 'rheumatoid arthritis' or 'cancer'.

Machine learning could be used to create a model for lupus diagnosis. For this purpose, available data from individuals with and without lupus, perhaps from electronic medical/health records (EMR/EHRs), would be input into a machine learning algorithm, which would be trained to discriminate lupus from nonlupus. The input data could comprise parameters of accepted clinical importance (e.g., organ involvement, autoantibody status, or complement status) or all available patient data could be employed (e.g., clinical data, gene expression data, 'omic' data, and demographic data) in model construction. In both situations, the model could be built on all input variables, or, the model could decide the subset of features that best discriminate between lupus and nonlupus, and then employ only select variables in final model construction. Both outputs could provide a standard, reproducible approach to lupus diagnosis that could be employed across centers and be widely useful. The latter circumstance, however, illustrates another powerful capability of machine learning, the ability to select parameters (i.e., features) that are most important in achieving the model outcome. One goal of many machine learning applications is to arrive at a model with high accuracy from the fewest number of features. This may help to discern the features that are of the greatest biological importance – potentially illustrating those that may act as drivers of disease. In addition, reducing necessary features allows for future data collection and application to be more efficient and cost-effective, as fewer inputs are needed.

In the same vein, this framework of training a model from input patient data can be used in multiple lupus schema, including the identification of biomarkers that predict flares or the classification of patients likely to experience disease progression. Overall, machine learning could contribute to standardization of lupus clinical practice across centers and heterogeneous patients. Moreover, machine learning can be used to determine patterns of potential biological importance in patients with lupus that may have been missed by human observation or not fully appreciated. Because of its great potential importance, considerable work has been devoted to applying machine learning to lupus diagnosis and management but this application of machine learning has not been fully developed or accepted.

MACHINE LEARNING IN THE DIAGNOSIS OF LUPUS

The diagnosis of lupus can be complex. Different symptom presentations across patients, nonstandard laboratory test results, and shared symptoms with other autoimmune or inflammatory diseases can complicate diagnosis [9]. As such, machine learning may offer a means of facilitating earlier or consistent diagnosis. To date, various machine learning models have employed different feature inputs to develop diagnostic models. Some of these features include those derived from American College of Rheumatology (ACR) or European Alliance of Associations in Rheumatology (EULAR) classification criteria, whereas others leverage gene expression analyses to provide alternate means for diagnosis.

A lupus diagnostic machine learning model employing clinical and serological features was recently developed by Adamichou et al. [10■■]. With the model, the authors developed a scoring system to diagnose adult lupus that includes a subset of parameters derived from Systemic Lupus International Collaborating Clinics (SLICC), ACR, and EULAR criteria. The model-derived scoring system is powerful as both a binary classifier of lupus as compared with other rheumatic diseases (94.2% accuracy), and also provides the probability of the sample being SLE (unlikely, possibly, likely, and definite SLE), which adds further clinical benefit. In addition, the scoring system, which provides weighted coefficients for each binary feature, increases the potential for clinical implementation of the model. Indeed, the same machine learning-derived scoring system was tested in a cohort of pediatric SLE and other rheumatic disease patients by independent authors and exhibited outstanding performance (AUC = 0.94) [11].

Similarly, another study aimed to enable earlier diagnoses of lupus or alert clinicians to 'red flags' that may suggest SLE [12]. Following the initial identification of 58 features, similar to those examined by Adamichou et al. [10■■], the authors evaluated the classification performance of three different machine learning models based on a final 12 features. However, in an effort to identify the strongest features that could further enable early diagnosis, a model with only the top three features (anti-dsDNA, low complement, and malar/maculopapular rash) was built and exhibited outstanding performance (AUC = 0.95 ± 0.02).
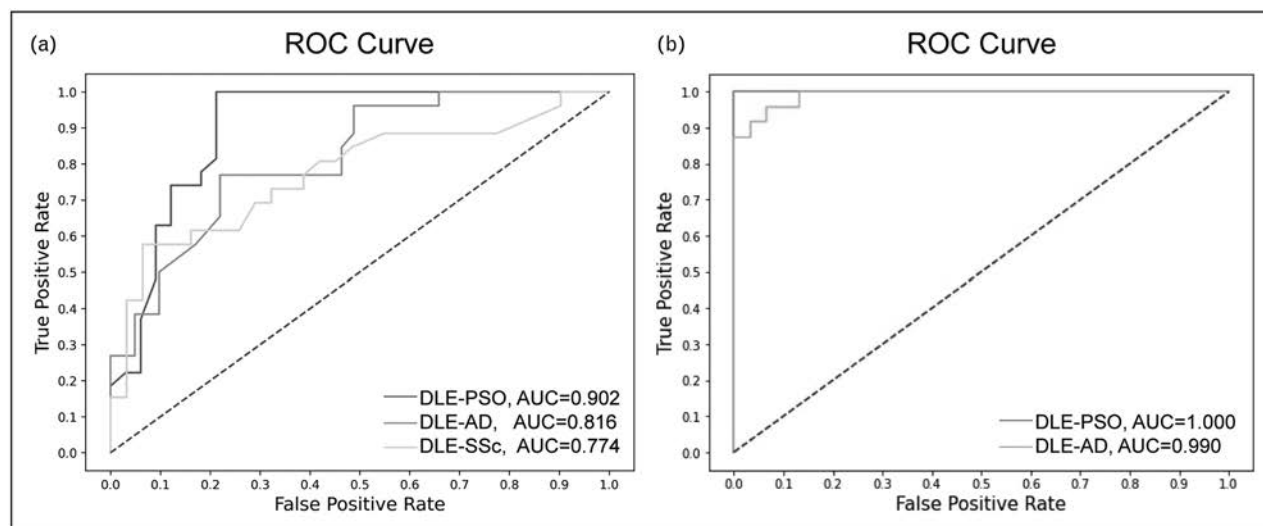
Gene expression-derived machine learning models have also been constructed to diagnose SLE or inflammatory skin diseases, including cutaneous lupus erythematosus (CLE). Ma et al. [13] examined machine learning models built with bulk or single-cell RNA-sequencing (RNA-seq) data for the ability to discriminate SLE from healthy controls. After identifying disease-specific differentially expressed genes from clustering of single-cell populations and analysis of receptor-ligand interactions, the expression of 67 genes was used as

input. Notably, the bulk RNA-seq model had better performance (AUC $= 0.998 \pm 0.004$).

Machine learning has also been used to classify CLE and other inflammatory skin diseases based on individual sample gene expression profiles [14■]. Using 48 input gene signatures related to inflammatory cells, resident skin cells, immune pathways, and cell processes, lesional CLE samples, specifically discoid lupus erythematosus (DLE), could be separated from healthy controls (AUC $= 0.977$) and other lesional diseases such as psoriasis (AUC $= 0.902$), atopic dermatitis (AUC $= 0.816$), and systemic sclerosis (AUC $= 0.774$) with outstanding to modest accuracy (Fig. 2a). In addition, machine learning was able to discriminate nonlesional CLE from nonlesional psoriasis (AUC $= 1.00$) and nonlesional atopic dermatitis (AUC $= 0.990$) with outstanding accuracy (Fig. 2b). Altogether, using methods to identify information about feature importance in model prediction, it was possible to determine the input elements that most contributed to proper disease classification, noting both distinct and overlapping features among the diseases. The identification of the unique features could be helpful in the future diagnoses and staging of inflammatory skin diseases, whereas identification of shared features may allow for the repurposing of treatments that are used in one disease to another indication based on similar gene expression. Machine learning has similarly been employed to determine the cellular gene signatures that most contribute to metabolic dysregulation in lupus nephritis kidneys [15].

An additional study did not directly build a diagnostic model but instead used machine learning to determine flow cytometry-identified T and B cell subsets that differentiate between SLE and primary Sjögren's syndrome (pSS) [16■]. With initial machine learning models and statistical tools, it was evident that SLE and pSS have very similar immune cell architectures – only five of the 29 measured immune cell subsets were different between diseases – and the performance of the machine learning classifier was modest (AUC $= 0.710$) [16■]. However, the authors next clustered the flow cytometry data from the patients with SLE and pSS and identified two endotypes – that is, molecular subsets of disease – and both endotypes comprised both SLE and pSS samples. Subsequent machine learning models built on T and B cell flow cytometry were able to accurately identify the two endotypes (AUC $= 0.994$), which the authors suggested may be more clinically important in predicting disease course than the disease label. A similar study also used immune cell flow cytometry, though not restricted to T and B cells, to first build models to classify SLE from controls, and then used the most important features determined from the classifiers as input into a clustering algorithm to determine patient subsets, which were correlated to clinical features [17].

FIGURE 2. Gene expression-derived machine learning models can classify lesional or nonlesional cutaneous lupus erythematosus from lesional or nonlesional samples from related inflammatory diseases. (a) Receiver operating characteristic curve of lesional discoid lupus erythematosus (DLE) samples compared with lesional psoriasis (PSO) (purple) samples, lesional DLE samples compared with lesional atopic dermatitis (AD) samples (orange), and lesional DLE samples compared with lesional systemic sclerosis (SSc) samples (green) using 48 specific cellular and pathway gene signatures. (b) Receiver operating characteristic curve of nonlesional DLE samples compared with nonlesional PSO (purple) samples and nonlesional DLE samples compared with nonlesional AD samples (orange) using the same 48 cellular and pathway gene signatures. Reproduced with permission from Martínez *et al.* Figs. 4a and 6a [14■].

Altogether, these diagnostic and classification models illustrate that using clinical, flow cytometry, or gene expression data, machine learning models may offer a means to classify systemic or cutaneous lupus from related diseases when other methods may be inconclusive. In addition, models with less discriminatory capacity may suggest that the two classes are more biologically similar than expected – such as lesional CLE and lesional systemic sclerosis, or B and T cells in SLE and pSS – or, perhaps, that different input data are necessary to discriminate groups. Indeed, an additional machine learning study was able to achieve AUCs between 0.83 and 0.96 for classifying SLE from pSS using DNA methylation data [18].

MACHINE LEARNING FOR FLARE PREDICTION AND THE TREATMENT OF LUPUS

The ability to accurately predict flares in SLE patients is a critical unmet medical need. Prediction of flares, disease activity, or disease progression with machine learning may help direct therapeutic plans for lupus and suggest specific prophylactic treatment. One study aimed to predict flares from EMRs [19]. Another study similarly estimated the score for different categories of lupus disease activity [20]. A third study used 59 demographic, clinical, immunological, pathological, and therapeutic characteristics as input to build a machine learning model to predict renal flares (AUC = 0.819) [21▪]. In addition, to increase clinical feasibility, a risk score prediction model (AUC = 0.746) was built from six important variables determined from the initial model.

Yones *et al.* [22▪] applied machine learning to blood gene expression data from pediatric patients with lupus with the aim of classifying patients into those with high and low disease activity. The authors repeatedly reduced the number of model input genes, by various feature selection techniques. Ultimately, they were able to classify patients with high and low disease activity using the expression of only 34 genes (accuracy = 81%). Examination of the genes that discriminated each subset provided useful information about underlying subset biology/pathogenesis, including the finding that the interferon response term was limited to low disease activity in this cohort [22▪]. Hierarchical clustering of the initial machine learning model output resulted in even further granularity about the disease activity subtypes, which were correlated with clinical data.

Another study employed gene expression data to determine the genes expressed by purified monocytes, NK, T, and B cells that were able to classify patients into high or low disease activity groups [23]. With patients of all ancestries, the models for the T

and B cells were able to achieve an AUC of 0.80, suggesting that T-cell and B-cell gene expression can modestly discriminate disease activity status. However, models that were restricted to specific ancestries (either European or Asian) did not perform as well, with the T-cell model in European ancestry-restricted patients achieving an AUC of 0.71, and the model in Asian-restricted patients achieving an AUC of 0.62. This suggests that there may be gene expression features related to ancestry that could be independent of disease activity, as previously reported [24].

Although machine learning has yet to be applied for matching individual patients with lupus with the most appropriate treatment or treatment dose clinically, advancements in machine learning-derived prediction of flares and subsetting of patients can help in the identification of specific treatments. For example, after a machine learning clustering model identified patient subsets using clinical data, the gene expression profiles of the subsets were input into a program [Connectivity Map Linked User Environment (CLUE)] that identifies drugs that reverse the disease gene expression signature of the subset [25].

MACHINE LEARNING IN IMAGING: MRI AND HISTOLOGY

Machine learning has frequently been applied as an image processing tool in medicine. The advantages of using machine learning for image processing include the ability to save time in both analysis of individual images and the thorough training required for analysis, increased objectivity and consistency, and the capacity to detect abnormalities that may be missed by the human eye [5]. Several studies have investigated the ability of machine learning to probe magnetic resonance (MR) and other medical images in lupus, especially as they relate to the classification of neuropsychiatric lupus [26,27], many of which were reviewed previously [5,6]. Indeed, machine learning analysis of radiologic images may be the most readily adaptable means to incorporate this tool to lupus clinical care, especially for the detection of small image perturbations and standardization of image analysis and grading across centers.

Machine learning can also be used to evaluate histologic findings [28–31,32▪▪,33]. As with MR image analysis, the use of the machine learning paradigm to evaluate histology could lead to better standardization of diagnoses, and, additionally, allow for the recognition or incorporation of features that were previously not considered in lupus nephritis or other lupus tissue pathologies. For example, current nephropathological classification

of lupus nephritis focuses on the proliferation of mesangial cells and endocapillary cells as well as the proportion of glomeruli with inflammatory lesions – whether they are local or diffuse [34]. Many of the recent studies using machine learning to evaluate lupus nephritis kidney biopsies focused on automating and thereby standardizing the classification of features from glomerular lesions biopsy [28–31]. However, tubulointerstitial inflammation, not glomerular inflammation, is a known predictor for end-stage renal disease (ESRD), but not all patients with this feature progress [32■■]. As such, Abraham *et al.* [32■■] employed confocal microscopy and machine learning to determine whether cellular compositions (T, B, myeloid dendritic, and plasmacytoid dendritic cells) in kidney regions could predict progression to ESRD. The machine learning algorithm was built to identify and classify the immune cell types, and then these frequencies were correlated with clinical features. Of note, they found that high B cell densities were protective, whereas high CD4$^-$ T cell densities (including CD8+, gamma/delta, and double negative T cells) were predictive of progressive disease.

Machine learning has also been employed to evaluate CLE histology. One study developed a smartphone app with a machine learning model for differentiating different skin diseases and subtypes of CLE using 9,241 input dermatologist-labeled images [33]. The model with the best performance achieved an AUC of 0.973. It is notable that the authors compared the diagnostic performance of their model for up to 197 randomly selected images to the diagnosis of 688 doctors. Among those doctors, the model outperformed nondermatologists and performed similarly to those with professorships in dermatology.

These studies illustrate the capacity of image analysis-machine learning frameworks, many of which employ the use of neural networks or deep learning to segment individual parts of the images, to advance our understanding of tissue pathogenesis and identify previously unidentified features or patterns.

MACHINE LEARNING IN CLINICAL TRIALS

Clinical trials in lupus are fraught with difficulties [35]. Although machine learning will not resolve all issues present in lupus clinical trials, there are specific areas where it could be utilized. Harrer *et al.* [8] wrote a comprehensive review about the means by which artificial intelligence could be utilized for clinical trial design and we aim to summarize their key points. Namely, clinical trials can fail because of difficulties identifying, recruiting, and enrolling both 'eligible' and 'suitable' patients [8]. 'Eligible'

patients are those who meet the eligibility criteria. If those patients are not readily identified, it can stall clinical trial progression, costing money, or even lead to trial termination [8]. For example, a phase I study of AMG 557 in cutaneous lupus (NCT01389895) was terminated because of slow enrollment [36]. 'Suitable' patients are those who have the potential to respond to the agent, meaning they exhibit upregulation of the targeted mechanism [8]. Ideally, machine learning models capable of identifying perspective patients from millions of datapoints could substantially impact the future of lupus clinical trials.

Machine learning mining of EMR/EHRs could allow for the identification of patients that meet the eligibility criteria for a trial. Once identified, these patients or their providers can be more easily contacted about potential interest in the trial. In addition, it may be important to select for patients with similar characteristics [8], that is, reducing trial population heterogeneity, which could also be achieved by machine learning mining of EMR/EHRs for eligible patients, and then clustering of those identified. To determine suitability, however, requires biomarkers, which may not be readily identifiable or have yet to be identified. Nevertheless, machine learning analysis may help to expedite the identification of disease or drug biomarkers that could then be employed for the identification of suitable participants, that is clinical trial enrichment. For lupus especially, machine learning identification of eligible members of minority populations could also help to address some of the difficulties with underrepresented minority populations in clinical trials [37].

Although machine learning has yet to be applied to clinical trial design in lupus, one trial (NCT04786431) made use of machine learning for outcomes analyses [38] – another appropriate use of the technique. Matthiesen *et al.* [39] employed supervised machine learning to classify patients with SLE from other cardiovascular disease patients based on their lipid profiles. Beyond clinical trial design, machine learning- and artificial intelligence-guided drug development and drug repurposing before the implementation of clinical trials are also promising [40,41]

WHAT CAN MACHINE LEARNING ACCOMPLISH AND WHERE WILL CLINICAL JUDGMENT STILL BE PREFERRED?

Although the definition of artificial intelligence suggests the existence of machines that can think like humans, artificial intelligence/machine learning should be considered supplemental tools in

clinical and scientific decision-making. Machine learning relies on the data it sees and the algorithms it employs. Therefore, even though a machine learning model may provide a finite outcome prediction, the clinician interacts with the patient and can use clinical judgment to override the machine learning-predicted outcome or use the machine learning outcome as one piece of information in the overall evaluation of the patient.

Importantly, the employment of machine learning as a clinician or scientist relies on both confidence and humility. One must have confidence to examine the machine learning results and choose to disregard or refine a machine learning model when the results are not biologically plausible, or in which model overfitting could be expected. Humility is required to consider the possibility that when there is a disagreement between current understanding and a specific machine learning outcome that 'truth' should be revisited, and the biological appropriateness of the outcome tested. Regardless, before any clinical impact can be made from a machine learning model, it is imperative that the model is well-trained, validated, and tested in an external dataset and has sufficient performance characteristics [2] to be clinically relevant.

BARRIERS TO THE APPLICATION OF MACHINE LEARNING IN LUPUS

There remain barriers that make the translation of machine learning models to the clinic complex [42]. Briefly, clinical personnel approval, including that of clinicians and stakeholders, of model implementation varies. Successful implementation of machine learning models was found to be more likely when clinicians and other stakeholders were involved in model development [42]. That is, adoption is more likely when clinicians played a key role in the identification of the clinical need and determination of how/where it would be best to intervene in the clinical care schema [42]. Collaboration between the model developer and the clinical personnel ensures that the model is clinically useful and relevant. In addition, some resistance to implementation can also occur because clinicians may consider machine learning to be a 'black box' in which the process to determine the model output may not be easily understood or they may not be versed on machine learning interpretation and the metrics used to evaluate model performance [42]. This too could be overcome through continued conversations designed to educate all parties about the principles, power, and shortcomings of machine learning.

Sufficient aggregation and collection of data can also be challenging for the implementation of machine learning models. Although many systems have moved to electronic formats to collect patient data, there are still differences between systems. And sometimes, health data is scattered across different physical locations and media [8]. Some medical records still exist on paper, which then must be translated to a form the computer can read.

CONCLUSION

The frequency with which articles on lupus that include machine learning are being reported is comparable with that of rheumatoid arthritis and cancer. There is momentum for a shift in clinical management to include advanced computational pipelines, including machine learning. This could include the use of machine learning-guided clinical practice for earlier diagnosis and flare prediction or identification of specific subsets of patients with lupus – and their defining features, which can then be treated with more precision. In addition, machine learning could be used to facilitate clinical trial enrollment, especially for easier identification of eligible patients, and possibly, with known biomarkers, enrich for patients that have a better chance of responding to the drug. Altogether, the future of lupus care likely includes machine learning, but to accomplish machine learning-supported clinical care, effective collaboration among experts from multiple disciplines will be necessary.

Conflicts of interest
K.M.K. was employed by AMPEL BioSolutions and the RILITE Research Institute during the preparation of this work. P.E.L. is a cofounder of AMPEL BioSolutions, LLC. The authors declare that the content of this article is not related to the commercial activities of AMPEL BioSolutions.

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