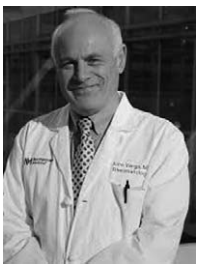
Editorial introductions

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

SECTION EDITORS

John Varga



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

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

Dr Varga is the author of more than 350 original articles, 40 book chapters and four books. His research has been funded by the National Institutes of Health and the Scleroderma Research Foundation. He is an elected member of the Association of American Physicians, and is Master of the American College of Rheumatology.

Anna Ghirardello

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

Andrea Doria

Andrea Doria, MD, 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 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).

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.

Gastrointestinal involvement in systemic sclerosis: diagnosis and management

Zsuzsanna H. McMahan and Laura K. Hummers

Purpose of review
This review provides important updates in systemic sclerosis (SSc)-related gastrointestinal disease, with a particular focus on the diagnosis and management of dysmotility.

Recent findings
In the past 2 years, several studies were published that present interesting diagnostic insights into SSc and gastrointestinal dysmotility. Studies focusing on new therapies and the novel application of existing therapies, both in SSc and non-SSc-associated gastrointestinal dysmotility syndromes, demonstrate progress in the management of these challenging complications.

Summary
SSc gastrointestinal disease is heterogeneous in its clinical presentation, which presents a challenge in diagnosis and management. Objective studies may help to identify patterns of gastrointestinal dysmotility and more specifically target therapy. A variety of drugs are now available or are under study in the management of gastrointestinal dysmotility, such as prucalopride, intravenous immunoglobulin, pyridostigmine, linaclotide, relamorelin, and others. These drugs may improve symptoms and quality of life in SSc gastrointestinal patients. Combination therapies are also under study. Electroacupuncture, dietary intervention (e.g. medical nutrition therapy, low FODmap diet), and medical cannibus may also play a role in alleviating patient symptoms; however, more data are needed to define the role of these interventions in SSc.

Keywords
diagnosis, dysmotility, gastrointestinal, management, systemic sclerosis

INTRODUCTION

The gastrointestinal tract is the most commonly affected internal organ in systemic sclerosis (SSc) [1,2]. Up to 90% of patients experience symptoms of upper and/or lower gastrointestinal dysmotility, which may be associated with significant morbidity and mortality [3]. Heterogeneity in the clinical presentation of SSc patients complicates risk stratification and diagnosis, and limited effective therapeutic options complicate treatment strategies [4].

In the past year, exciting updates in the field of gastrointestinal disease related to the diagnosis and management of patients with or without SSc were published. In addition, expert driven recommendations for the treatment of gastrointestinal complications relevant to SSc were updated, providing useful information for practicing rheumatologists, gastroenterologists, and general practitioners [5–7]. This review will focus on gastrointestinal dysmotility, highlighting new diagnostic and therapeutic data with relevance to this important complication in SSc.

METHODS

The following initial search terms (01/07/2016–05/10/2018) were used in PubMed: 'scleroderma gastrointestinal' (denominator 77); 'systemic sclerosis gastrointestinal' (denominator 107), 'treatment gastrointestinal dysmotility' (denominator 85), 'fecal incontinence systemic sclerosis' (denominator 11), and 'fecal incontinence scleroderma' (denominator 7) and 'treatment gastrointestinal motility' (denominator 625). We identified 81 studies of interest based on their relevance to the diagnosis and treatment of gastrointestinal disease in systemic sclerosis, including key review articles whose

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KEY POINTS

- Systemic sclerosis-associated gastrointestinal dysmotility is a heterogeneous complication.
- Objective imaging studies are important in identifying distinct characteristics of the disease and localizing the involved regions of the gut.
- Novel promotility agents and the novel application of existing agents are expanding our options for the management of these challenging patients.
- More data are needed in the role of alternative medicine and specific diets in the modification of SSc gastrointestinal disease symptoms.

reference lists were screened for additional relevant manuscripts.

DIAGNOSIS

Esophagus

Esophageal disease in SSc is the most common gastrointestinal complication, as it affects up to 90% of patients. It is often one of the earliest features of SSc, and may present with symptoms of dysphagia, heartburn, and regurgitation. An international gastroesophageal reflux disease (GERD) working group recently published a consensus statement, incorporating published data with expert consensus opinion [8]. This working group suggests that a diagnosis of GERD is initially dependent on response to an empiric trial of antisecretory therapy. When symptoms persist, despite initial therapy, and/or if alarm symptoms are present (e.g. unintentional weight loss, anemia), then esophageal motor physiology and reflux burden may be investigated through high-resolution esophageal manometry and ambulatory pH monitoring, respectively [8]. These additional data can more specifically define the clinical problem and direct patient care towards targeted therapeutic interventions.

Esophageal manometry is the gold standard for diagnosing esophageal dysmotility in SSc [8]. Several new studies have sought to define the distribution of manometric findings in SSc. Manometric findings in SSc are heterogeneous, with recent studies suggesting that absent contractility is the most frequent finding (56%), followed by normal motility in 26%, and ineffective esophageal motility in 10% [9]. The characteristic esophageal findings in SSc of hypotensive lower esophageal sphincter and absent contractility coexist in approximately 33% of patients [9,10]. Significant overlap between the presence of esophageal involvement (defined by high-resolution manometry) and anorectal dysfunction (defined by anorectal manometry) was also recently reported, suggesting that there may be a mechanistic link between the esophageal and anorectal complications in SSc [11]. Others sought to determine whether high-resolution manometry correlates with upper gastroesophageal reflux disease symptoms [12], and found that a negative correlation between reflux scores from the UCLA GIT 2.0 questionnaires (higher = more severe), suggesting that esophageal symptoms may serve as a good surrogate for objective esophageal data in SSc.

Stomach

Gastroparesis is reported in 38–50% of SSc patients. Common presenting symptoms include early satiety, bloating, and regurgitation, though a strong association between symptoms and objective motility testing has not yet been demonstrated even in the general population. Stanford investigators demonstrated that non-SSc patients with symptoms suggestive of a functional dyspepsia and/or gastroparesis (such as postprandial distress) also had concomitant esophageal motility abnormalities. [13]. Seventy-two percentage (44/61) of these symptomatic patients demonstrated evidence of gastroparesis by scintigraphy. Concomitant esophageal motility disorders were more common among patients with evidence of gastroparesis (68%) than among patients without evidence of gastroparesis (42%). Importantly, symptoms of heartburn, regurgitation, bloating, nausea, vomiting, dysphagia, and belching could not distinguish between patients with and without gastroparesis, though weight loss was more prevalent and severe in the gastroparesis group. This suggests that symptoms alone are not effective in distinguishing between the presence and absence of gastroparesis in SSc.

Small intestine

The small intestine is affected in 12–55% of SSc patients [14–16]. Small intestinal involvement may present with symptoms of small intestinal dysmotility (e.g. distention, bloating), small intestinal bacterial overgrowth (SIBO), or both. Though the etiopathogenesis of SIBO is poorly understood, investigators recently determined that non-SSc patients with SIBO have significantly lower ileocecal junction pressure, prolonged small bowel transit time, and a higher pH as compared to those without SIBO, and it was thought that these abnormalities may play a role in SIBO pathogenesis [17].

Investigating these measures in SSc patients may provide insight into SIBO pathogenesis in SSc.

Colon and anorectum

Constipation and fecal incontinence are reported in 50% or more of SSc patients [18,19]. A French study recently examined the prevalence of fecal incontinence in 77 SSc patients and identified risk factors associated with this complication. Findings from this cohort were compared with a historical cohort from the general population in the Rhone-Alpes region of France [20]. They found that 38% of SSc patients, and only 6% of patients in the general population had fecal incontinence. Clinical associations between SSc-related fecal incontinence and longer disease duration, loose stools, SIBO, and constipation, were also reported [20,21]. These findings suggest that control of lower bowel symptoms may improve fecal incontinence in SSc.

THERAPY

While data on targeted therapies for gastrointestinal dysmotility specifically in SSc is somewhat lacking, data evaluating novel therapeutic targets from other gastrointestinal dysmotility disorders may help physicians manage these patients. A list of possibly beneficial prokinetic therapies and the level of available data in SSc are listed in Table 1. In this section, new data on therapies will be examined in detail and divided by gastrointestinal regions.

Gastroesophageal reflux disease

Gastroesophageal reflux disease, in patients with or without SSc, is often initially managed with lifestyle modification, proton pump inhibitors (PPI), and H2 blockers. However, data related to the best first-line agent and the efficacy of combination therapies in SSc are limited. The efficacy of combination therapy for GERD was recently studied in a double-blind randomized multicenter clinical trial in non-SSc patients with esophageal reflux disease. Investigators sought to determine whether esomeprazole single therapy or esomeprazole and mosapride (gastroprokinetic agent that increases decreases gastric emptying time and decreases esophageal reflux) combination therapy was more effective [44]. Combination therapy improved upper abdominal pain, belching, and contributed to more rapid improvement in GERD symptom scores.

In a SSc population, a randomized placebo-controlled trial was recently completed, in which SSc patients with GERD who partially responded to PPI were randomly assigned to add domperidone or algycon (a medication for indigestion/heartburn made of alginic acid, aluminum hydroxide, and magnesium carbonate) therapy. Domperidone and algycon were equally effective treatments in combination with omeprazole, though approximately 20% of patients were nonresponders in each group [22].

Esophageal dysmotility

Esophageal dysmotility is often associated with symptoms of regurgitation, heartburn, dysphagia, and chest discomfort. Treating these symptoms can significantly improve quality of life. An open-label trial was used to evaluate the effects of 20 mg daily buspirone on esophageal motor function and symptoms in SSc patients. Of the 22 patients who tolerated the drug, lower esophageal sphincter pressure increased from 7.7 ± 3.9 to 12.2 ± 4.6 mmHg ($P = 0.00002$) after buspirone administration in the absence of change in other manometric parameters. Improvements in heartburn and regurgitation were noted over the 4-week follow-up period [23].

Gastroparesis

Gastroparesis can be mild, and such cases may be managed by lifestyle/dietary modification. Patients with more significant symptoms, however, often require prokinetic agents, such as metoclopramide or domperidone, to decrease gastric emptying time; however, the tolerability and adverse effects (e.g. tarditive dyskinesia, long QT interval) associated with these medications can limit their use. Novel therapies, under study in the gastroparesis population, aim to control symptoms and limit toxicity.

Studies suggest that intravenous immunoglobulin (IVIG) may benefit patients with gastroparesis [45▪▪]. Both an open-label study and a retrospective chart review determined that IVIG may improve gastroparesis symptoms refractory to standard treatments. Although there was no placebo group in the open-label trial, significant improvement was noted in nausea, vomiting, early satiety, and abdominal pain symptom scores [24,25,46]. The application of IVIG therapy in gastrointestinal dysmotility is particularly interesting in the context of functional antimuscarinic 3 receptor (M3R) antibodies, which are reported in scleroderma, and known to have negative effects on gastrointestinal motility.

Other novel approaches in the management of gastroparesis are also under study. Selective 5HT4 receptor agonists, such as prucalopride, have a higher affinity for the 5HT4 receptor, and are therefore thought to be less cardiotoxic than prior

Table 1. Promotility agents and available data in scleroderma

Drug	Use in GI disease	Mechanism of action	Common or significant adverse events	Data for use in SSc GI disease
Metoclopramide	Esophageal dysmotility, hypotensive LES, gastroparesis, small bowel dysmotility	Dopamine 2 receptor antagonist, 5HT4 agonist, weak 5HT3 antagonist	Dyskinesia and acute dystonia	Case series [28–30]
Domperidone	Esophageal dysmotility, hypotensive LES, gastroparesis	Dopamine 2 antagonist	Cardiac arrhythmia (QT prolongation), hyperprolactinemia	RCT; open-label trial [22,31]
Pyridostigmine	Esophageal dysmotility, gastroparesis, small bowel and large bowel dysmotility	Acetylcholinesterase inhibitor	Abdominal pain and diarrhea	Case series [27]
Bethanechol	Esophageal dysmotility,	Parasympathetic choline carbamate that selectively stimulates muscarinic receptors	Abdominal pain and diarrhea	None
Buspirone	Esophageal dysmotility, hypotensive LES	5HT1 receptor agonist; preferential full agonist of presynaptic 5HT1A receptors which are inhibitor autoreceptors and a partial agonist of postsynaptic 5HT1A	Dizziness, drowsiness, nausea	Open label trial [23,31]
Sildenafil	Hypotensive LES, colonic dysmotility	Phosphodiesterase inhibitor	Headache, lightheadedness, dizziness	None
Cisapride	Esophageal dysmotility, gastroparesis, small bowel dysmotility	Serotonin 5HT4 receptor agonist; indirectly acts as a parasympathomimetic	Cardiac arrhythmia, headache, rash, abdominal cramps, diarrhea,	Double-blind, placebo-controlled trial; case series [32–35]
Prucalopride	Large bowel dysmotility	Selective high affinity 5HT4 receptor agonist	Headache, nausea, abdominal pain, diarrhea	Randomized cross-over study, case series [26,36]
IVIG	Gastroparesis	May inhibit pathogenic antibodies to M3R receptors	Aseptic meningitis	Case series [24,25,37–40]
Remorelin	Gastroparesis	Ghrelin agonist	Flushing, upset stomach, nausea, fatigue	None
Octreotide	Small bowel dysmotility	Somatostatin mimic	Arrhythmia, tachyphylaxis, hyperglycemia, dizziness, abdominal pain, diarrhea, flatulence	RCT; case series [40,41]
Neostigmine	Small/large bowel dysmotility	Anticholinesterase agent	Arrhythmia, dizziness, diarrhea, dysphagia, nausea, crams, salivation, lacrimation, bronchospasm	Randomized placebo crossover study; case series [42,43]
Linaclotide	Colonic dysmotility	Guanylate cyclase 2C agonist on intestinal epithelial cells	Diarrhea, abdominal pain flatulence, URI	None
Plecanatide	Colonic dysmotility	Activates guanylate cyclase C on intestinal endothelial cells	Dizziness, diarrhea, abdominal distention	None
Lubiprostone	Colonic dysmotility	Activates CIC-2 chloride channels in GI epithelial cells	Headache, nausea, diarrhea	None
Misoprostol	Colonic dysmotility	Prostaglandin analogue	Diarrhea, abdominal pain, headache	None
Colchicine	Colonic dysmotility	Inhibitors microtubule polymerization;	Diarrhea, nausea, vomiting	None

GI, gastrointestinal; SSc, systemic sclerosis.

versions such as cisapride. These drugs decrease gastric emptying time [47], and improve motility in other parts of the gut (e.g. colon) [47–52]. However, trials with revexepride (M0003 or R149402) – a different 5HT4 receptor agonist – demonstrated no significant improvement in symptoms or gastric emptying in non-SSc patients with gastroparesis [53]. In addition, while prucalopride demonstrated efficacy in chronic constipation, data showing benefit in gastroparesis are lacking [54]. In summary, these data suggest that selective 5HT4 receptor agonists have a role in the management of refractory constipation; there may not be a significant role from them in the management of gastroparesis.

Relamorelin is a synthetic peptide and selective agonist of the ghrelin/growth hormone secretogogue receptor [55,56]. It increases growth hormone levels and accelerates human gastric emptying time. In phase IIB studies, it decreased nausea, fullness, bloating, and abdominal pain associated with diabetic gastroparesis. Cardiac and neurological side effects were not reported [55]. At present, it is under investigation in phase III studies, but is unstudied in SSc. Relamorelin may also play a role in the management of constipation as it can stimulate defecation and improve lower gastrointestinal transit [57,58].

Small bowel

Small bowel involvement associated with distention, diarrhea, and bloating can be severe is SSc and result in malabsorption, recurrent pseudoobstruction, hospitalization, and unintentional weight loss. A range of therapeutic approaches is often integrated in the management of such patients, including dietary modification [59], antibiotics for suspected bacterial overgrowth, and promotility agents.

A systematic review and meta-analysis recently evaluated the evidence related to the efficacy and safety of rifaximin for the eradication of SIBO in adults [60▪▪]. Thirty-two studies and 1331 patients were included. The overall eradication rate was 70.8%, whereas the overall rate of adverse events was 4.6%. Improvement or resolution of symptoms occurred in 67.7% of patients with eradicated SIBO, though this subset analysis only included 10 studies. Overall, it was determined that rifaximin therapy is well tolerated and effective for SIBO, though RCTs need to confirm these findings and define the optimal regimen.

Another systematic review assessed the evidence for the efficacy of probiotics in preventing or treating SIBO [61▪▪]. Fourteen full-text articles and eight abstracts were included. Probiotics did not prevent SIBO, but contributed to decontaminating SIBO, decreasing H2 concentration and relieving abdominal pain.

Recent studies examined the prevalence of lactose malabsorption and correlation between lactose malabsorption and SSc gastrointestinal disease. Lactose malabsorption was more prevalent in SSc patients than in controls, and the presence of lactose malabsorption correlated strongly with severe esophageal and small intestinal motor disorders [59]. A trial of lactose elimination in such patients may improve quality of life and reduce the need for extensive motility testing and promotility medications.

Studies using animal models and human engineered tissue have identified potentially novel therapies for patients with small bowel dysmotility [62,63▪]. Electroacupuncture at the L11 stimulation point promoted jejunal motility through the parasympathetic pathway in rats [63▪], and the implantation of neural crest cells restored enteric nervous system function in human tissue engineered small intestine [64]. Further studies are needed to determine the efficacy and safety of such interventions in patients.

A case series involving five SSc patients treated successfully with Abatacept in the treatment of chronic intestinal pseudo-obstruction (CIPO) was also recently described. Improvement in symptoms and a reduction in the episodes of pseudoobstruction suggested that further studies might be important [65]. A multicentered clinical trial evaluating the effects of abatacept in patients with diffuse SSc is underway, and will hopefully provide additional data in this area.

Colonic hypomotility

Mild to moderate colonic hypomotility is often managed with dietary modification, stool softeners and laxatives; however, these interventions are inadequate in more severe disease. Prior studies demonstrated that serotonin receptor agonists are effective promotility agents; however, cardiotoxicity resulted in their removal from the market. Recently, in both the SSc and general gastrointestinal literature, several studies evaluated the safety and efficacy of prucalopride for refractory constipation [47–52]. Vigone and Beretta [26] reported the results of an open-label crossover study (PROGASS) evaluating the safety and efficacy of prucalopride in SSc patients' gastrointestinal disease. Patients on prucalopride had a higher number of complete bowel movements, and reductions in reflux and bloating, as determined by the UCLA GIT 2.0. In the non-SSc population, prucalopride reduced abdominal symptoms in patients with functional bowel disorders [66]. Prucalopride had a favorable safety and tolerability profile in an analysis of six randomized controlled clinical trials [67]. It is a promising drug for the management of SSc gastrointestinal dysmotility.

Pyridostigmine – an acetylcholinesterase inhibitor – may also be applied in the context of gastrointestinal dysmotility [68–71]. Most recently, a retrospective series evaluated 31 symptomatic SSc patients who were treated for at least 4 weeks with at least 30 mg three times daily pyridostigmine. Constipation was the most commonly improved symptom [27]. Symptom improvement from pyridostigmine was also reported in prior case series focused on non-SSc patients with autoimmune gastrointestinal dysmotility [69,72,73]. In our experience, pyridostigmine generally has a milder effect on the colon,

relative to linaclotide or lubiprostone, and is a good option for patients who do not tolerate the intensity of these drugs.

Guanylyl cyclase agonists, such as sildenafil, increase cGMP levels in the intestinal epithelium to promote secretion and may normalize bowel transit in preclinical models [74]. Further clinical studies in humans are needed to define the potential benefit of cGMP agonists in the management of refractory constipation.

An expert panel in functional digestive disorders convened to review the efficacy and safety of linaclotide and to develop an updated consensus report for the treatment of patients with constipation-predominant irritable bowel syndrome (CP-IBS) [7]. The panel recommended linaclotide for the treatment of moderate to severe CP-IBS in adults. They recommended that patients take it continuously, and that tachyphylaxis is unlikely to occur based on existing data. While this drug has not yet been studied in SSc, it could be considered in patients with refractory constipation.

Fecal incontinence

The management of fecal incontinence can be challenging in SSc. Importantly, women with SSc have an increased prevalence of lower gastrointestinal and pelvic floor symptoms than what is observed in the general population [75]. A recent study evaluated whether anorectal feedback improves symptoms of fecal incontinence in SSc compared to patients with functional fecal incontinence [76]. Both groups of patients benefitted equally from biofeedback therapy as measured by Fecal Incontinence Severity Index (FISI) scores, feelings of control over bowel movements, and quality of life. Anorectal feedback is a noninvasive intervention that should be considered early in the management of SSc-associated fecal incontinence.

Other therapies under investigation

Medical cannabis is gaining increased attention in the literature for the management of autoimmune and gastrointestinal diseases [77]. In the context of gastrointestinal motility, endocannabinoids exert marked antipropulsive effects, mainly mediated by the reduction in acetylcholine release through the activation of presynaptic cannabinoid receptor-1 (CB1). Collectively, the evidence suggests that endocannabinoids significantly reduce smooth muscle contractility though the binding of CB1 [78,79]. Further studies in humans with SSc will be important in determining the role of endocannabinoids in the management of SSc gastrointestinal symptoms and further defining their effects on gastrointestinal dysmotility.

Dietary modification

Medical nutrition therapy was evaluated in eighteen SSc patients with gastrointestinal involvement and unintentional weight loss were consented and recruited for a 6-week intervention. Individually tailored medical nutrition therapy improved symptom burden and appendicular lean height (a measure of sarcopenia) in this population [80]. Specific diets, such as the low FODmap diet, may benefit IBS patients and should be evaluated in SSc [81].

CONCLUSION

The diagnosis and management of gastrointestinal dysmotility in patients with and without SSc is an area of active research. Increasingly sensitive diagnostic tools are available, allowing investigators to identify important clinical subsets within this disease. New therapies may significantly transform our approach to this significant complication of SSc in the coming years.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Jaeger VK, Wirz EG, Allanore Y, *et al.* Incidences and risk factors of organ manifestations in the early course of systemic sclerosis: a longitudinal EUSTAR study. PLoS One 2016; 11:e0163894.
2. Nazarinia MA, Esmaeilzadeh E, Emami Y, Salehi A. One decade distinct features, morbidity and mortality of scleroderma: a cross-sectional study. Clin Exp Rheumatol 2016; 34(Suppl 100):74–78.

3. Santosa A, Tan CS, Teng GG, *et al.* Lung and gastrointestinal complications are leading causes of death in SCORE, a multiethnic Singapore systemic sclerosis cohort. Scand J Rheumatol 2016; 45:499–506.
4. Alastal Y, Hammad TA, Renno A, *et al.* Gastrointestinal manifestations associated with systemic sclerosis: results from the nationwide inpatient sample. Ann Gastroenterol 2017; 30:498–503.
5. Kowal-Bielecka O, Fransen J, Avouac J, *et al.* Update of EULAR recommendations for the treatment of systemic sclerosis. Ann Rheum Dis 2017; 76:1327–1339.
6. Savarino E, Bredenoord AJ, Fox M, *et al.* Expert consensus document: advances in the physiological assessment and diagnosis of GERD. Nat Rev Gastroenterol Hepatol 2017; 14:665–676.
7. Rey E, Mearin F, Alcedo J, *et al.* Optimizing the use of linaclotide in patients with constipation-predominant irritable bowel syndrome: an expert consensus report. Adv Ther 2017; 34:587–598.
8. Savarino E, Bredenoord AJ, Fox M, *et al.* Expert consensus document: advances in the physiological assessment and diagnosis of GERD. Nat Rev Gastroenterol Hepatol 2017; 14(11):665–676.
9. Crowell MD, Umar SB, Griffing WL, *et al.* Esophageal motor abnormalities in patients with scleroderma: heterogeneity, risk factors, and effects on quality of life. Clin Gastroenterol Hepatol 2017; 15:207–213; e1.
10. Perez Y, Lopez N, Lugo-Zamudio G, Barbosa-Cobos RE, *et al.* Frequency of motor alterations detected through manometry in patients with esophageal symptoms and scleroderma. Rev Gastroenterol Mex 2017; 82:193–195.
11. Luciano L, Granel B, Bernit E, *et al.* Esophageal and anorectal involvement in systemic sclerosis: a systematic assessment with high resolution manometry. Clin Exp Rheumatol 2016; 34(Suppl 100):63–69.
12. Abozaid HSM, Imam HMK, Abdelaziz MM, *et al.* High-resolution manometry compared with the University of California, Los Angeles Scleroderma Clinical Trials Consortium GIT 2.0 in Systemic Sclerosis. Semin Arthritis Rheum 2017; 47:403–408.
13. Triadafilopoulos G, Nguyen L, Clarke JO. Patients with symptoms of delayed gastric emptying have a high prevalence of oesophageal dysmotility, irrespective of scintigraphic evidence of gastroparesis. BMJ Open Gastroenterol 2017; 4:e000169.
14. Savarino E, Mei F, Parodi A, *et al.* Gastrointestinal motility disorder assessment in systemic sclerosis. Rheumatology (Oxford) 2013; 52:1095–1100.
15. Marie I, Ducrotte P, Denis P, *et al.* Small intestinal bacterial overgrowth in systemic sclerosis. Rheumatology (Oxford) 2009; 48:1314–1319.
16. Tauber M, Avouac J, Benahmed A, *et al.* Prevalence and predictors of small intestinal bacterial overgrowth in systemic sclerosis patients with gastrointestinal symptoms. Clin Exp Rheumatol 2014; 32; S-82-7.
17. Chander Roland B, Mullin GE, Passi M, *et al.* A prospective evaluation of ileocecal valve dysfunction and intestinal motility derangements in small intestinal bacterial overgrowth. Dig Dis Sci 2017; 62:3525–3535.
18. Martin M, Meaux-Ruault N, Magy-Bertrand N, *et al.* Anal incontinence and vesico-sphincter events in systemic sclerosis: an epidemiologic bicentric cohort study. Semin Arthritis Rheum 2016; 46:124–132.
19. Forbes A, Marie I. Gastrointestinal complications: the most frequent internal complications of systemic sclerosis. Rheumatology (Oxford) 2009; 48(Suppl 3):iii36–iii39.
20. Garros A, Marjoux S, Khouatra C, *et al.* Prevalence of fecal incontinence in a cohort of systemic sclerosis patients within a regional referral network. United European Gastroenterol J 2017; 5:1046–1050.
21. Richard N, Hudson M, Gyger G, *et al.* Clinical correlates of faecal incontinence in systemic sclerosis: identifying therapeutic avenues. Rheumatology (Oxford) 2017; 56:581–588.
22. Foocharoen C, Chunlertrith K, Mairiang P, *et al.* Effectiveness of add-on therapy with domperidone vs. alginic acid in proton pump inhibitor partial response gastro-oesophageal reflux disease in systemic sclerosis: randomized placebo-controlled trial. Rheumatology (Oxford) 2017; 56:214–222.
23. Karamanolis GP, Panopoulos S, Denaxas K, *et al.* The 5-HT1A receptor agonist buspirone improves esophageal motor function and symptoms in systemic sclerosis: a 4-week, open-label trial. Arthritis Res Ther 2016; 18:195.
24. Clark KE, Etomi O, Denton CP, *et al.* Intravenous immunogobulin therapy for severe gastrointestinal involvement in systemic sclerosis. Clin Exp Rheumatol 2015; 33:S168–S170.
25. Raja J, Nihtyanova SI, Murray CD, *et al.* Sustained benefit from intravenous immunoglobulin therapy for gastrointestinal involvement in systemic sclerosis. Rheumatology (Oxford) 2016; 55:115–119.
26. Vigone B, Caronni M, Severino A, *et al.* Preliminary safety and efficacy profile of prucalopride in the treatment of systemic sclerosis (SSc)-related intestinal involvement: results from the open label cross-over PROGASS study. Arthritis Res Ther 2017; 19:145.
27. Ahuja NK, Mische L, Clarke JO, *et al.* Pyridostigmine for the treatment of gastrointestinal symptoms in systemic sclerosis. Semin Arthritis Rheum 2018; 48(1):111–116.
28. Mercado U, Arroyo de Anda R, Avendano L, *et al.* Metoclopramide response in patients with early diffuse systemic sclerosis. Effects on esophageal motility abnormalities. Clin Exp Rheumatol 2005; 23:685–688.
29. Johnson DA, Drane WE, Curran J, *et al.* Metoclopramide response in patients with progressive systemic sclerosis. Effect on esophageal and gastric motility abnormalities. Arch Intern Med 1987; 147:1597–1601.
30. Drane WE, Karvelis K, Johnson DA, *et al.* Scintigraphic detection of metoclopramide esophageal stimulation in progressive systemic sclerosis. J Nucl Med 1987; 28:810–815.
31. Karamanolis GP, Panopoulos S, Karlaftis A, *et al.* Beneficial effect of the 5-HT1A receptor agonist buspirone on esophageal dysfunction associated with systemic sclerosis: a pilot study. United European Gastroenterol J 2015; 3:266–271.
32. Kahan A, Chaussade S, Gaudric M, *et al.* The effect of cisapride on gastro-oesophageal dysfunction in systemic sclerosis: a controlled manometric study. Br J Clin Pharmacol 1991; 31:683–687.
33. Horowitz M, Maddern GJ, Maddox A, *et al.* Effects of cisapride on gastric and esophageal emptying in progressive systemic sclerosis. Gastroenterology 1987; 93:311–315.
34. Wang SJ, Lan JL, Lan JL, *et al.* Effects of cisapride on colonic transit in patients with progressive systemic sclerosis. Clin Rheumatol 2002; 21:271–274.
35. Linke R, Meier M, Muenzing W, *et al.* Prokinetic therapy: what can be measured by gastric scintigraphy? Nucl Med Commun 2005; 26:527–533.
36. Boeckxstaens GE, Bartelsman JF, Lauwers L, Tytgat GN. Treatment of GI dysmotility in scleroderma with the new enterokinetic agent prucalopride. Am J Gastroenterol 2002; 97:194–197.
37. Kumar S, Singh J, Kedika R, *et al.* Role of muscarinic-3 receptor antibody in systemic sclerosis: correlation with disease duration and effects of IVIG. Am J Physiol Gastrointest Liver Physiol 2016; 310:G1052–G1060.
38. Singh J, Cohen S, Mehendiratta V, *et al.* Effects of scleroderma antibodies and pooled human immunoglobulin on anal sphincter and colonic smooth muscle function. Gastroenterology 2012; 143:1308–1318.
39. Singh J, Mehendiratta V, Del Galdo F, *et al.* Immunoglobulins from scleroderma patients inhibit the muscarinic receptor activation in internal anal sphincter smooth muscle cells. Am J Physiol Gastrointest Liver Physiol 2009; 297:G1206–G1213.
40. Perlemuter G, Cacoub P, Chaussade S, *et al.* Octreotide treatment of chronic intestinal pseudoobstruction secondary to connective tissue diseases. Arthritis Rheum 1999; 42:1545–1549.
41. Soudah HC, Hasler WL, Owyang C. Effect of octreotide on intestinal motility and bacterial overgrowth in scleroderma. N Engl J Med 1991; 325: 1461–1467.
42. Menys A, Butt S, Emmanuel A, *et al.* Comparative quantitative assessment of global small bowel motility using magnetic resonance imaging in chronic intestinal pseudo-obstruction and healthy controls. Neurogastroenterol Motil 2016; 28:376–383.
43. Battle WM, Snape WJ Jr, Wright S, *et al.* Abnormal colonic motility in progressive systemic sclerosis. Ann Intern Med 1981; 94:749–752.
44. Lee JY, Kim SK, Cho KB, *et al.* A double-blind, randomized, multicenter clinical trial investigating the efficacy and safety of esomeprazole single therapy versus mosapride and esomeprazole combined therapy in patients with esophageal reflux disease. J Neurogastroenterol Motil 2017; 23:218–228.
45. ■■ Ashat M, Lewis A, Liaquat H, *et al.* Intravenous immunoglobulin in drug and device refractory patients with the symptoms of gastroparesis-an open-label study. Neurogastroenterol Motil 2018; 30. doi: 10.1111/nmo.13256.

In this study, the authors explored the efficacy of IVIG in the management of gastroparesis and found potential benefit for patients with refractory disease.

46. Sanges S, Riviere S, Mekinian A, *et al.* Intravenous immunoglobulins in systemic sclerosis: data from a French nationwide cohort of 46 patients and review of the literature. Autoimmun Rev 2017; 16:377–384.
47. Jeong EJ, Chung SY, Hong HN, *et al.* The novel, potent and highly selective 5-HT4 receptor agonist YH12852 significantly improves both upper and lower gastrointestinal motility. Br J Pharmacol 2018; 175:485–500.
48. Pauwelyn V, Ceelen W, Lefebvre RA. Synergy between 5-HT4 receptor stimulation and phosphodiesterase 4 inhibition in facilitating acetylcholine release in human large intestinal circular muscle. Neurogastroenterol Motil 2018; 30:10.
49. Pauwelyn V. Lefebvre RA 5-HT4 receptors facilitate cholinergic neurotransmission throughout the murine gastrointestinal tract. Neurogastroenterol Motil 2017; 29:10.
50. Miner PB Jr, Camilleri M, Burton D, *et al.* Prucalopride induces high-amplitude propagating contractions in the colon of patients with chronic constipation: a randomized study. Neurogastroenterol Motil 2016; 28:1341–1348.
51. Corleto VD, Antonelli G, Coluccio C, *et al.* Efficacy of Prucalopride in bowel cleansing before colonoscopy: results of a pilot study. World J Gastrointest Endosc 2017; 9:558–560.
52. Gong J, Xie Z, Zhang T, *et al.* Randomised clinical trial: prucalopride, a colonic pro-motility agent, reduces the duration of postoperative ileus after elective gastrointestinal surgery. Aliment Pharmacol Ther 2016; 43:778–789.
53. Tack J, Rotondo A, Meulemans A, *et al.* Randomized clinical trial: a controlled pilot trial of the 5-HT4 receptor agonist revexepride in patients with symptoms suggestive of gastroparesis. Neurogastroenterol Motil 2016; 28:487–497.
54. Stevens JE, Jones KL, Rayner CK, Horowitz M. Pathophysiology and pharmacotherapy of gastroparesis: current and future perspectives. Expert Opin Pharmacother 2013; 14:1171–1186.
55. Chedid V, Camilleri M. Relamorelin for the treatment of gastrointestinal motility disorders. Expert Opin Investig Drugs 2017; 26:1189–1197.
56. Sanger GJ, Pasricha PJ. Investigational drug therapies for the treatment of gastroparesis. Expert Opin Investig Drugs 2017; 26:331–342.

57. Acosta A, Camilleri M, Kolar G, *et al.* Relamorelin relieves constipation and accelerates colonic transit in a phase 2, placebo-controlled, randomized trial. Clin Gastroenterol Hepatol 2015; 13:2312–2319.e1.

58. Acosta A, Camilleri M, Busciglio I, *et al.* Short-term effects of relamorelin on descending colon motility in chronic constipation: a randomized, controlled trial. Dig Dis Sci 2016; 61:852–860.

59. Marie I, Leroi AM, Gourcerol G, *et al.* Lactose malabsorption in systemic sclerosis. Aliment Pharmacol Ther 2016; 44:1123–1133.

60. ■■ Gatta L, Scarpignato C. Systematic review with meta-analysis: rifaximin is effective and safe for the treatment of small intestine bacterial overgrowth. Aliment Pharmacol Ther 2017; 45:604–616.

In this study, the authors determined that rifaxamin is effective and well tolerated in the management of SIBO, but that controlled trials are needed and the optimal regimen still needs to be established.

61. ■■ Zhong C, Qu C, Wang B, *et al.* Probiotics for preventing and treating small intestinal bacterial overgrowth: a meta-analysis and systematic review of current evidence. J Clin Gastroenterol 2017; 51:300–311.

In this study, the authors conducted a systematic review and meta-analysis to determine whether probiotics have a role in preventing and treating SIBO. They found that probiotics do not prevent SIBO, but that they do play a role in alleviating symptoms.

62. Moore JS, Gibson PR, Burgell RE. Neuromodulation via interferential electrical stimulation as a novel therapy in gastrointestinal motility disorders. J Neurogastroenterol Motil 2018; 24:19–29.

63. ■ Hu X, Yuan M, Yin Y, *et al.* Electroacupuncture at LI11 promotes jejunal motility via the parasympathetic pathway. BMC Complement Altern Med 2017; 17:329.

In this study, the authors report that electroacupuncture may have a role in promoting jejunal motility through the parasympathetic pathway.

64. Schlieve CR, Fowler KL, Thornton M, *et al.* Neural crest cell implantation restores enteric nervous system function and alters the gastrointestinal transcriptome in human tissue-engineered small intestine. Stem Cell Rep 2017; 9:883–896.

65. Vigone B, Beretta L. Abatacept to treat chronic intestinal pseudo-obstruction in five systemic sclerosis patients with a description of the index case. J Scleroderma Relat Disord 2018. doi:10.1177/2397198318766819.

66. Malagelada C, Nieto A, Mendez S, *et al.* Effect of prucalopride on intestinal gas tolerance in patients with functional bowel disorders and constipation. J Gastroenterol Hepatol 2017; 32:1457–1462.

67. Camilleri M, Piessevaux H, Yiannakou Y, *et al.* Efficacy and safety of prucalopride in chronic constipation: an integrated analysis of six randomized, controlled clinical trials. Dig Dis Sci 2016; 61:2357–2372.

68. Manini ML, Camilleri M, Grothe R, Di Lorenzo C. Application of pyridostigmine in pediatric gastrointestinal motility disorders: a case series. Paediatr Drugs 2017. doi:10.1007/s40272-017-0277-6.

69. O'Dea CJ, Brookes JH, Wattchow DA. The efficacy of treatment of patients with severe constipation or recurrent pseudo-obstruction with pyridostigmine. Colorectal Dis 2010; 12:540–548.

70. Choudhury A, Rahyead A, Kammermeier J, Mutalib M. The use of pyridostigmine in a child with chronic intestinal pseudo-obstruction. Pediatrics 2018; 141:S404–S407.

71. Soufi-Afshar I, Moghadamnia A, Bijani A, *et al.* Comparison of pyridostigmine and bisacodyl in the treatment of refractory chronic constipation. Caspian J Intern Med 2016; 7:19–24.

72. Bharucha AE, Low PA, Camilleri M, *et al.* Pilot study of pyridostigmine in constipated patients with autonomic neuropathy. Clin Auton Res 2008; 18:194–202.

73. Pasha SF, Lunsford TN, Lennon VA. Autoimmune gastrointestinal dysmotility treated successfully with pyridostigmine. Gastroenterology 2006; 131: 1592–1596.

74. Sharman SK, Islam BN, Hou Y, *et al.* Sildenafil normalizes bowel transit in preclinical models of constipation. PLoS One 2017; 12:e0176673.

75. Umar SB, Griffing L, Garcia H, *et al.* The impact of pelvic floor and lower gastrointestinal symptoms on quality of life in women with systemic sclerosis. J Clin Gastroenterol 2016; 50:e55–e59.

76. Collins J, Mazor Y, Jones M, *et al.* Efficacy of anorectal biofeedback in scleroderma patients with fecal incontinence: a case-control study. Scand J Gastroenterol 2016; 51:1433–1438.

77. Katz D, Katz I, Porat-Katz BS, Shoenfeld Y. Medical cannabis: Another piece in the mosaic of autoimmunity? Clin Pharmacol Ther 2017; 101:230–238.

78. Goyal H, Singla U, Gupta U, May E. Role of cannabis in digestive disorders. Eur J Gastroenterol Hepatol 2017; 29:135–143.

79. Pesce M, D'Alessandro A, Borrelli O, *et al.* Endocannabinoid-related compounds in gastrointestinal diseases. J Cell Mol Med 2018; 22:706–715.

80. Doerfler B, Allen TS, Southwood C, *et al.* Medical Nutrition Therapy for Patients With Advanced Systemic Sclerosis (MNT PASS): a pilot intervention study. JPEN J Parenter Enteral Nutr 2017; 41:678–684.

81. Eswaran S. Low FODMAP in 2017: lessons learned from clinical trials and mechanistic studies. Neurogastroenterol Motil 2017; 29. doi:10.1111/nmo.13055.

Hematopoietic stem-cell transplantation in systemic sclerosis: an update

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Purpose of review
To provide an overview of recently published work on autologous hematopoietic stem-cell transplantation (HSCT) in patients with systemic sclerosis (SSc).

Recent findings
Superiority of HSCT vs. intravenous cyclophosphamide pulses was demonstrated in the randomized controlled American Scleroderma: Cyclophosphamide or Transplantation (SCOT) Trial ($n = 75$), supporting the results from earlier studies. In the SCOT Trial, total body irradiation was used instead of the nonmyeloablative regimens used in other trials, and considered well tolerated during a follow-up time of 4.5 years. Three small uncontrolled prospective cohorts ($n = 4$, 14 and 18) and one retrospective analyses ($n = 18$), using various nonmyeloablative regimens, also showed improvement in skin involvement and lung volumes post-HSCT. Transplant-related toxicity and mortality remain an essential issue in HSCT. High treatment-related mortality was reported in one prospective cohort ($n = 18$), using alemtuzumab as a conditioning agent. Furthermore, cardiac complications, either treatment or disease related, require special attention. In translational studies, trends are reported in number of regulatory T cells and diversity of T-cell receptor repertoire at baseline and post-HSCT correlating with treatment response.

Summary
There is increasing evidence that patients with rapidly progressive SSc may benefit from HSCT. However, optimal patient selection, pretransplantation workup and posttransplant management, still have to be established.

Keywords
autologous hematopoietic stem-cell transplantation, bone marrow transplantation, diffuse cutaneous systemic sclerosis, poor prognosis, scleroderma

INTRODUCTION

Diffuse cutaneous systemic sclerosis (dcSSc) is a rare, autoimmune disease characterized by multiorgan involvement and is associated with poor outcomes. Autologous hematopoietic stem-cell transplantation (HSCT) is the only therapy in SSc that has shown long-term clinical benefit and has been increasingly performed in SSc in the last decades [1]. The 2017 update of the European League Against Rheumatism guideline for the treatment of SSc now recommends consideration of HSCT in rapidly progressive SSc at risk of organ damage, a recommendation adopted in various national guidelines [2▪,3–5]. These recommendations were based on the results of the first randomized controlled trials (ASSIST and ASTIS) [6,7]. In these studies, significant improvement in skin involvement and lung function after HSCT were reported. In addition, the ASTIS trial reported improvement in event-free survival compared with treatment with cyclophosphamide (CYC) only.

In the past few years, research has focused on short-term and long-term efficacy and safety of HSCT and identification of determinants of clinical response. In this review, we summarize the results of studies published last year and discuss their

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KEY POINTS

- HSCT has long-term treatment benefits in patients with early diffuse cutaneous SSc.
- Attention to cardiac functioning before and during treatment is of key importance.
- Research priorities are identification of predictors for remission and severe adverse events and posttransplantation management.

implications for clinical practice in the context of established evidence.

MECHANISM OF ACTION AND PREDICTORS FOR CLINICAL RESPONSE

The exact mechanism of action in HSCT is unknown. Additional evidence was provided last year about parameters that delineated responders from nonresponders. A visual summary is provided in Fig. 1. In an earlier study, serum titers of circulating antitopoisomerase I antibodies were associated with skin improvement post-HSCT [8]. Subsequently, binding of this autoantibody with several epitopes associated with SSc was assessed and published last year. Although the number of bound antibodies post-HSCT was decreased significantly in 18 transplanted SSc patients, there was no correlation with clinical response [9]. Then, the same authors evaluated molecular recognition patterns of antitopoisomerase I antibodies toward 45 peptides and compared sera from SSc transplant patients with healthy controls and patients with SSc or other connective tissue diseases [10]. A new epitope was identified to be recognized predominantly by antitopoisomerase I positive sera. The number of antibodies bound to this specific epitope was significantly lower at baseline in responders compared with nonresponders.

Thymic size, as a proxy for thymopoiesis, was assessed in a study with ($N = 28$) SSc patients treated with HSCT. Surprisingly, thymic size decreased 11-month post-HSCT, but did not correlate with treatment response [11]. This finding is incompatible with previously reported enlargement of the thymus and T-cell repopulation after HSCT [12]. However,

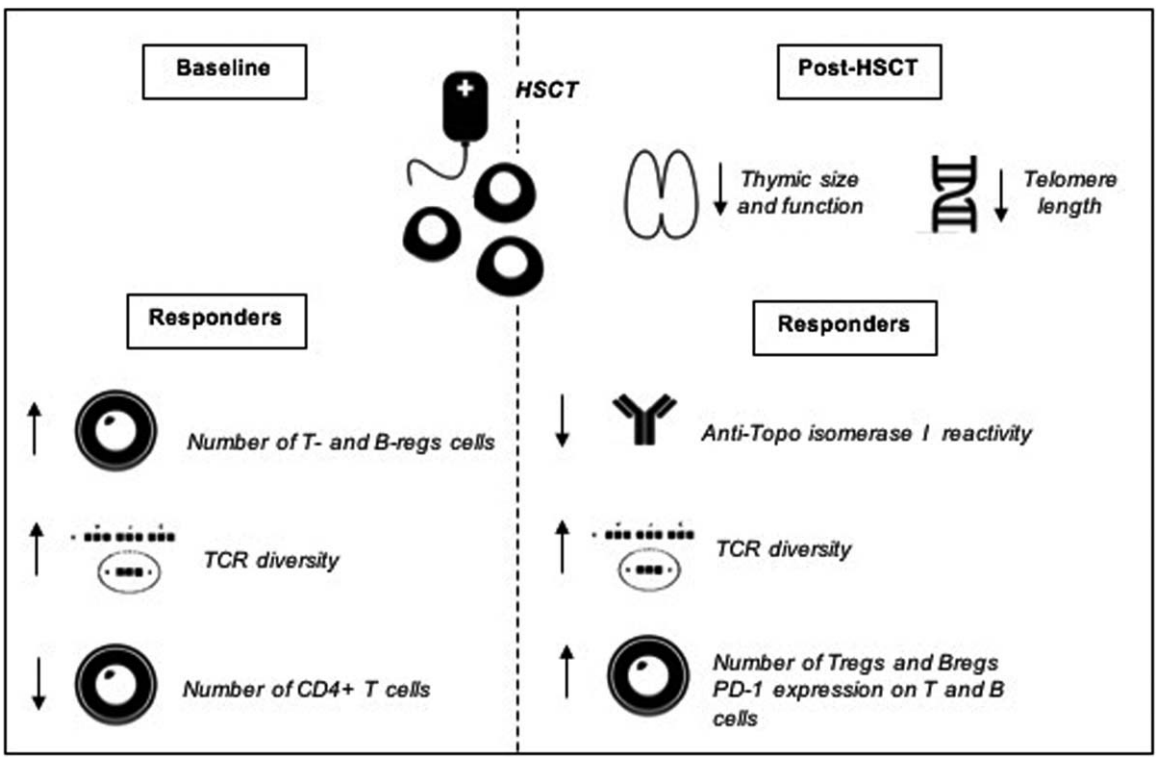

FIGURE 1. Summary of recently reported factors associated with response. In patients responding to hematopoietic stem-cell transplantation, numbers of circulating T-regulatory and B-regulatory cells were higher and the T-cell receptor repertoire was more diverse at baseline and after hematopoietic stem-cell transplantation, compared with nonresponders. The number of CD4+ T cells was lower in responders at baseline. Posthematopoietic stem-cell transplantation there was lower reactivity of antitopoisomerase I against certain epitopes and higher programed death-1 expression on T and B cells in responders. After hematopoietic stem-cell transplantation thymic function was increased and telomere length was shorter.

we could not find any sound explanation for this inconsistent observation.

Alterations in telomeres were studied in 25 SSc patients post-HSCT retrospectively [13]. Patients had high rates of homeostatic proliferation, resulting in increased number of senescent and exhausted cells and telomere attritition uptil 3 years post-HSCT, reflecting physiological immunological reconstitution also reported in other conditions after HSCT. There were no differences between responders and nonresponders with regard to telomere length. PD-1+ (programed death-1 positive) expression on T cells, and the number of CD8(+)CD28(−) cells expressing CD57 and FoxP3 were increased post-HSCT and PD-1 expression on T and B cells were higher in responders ($n = 19$). These factors are essential for inducing tolerance, and build on the existing evidence that HSCT induces immunological tolerance.

Another important aspect of a recovering immune system is the T-cell receptor (TCR) repertoire which was associated with clinical response in a few small studies published earlier [9]. A recent study with a long follow-up period (median 6 years) confirmed these findings; post-HSCT, differences in clonality of TCR repertoire were observed between responders and nonresponders [14]. This observation was also made in one larger study, including 31 patients [15▪▪]. This last study sheds some new light on this subject, additionally reporting an increased number to B-regulatory cells in responders compared with nonresponders. In conclusion, several studies have tried to identify the immunological mechanism most relevant for remission and to find parameters predictive for clinical response, but so far, the relationship between specific parameters such as immune cell counts, autoantibodies and cytokines and clinical response is unclear [16]. The diversity of the recently reported findings emphasizes the need for hypothesis-generating studies to unravel the mechanism of action of HSCT.

UPDATE CLINICAL TRIALS

In the past year, additional evidence for the beneficial effect of HSCT was reported (Table 1).

The studies discussed here also reflect the increased use of HSCT in SSc all over the world and the large variety of conditioning regimens applied. A small Indian single-center study reported the long-term results of their first experiences with HSCT in SSc [17]. Four patients with no preexisting internal organ involvement were treated with a conditioning regimen containing CYC and fludarabine, after which they received unselected autologous stem cells. At 4 years post HSCT, skin thickening and the frequency of Raynaud's symptoms were significantly decreased in all patients, pulmonary function improved and gastrointestinal complaints disappeared. No treatment-related adverse events were reported. A small Japanese uncontrolled clinical trial assessed 14 patients treated with CYC followed by HSCT [18]. Five patients received a CD34+ selected graft, the other nine received an unselected graft. Patients did not receive rabbit antithymocyte globulin (rATG). In the follow-up period of 137 months, overall survival (OS) was 93% and event-free survival was 40% at 10 years. A relatively high number of six patients (43%) required additional immunosuppressive treatments due to progression of skin sclerosis and/or interstitial lung disease during the follow-up period. This raises the question whether the lack of rATG and CD34+ selection contributed to this high number of relapses and which conditioning regimen is most effective. A Polish prospective cohort study followed 18 dcSSc with a median age of 52 years and disease duration of 14 months [19]. Eleven patients received CYC (median dose 3 g) and granulocyte-colony stimulating factor for mobilization, and CYC (median dose 12.3 g) and alemtuzumab (median dose, 60 mg) for conditioning. Two patients received melphalan (140 mg/m^2) and alemtuzumab for conditioning, four received CYC and rATG (7.5 mg/kg) and one only received CYC. Eleven patients were alive after a median follow-up of 42 months and experienced a significant decrease in modified Rodnan skin score, but no change in lung function. On the contrary, 45% experienced disease progression requiring immunosuppressive therapy.

Second, support for the superiority of high-dose immunosuppressive therapy followed by HSCT over CYC alone was provided in two other studies. In an Italian retrospective study, treatment effects of eighteen dcSSc patients treated with HSCT were compared with 36 dcSSc patients treated with CYC ($n = 25$, 1 g monthly for 6 months) or other conventional therapies ($n = 11$, methotrexate, azathioprine and/or methylprednisolone) [20]. Again improvement of skin involvement and pulmonary volumes was observed in long-term (60 months) follow-up and diffusing capacity remained stable. There was a survival benefit in the patients who received HSCT.

The long awaited results of the American Scleroderma: Cyclophosphamide or Transplantation (SCOT) Trial underscore the previously demonstrated efficacy of HSCT. Seventy-four dcSSc patients were randomized to undergo either treatment with 12 monthly intravenous CYC pulses or a myeloablative regimen, using total body irradiation and CYC at a lower than usual dose (120 mg/kg), followed by HSCT. Thirty-six participants received HSCT and 39 were only treated with CYC pulses

Table 1. Overview of recently published clinical studies and results

Study	Design, *N*	Median age (years)	Median disease duration (years)	Follow-up (years)	Received treatment	CD34+ selection	TRM, *n* (%)
Randomized controlled trials							
Sullivan *et al.* (2018), USA [21▪▪]	RCT, $N=75$	45.9	2.3	4.5	(1) Mob: G-CSF. Con: TBI, CYC (120 mg/kg), rATG (90 mg/kg) (2) Monthly CYC (750 mg/m^2 × 12)	Yes	1 (3)
Other							
Nair *et al.* (2018), India [17]	Prospective observational study, $N=4$	30	2.8	4	Mob: CYC (2 g/m^2) + G-CFS. Con: 2 days fludarabine 30 mg, CYC (60 mg/kg) + rATG (3.5 mg/kg)	No	0 (0)
Nakamura *et al.* (2018), Japan [18]	Phase II study, $N=14$	44.5	1.8	11.4	Unknown	No $n=9$; Yes $n=5$	1 (7)
Helbig *et al.* (2017), Poland [19]	Prospective observational study, $N=18$	52	1.2	3.5	HSCT group: Mob: CYC (2 g/m^2 2 days) + G-CFS 10 μg/kg). Con: CYC (200 mg/kg) + alemtuzumab (med 60 mg) ($N=11$), MEL (140 mg/m^2) + alemtuzumab ($N=2$), CYC + rATG (7.5 mg/kg) ($N=4$), CYC ($N=1$)	Yes	4 (22)
Del Papa *et al.* (2017), Italy [20]	Retrospective study, $N=18$ Matched controls 36	41	2.0	5	HSCT group: Mob: CYC (2 g/m^2 2 days) + G-CFS 10 μg/kg). Con: CYC (60 mg/kg) + rATG (3.5 mg/kg) Control group: 1 g × 6 ($N=25$), MTX, AZA and/or MP ($N=11$)	Yes	1 (5.6)

AZA, azathioprine; Con, conditioning; CYC, cyclophosphamide; G-CSF, granulocyte-colony stimulating factor; GRCS, global rank composite score; HSCT, hematopoietic stem-cell transplantation; MEL, melphalan; Mob, mobilization; MP, methylprednisolone; mRSS, modified Rodnan skin score; MTX, methotrexate; rATG, rabbit antithymocyte globulin; RCT, randomized controlled trial; TBI, total body irradiation; TRM, treatment-related mortality.

[21▪▪]. On the contrary, accrual of patients in the study was more difficult than expected, and it was anticipated that the adjusted projected enrollment of patients in this study would not allow analysis using established endpoints, such as event-free survival and OS. Thus, a composite score was composed to compare both treatment groups. The authors conclude that with regard to this global rank composite score, myeloablative conditioning followed by HSCT is superior to CYC. The use of posttransplantation immunosuppression (disease modifying anti-rheumatic drugs) was also significantly higher in the CYC group (44 vs. 9%, $P=0.001$). The use of a different conditioning regimen and different, more stringent criteria for patient selection (all patients with cardiac involvement were excluded in the SCOT) complicate comparison with earlier trials. Furthermore, the study was underpowered to draw any firm conclusions with regard to event free survival and OS.

No less than three systematic reviews were published last year [22–24]. Two reviews concluded that the high heterogeneity prevented meta-analysis [23,24]. Still, one meta-analysis was performed, only including three randomized controlled trials (RCTs) and one observational study (total $N=304$). A reduction in all-cause mortality [risk ratio of 0.5 (confidence interval (CI), 0.33–0.75)] and significant improvement of pulmonary function and quality of life was reported [24]. However, for pulmonary function and quality of life, data from only two RCTs (ASSIST and ASTIS) was pooled.

SAFETY ISSUES

Transplant-related toxicity and mortality remain an essential issue in HSCT. Significantly, most of the studies published last year reported lower rates of treatment-related mortality (TRM), compared with previously performed studies. In the small Italian retrospective study TRM was 5.6% ($n = 1$) due to interstitial pneumonitis [20]. There was a survival benefit in the patients who received HSCT, compared with 36 matched controls. In the Japanese phase two trial, adverse events related to HSCT occurred in six patients (43%), fatal cardiomyopathy was reported in one patient [18]. TRM was highly variable in the meta-analysis [risk ratio 9.00 (95% CI, 1.57–51.69)] [22].

The SCOT reported a TRM of 3% ($n = 1$, $P = 0.48$) at 54 months and 6% ($n = 4$) at 72 months, as compared with 0% in the CYC group. This relatively low percentage in the HSCT group, could probably partly be attributed to the exclusion of patients with cardiac involvement. Comparison with earlier RCTs is again difficult, because of the relatively small number of patients in the SCOT and the low occurrence of TRM in both trials (four in the SCOT, eight in the ASTIS). Furthermore, it is not clear if definitions for TRM were applied the same in all studies.

In contrast with the reported decreased TRM, the Polish prospective cohort study reported TRM of 22% ($n = 4$) after median follow-up of 42 months [19]. Four patients died early after transplant due to bilateral pneumonia followed by multiorgan failure in three patients and myocardial infarction in one. Authors attribute the high mortality rate to the administration of alemtuzumab causing life-threatening infectious complications.

Overall, toxicity related to treatment can be attributed to the different immunosuppressant treatments used during the mobilization and conditioning prior to HSCT and is mainly caused by cardiotoxicity and infections.

Cardiotoxicity

The heart is commonly involved in SSc, often subclinically and cardiac failure is an important cause of death in patients with rapidly progressive dcSSc [25]. Furthermore, treatment-related cardiotoxicity is a major cause of mortality and morbidity, and discussion about cause, treatment and prevention is still ongoing.

Although there are some reports about CYC-induced cardiotoxicity after HSCT in SSc patients, it is not clear how to prevent it or identify patients at risk. No study clearly reported that CYC itself leads to more complications in patients with known cardiac involvement prior to HSCT. Importantly, it cannot always be predicted, as was illustrated by a report describing a case of fatal heart failure post-HSCT in a patient without cardiac disease pretreatment [25]. The patient developed CYC-induced hemorrhagic pericarditis with an intracavity thrombus shortly after the conditioning regimen (histological evidence was obtained after autopsy).

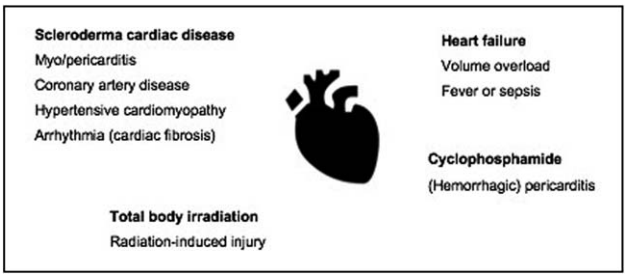

FIGURE 2. Causes of cardiac complications during and after hematopoietic stem-cell transplantation. Several factors contribute to the higher risk of cardiac complication during and after hematopoietic stem-cell transplantation. Scleroderma heart disease is diverse, including myocarditis and pericarditis and hypertensive cardiomyopathy as a result of scleroderma renal crisis. Furthermore, cardiac fibrosis can lead to life-threatening arrhythmia. At last, chronic inflammatory diseases are associated with coronary heart disease. Although rare, toxicity caused by treatment regimens includes injury related to cyclophosphamide or total body irradiation. In patients with decreased cardiac function, heart failure can develop following the higher cardiac demand in conditions such as fever and volume overload by infusion during the procedure.

Still, the risk of cardiac complications in HSCT in patients with decreased cardiac function should be underlined due to the higher cardiac demand in conditions such as transplant-related fever, neutropenic infection and volume overload by infusion during the procedure (Fig. 2) [26]. Furthermore, presence of cardiac disease might reflect extensive organ involvement, longer disease duration and/or a poor overall performance state, which can all be associated with poor outcome.

Thus, probably to prevent complications, in the SCOT, all patients with cardiac involvement were excluded, though it was not clear what their definition of 'cardiac involvement' was. Moreover, in a recently published review, extensive cardiac screening before HSCT was recommended, including echocardiogram with tissue Doppler and dobutamine stress test, cardiac MRI with contrast, cardiac catheterization with fluid challenge and Holter [27▪]. If any of these investigations are abnormal, exclusion from HSCT was suggested. In addition to the invasiveness of the tests and the financial costs of this exhausting screening program, there is insufficient evidence that patient with mildly decreased cardiac function should be excluded from treatment.

Recent literature thus does not shed much light on the quintessential dilemma of which specific patients should be offered the treatment; the adage that treatment risks have to be balanced against the considerable benefits of HSCT remains true. To move the field forward, and provide patients with the best possible treatment, the exclusion of patient groups for whom no other option exists without solid evidence, warrants open discussion.

Infections

With regard to infectious complications of HSCT, earlier trials mostly reported (mild) viral reactivation [including Epstein–Barr virus (EBV)]. In the SCOT more severe infections were reported in the HSCT group (rate 0.21 vs. 0.13, $P = 0.09$). There was one case of fatal enterococcal meningitis. Viral infections included Varicella Zoster infections and cytomegalovirus reactivation occurred more often after transplantation (13 vs. 1 and 5 vs. 0, respectively) [21▪▪]. In the retrospective study performed in Italy, five patients developed fever with positive blood culture and three had pneumonia, all treated adequately with antibiotics [20]. In the Polish cohort, three patients experienced fatal bilateral pneumonia, early after HSCT [19]. Viral infections were seen in 28% of patients post-HSCT in this study. In this group, two (11%) patients developed EBV-related lymphoproliferative disease. This complication was also reported in a small number of patients in the ASTIS trial. These findings, while not unexpected, emphasize the necessity of adequate antiviral prophylaxis and regular screening for EBV reactivation.

IMPLICATIONS FOR FURTHER RESEARCH

Aside from enabling directed research into HSCT in SSc, more insight in the cellular and molecular actors responsible for the beneficial effect of HSCT may aid in predicting response and subsequently providing personalized care, such as preemptive posttransplant immunosuppression for patients who are predicted to respond poorly. Posttransplantation progression rate is still remarkably high, further research on optimal posttransplantation management is therefore needed and is currently investigated in two studies (NCT01413100 and NCT02516124). In addition, given the finding in both ASTIS trial and SCOT that prior treatment with CYC may be associated with worse outcomes after HSCT, it can be questioned whether HSCT should be offered rather as a first-line treatment in early rapidly progressive dcSSc patients. Larger studies comparing different timing of HSCT, different and less toxic conditioning regimens (currently investigated: NCT00622895 and NCT01445821), investigating adapted conditioning regimens for patients with preexisting cardiac involvement and use of post-HSCT immunosuppression, could support decision making in clinical practice.

CONCLUSION

Overall, last year additional evidence was provided confirming the long-term treatment benefits of HSCT in dcSSc. TRM should be weighed against the severity of the disease and remains an important factor in decision making. Small sample sizes, differences in patient selection, follow-up duration and treatment regimens complicate comparison between the studies performed. Further studies should help to identify subgroups in whom HSCT would be most beneficial, optimal cardiac screening and optimal conditioning regimens, especially in patients with cardiac involvement.

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REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Snowden JA, Badoglio M, Labopin M, *et al.* Evolution, trends, outcomes, and economics of hematopoietic stem cell transplantation in severe autoimmune diseases. Blood Adv 2017; 1:2742–2755.
2. Kowal-Bielecka O, Fransen J, Avouac J, *et al.* Update of EULAR recommen-
▪ dations for the treatment of systemic sclerosis. Ann Rheum Dis 2017; 76:1327–1339.

Adoption of hematopoietic stem-cell transplantation (HSCT) in the European treatment guidelines is a major step for the position of HSCT in clinical practice in systemic sclerosis (SSc).

3. van Rhijn-Brouwer FCC, Vonk MC, Voskuyl AE, *et al.* Consensus-based recommendations on autologous hematopoietic stem cell transplantation in poor prognosis diffuse cutaneous systemic sclerosis. Ned Tijdschr Reumatol 2016; 3:17–23.
4. Pugnet G, Castilla-Llorente C, Puyade M, *et al.* Indications and follow-up for autologous hematopoietic stem cell transplantation in autoimmune and autoinflammatory diseases: Guidelines from the Francophone Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC). Bull Cancer 2017; 104(12S):S169–S180.
5. Denton CP, Hughes M, Gak N, *et al.* BSR and BHPR guideline for the treatment of systemic sclerosis. Rheumatology (Oxford) 2016; 55:1906–1910.
6. van Laar JM, Farge D, Sont JK, *et al.* Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. JAMA 2014; 311:2490–2498.
7. Burt RK, Shah SJ, Dill K, *et al.* Autologous nonmyeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised phase 2 trial. Lancet 2011; 378:498–506.

8. Tsukamoto H, Nagafuji K, Horiuchi T, *et al.* Analysis of immune reconstitution after autologous CD34+ stem/progenitor cell transplantation for systemic sclerosis: predominant reconstitution of Th1 CD4+ T cells. Rheumatology (Oxford) 2011; 50:944–952.
9. Henes J, Glaeser L, Kotter I, *et al.* Analysis of antitopoisomerase I antibodies in patients with systemic sclerosis before and after autologous stem cell transplantation. Rheumatology (Oxford) 2017; 56:451–456.
10. Glaeser L, Henes J, Kotter I, *et al.* Molecular recognition patterns of anti-topoisomerase I-antibodies in patients with systemic sclerosis before and after autologous stem cell transplantation. Clin Exp Rheumatol 2018. [Epub ahead of print]
11. Henes JC, Wirths S, Vogel W, *et al.* Thymic findings before and after autologous stem cell transplantation for severe systemic sclerosis: a retrospective study using computed tomography in the pre and posttransplantation setting. Clin Exp Rheumatol 2017; 35(Suppl 104):11–12.
12. Storek J, Zhao Z, Lin E, *et al.* Recovery from and consequences of severe iatrogenic lymphopenia (induced to treat autoimmune diseases). Clin Immunol 2004; 113:285–298.
13. Arruda LCM, Lima-Junior JR, Clave E, *et al.* Homeostatic proliferation leads to telomere attrition and increased PD-1 expression after autologous hematopoietic SCT for systemic sclerosis. Bone Marrow Transplant 2018. [Epub ahead of print]
14. Farge D, Arruda LC, Brigant F, *et al.* Long-term immune reconstitution and T cell repertoire analysis after autologous hematopoietic stem cell transplantation in systemic sclerosis patients. J Hematol Oncol 2017; 10:21.
15. ■■ Arruda LCM, Malmegrim KCR, Lima-Junior JR, *et al.* Immune rebound associates with a favorable clinical response to autologous HSCT in systemic sclerosis patients. Blood Adv 2018; 2:126–141.

Analysis that identified immunological parameters correlating with clinical response in SSc patients treated with HSCT.

16. van Rhijn-Brouwer FCC, Spierings J, van Laar JM. Autologous hematopoietic stem cell transplantation in systemic sclerosis: a reset to tolerance? Immunol Lett 2018; 195:88–96.
17. Nair V, Vasdev V, Kumar A, *et al.* Stem cell transplant in systemic sclerosis: an Indian experience. Int J Rheum Dis 2018; 21:859–865.
18. Nakamura H, Odani T, Yasuda S, *et al.* Autologous haematopoietic stem cell transplantation for Japanese patients with systemic sclerosis: long-term follow-up on a phase II trial and treatment-related fatal cardiomyopathy. Mod Rheumatol 2018; 1–6. [Epub ahead of print]
19. Helbig G, Widuchowska M, Koclega A, *et al.* Safety profile of autologous hematopoietic stem cell mobilization and transplantation in patients with systemic sclerosis. Clin Rheumatol 2018; 37:1709–1714.
20. Del Papa N, Onida F, Zaccara E, *et al.* Autologous hematopoietic stem cell transplantation has better outcomes than conventional therapies in patients with rapidly progressive systemic sclerosis. Bone Marrow Transplant 2017; 52:53–58.
21. ■■ Sullivan KM, Goldmuntz EA, Keyes-Elstein L, *et al.* Myeloablative autologous stem-cell transplantation for severe scleroderma. N Engl J Med 2018; 378:35–47.

Large randomized controlled trial comparing myeloablative HSCT with cyclophosphamide pulse therapy in systemic sclerosis.

22. Shouval R, Furie N, Raanani P, *et al.* Autologous hematopoietic stem cell transplantation for systemic sclerosis: a systematic review and meta-analysis. Blood Marrow Transplant 2018; 24:937–944.
23. Host L, Nikpour M, Calderone A, *et al.* Autologous stem cell transplantation in systemic sclerosis: a systematic review. Clin Exp Rheumatol 2017; 35(Suppl 106):198–207.
24. Eyraud A, Scouppe L, Barnetche T, *et al.* Efficacy and safety of autologous haematopoietic stem cell transplantation in systemic sclerosis: a systematic review of literature. Br J Dermatol 2018; 178:650–658.
25. Martin M, Fornecker LM, Marcellin L, *et al.* Acute and fatal cardiotoxicity following high-dose cyclophosphamide in a patient undergoing autologous stem cell transplantation for systemic sclerosis despite satisfactory cardiopulmonary screening. Bone Marrow Transplant 2017; 52:1674–1677.
26. Burt RK, Oliveira MC, Shah SJ, *et al.* Cardiac involvement and treatment-related mortality after nonmyeloablative haemopoietic stem-cell transplantation with unselected autologous peripheral blood for patients with systemic sclerosis: a retrospective analysis. Lancet 2013; 381:1116–1124.
27. ■ Farge D, Burt RK, Oliveira MC, *et al.* Cardiopulmonary assessment of patients with systemic sclerosis for hematopoietic stem cell transplantation: recommendations from the European Society for Blood and Marrow Transplantation Autoimmune Diseases Working Party and collaborating partners. Bone Marrow Transplant 2017; 52:1495–1503.

The opinion article draws special attention to cardiac complications associated with HSCT and emphasizes the importance of cardiopulmonary screening.

An update on autoantibodies in scleroderma

Christopher A. Mecoli and Livia Casciola-Rosen

Purpose of review
New research continues to provide important insights into the utility of antibody specificities. This review provides an update of recent findings, and the important insights they provide into disease mechanism.

Recent findings
A growing number of autoantibodies have been discovered in scleroderma patients with unique clinical associations. A subgroup of these antibodies may have functional consequences and contribute to disease pathogenesis, driving the vascular and fibrotic phenotype. Recent research into the relationship between malignancy and scleroderma onset provides important new insights into disease mechanism, and highlights the utility of autoantibodies as unique research probes.

Summary
Continued advances in the study of scleroderma antibody specificities has led to important insights into disease pathogenesis and clinical subgrouping. These advances include newly described specificities, functional antibodies and an emerging understanding of the cancer–scleroderma relationship.

Keywords
antibodies, phenotype, scleroderma

INTRODUCTION

The presence of scleroderma-specific autoantibodies has long been recognized, as has their association with distinct clinical phenotypes and utility for assessing risk and long-term prognosis [1]. New research continues to provide important insights into the utility of antibody specificities. In this review, we will highlight recent studies that define novel clinical associations for existing and new autoantibody specificities; these are summarized in Table 1 and discussed in the text below. We will also review emerging findings that illuminate the relationship between cancer and scleroderma, and provide tantalizing suggestions about the mechanism by which scleroderma arises.

NEW INSIGHTS FROM EXISTING AUTOANTIBODY SPECIFICITIES

The majority of patients with scleroderma (~60–80%) have one of the following well-defined scleroderma autoantibodies: centromere (ACA), topoisomerase-1, or RNA polymerase III (RNApol3). Despite the fact that these antibodies have been recognized for decades, exciting new studies are just beginning to define their biologic underpinnings.

Cancer, scleroderma and RNA polymerase III antibodies

One of the most insightful recent observations into the relationship between cancer and autoimmunity emerged from a study investigating whether clinical features differed by autoantibody status in a small, well-defined cohort of patients with scleroderma and an associated malignancy [2]. In this work, Shah *et al.* observed that in patients with RNApol3 antibodies, the emergence of cancer and the clinical onset of scleroderma occurred very close together in time. This key observation subsequently led to a groundbreaking study showing that in some cases, scleroderma may be initiated by autoantigen mutation within the patient's cancer [3,4▪▪]. Notably, most anti-RNApol3-positive patients do not have an identifiable cancer. Despite these new insights into mechanism, the optimal approaches for cancer screening and detection in scleroderma patients

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KEY POINTS

- Scleroderma patients with RNA polymerase III (RNApol3) antibodies are at increased risk of cancer within 3 years of diagnosis, most notably for breast and lung cancers.
- New scleroderma-specific antibodies such as eIF2B, RuvBL1/2, and anti-BICD2 are infrequent, but are associated with unique clinical phenotypes.
- Autoantibodies against angiotensin II type I receptor (AT1R) and endothelin-1 type A receptor (ETAR) may have functional consequences in scleroderma.

with RNApol3 antibodies remain undefined, and are a high research priority.

The EULAR Scleroderma Trials and Research Cohort performed a large case–control study of patients with RNApol3 antibodies to begin to address this issue [5]. The study consisted of 158 anti-RNApol3-positive patients matched by sex, disease duration, age at disease onset and cutaneous subset to 199 scleroderma patients lacking this antibody. Consistent with earlier studies from our group and others [2,6,7], these authors found that patients with RNApol3 antibodies were more likely to be diagnosed synchronously (−6 months to +12 months) with malignancy, odds ratio (OR) 7.38 [95% confidence interval [CI] 1.61–33.8]. This association appeared to be driven by the magnitude of breast cancer risk, OR 20.2 (95% CI 1.41–355). On the basis of these results, for every 17 patients screened, one synchronous malignancy would be detected.

New studies on the risk of cancer in an observational cohort study of 2383 scleroderma patients followed at the Johns Hopkins Scleroderma Center relative to the general population shed important insights into the cancer-screening issue [8▪]. Cancer risk was determined by comparing the incidence in the Johns Hopkins Scleroderma cohort to the Surveillance, Epidemiology and End Results (SEER) registry, a nationally representative sample of the US population. A total of 205 (8.6%) patients were diagnosed with cancer over 37,686 person-years. The standardized incidence ratio (SIR) of cancer in anti-RNApol3 antibody-positive patients within 3 years of scleroderma diagnosis was 2.84 (95% CI 1.89–4.10). Interestingly, among anti-RNApol3-positive patients, the risk of different cancer types differed based on skin subtype. Those with diffuse scleroderma had an increased breast cancer risk (SIR 5.14, 95% CI 2.66–8.98), whereas those with limited scleroderma had a high-lung cancer risk (SIR 10.4, 95% CI 1.26–37.7). For patients with anticentromere antibodies, a lower risk of cancer was observed throughout follow-up (SIR 0.59, 95% CI 0.44–0.76). These data suggest that enhanced screening of breast cancer with MRI imaging may be warranted in women with diffuse scleroderma and antibodies against RNApol3. Additional studies are needed to confirm these tantalizing findings, and to define evidence-based guidelines for optimal screening practices.

RNA polymerase III antibodies are associated with a short cancer–scleroderma interval

In a recent study, our group identified autoantibodies to RNA Binding Region Containing 3 (RNPC3) in a cohort of 'antibody-negative' (that is, lacking the three most prominent antibody specificities in scleroderma: centromere, topoisomerase-1 and RNApol3) scleroderma patients with

Table 1. Newly described clinical characteristics associated with both well-defined and novel autoantibodies found in patients with systemic sclerosis

Antibodies in scleroderma	Antibody abbreviation	Salient features and clinical associations
RNA polymerase III	RNApol3	Malignancy (notably breast and lung cancer) [2,6,7]
RNA Binding Region Containing 3	RNPC3	Malignancy, ILD, GI dysmotility, myopathy [9,10]
Ribonuclease P protein subunit 25	Rpp25	Antigen target of anti-Th/To immune response [12]
Eukaryotic initiation factor 2B	eIF2B	Diffuse cutaneous disease, ILD [13]
RuvBL1 and RuvBL2	RuvBL1/2	Diffuse cutaneous disease, inflammatory myositis overlap [14,16]
Bicaudal D homolog 2	BICD2	Inflammatory myositis, ILD [17]
Interferon-inducible protein 16	IFI16	Digital ischemia [20–22]
Angiotensin II type I receptor	AT1R	Vascular disease (digital ischemia, PAH) [26,27]
Endothelin-1 type A receptor	ETAR	Vascular disease (digital ischemia, PAH) [26,27]
Muscarinic-3 receptor	M3R	GI dysmotility [29,30]
Platelet-derived growth factor receptor	PDGFR	Controversial, possibly profibrotic [31,32]

GI, gastrointestinal; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension.

short-interval malignancy detection, using phage-immunoprecipitation sequencing [9]. We subsequently described a close temporal association between anti-RNPC3-positive scleroderma onset and malignancy detection [10]. The study cohort consisted of 318 patients with scleroderma and cancer; of these, 12 patients had RNPC3 antibodies. Interestingly, a short cancer–scleroderma interval (<1 year) was described for the 12 anti-RNPC3-positive patients, similar to the findings with anti-RNA-pol3 antibodies. Relative to scleroderma patients with anticentromere antibodies, those with anti-RNPC3 antibodies had a more than four-fold increased risk of cancer within 2 years of scleroderma onset (OR 4.3 95% CI 1.1–16.9, $P=0.037$). In this study, it was also noted that aside from the short-interval cancer relationship, RNPC3 antibodies associated with other clinical features including severe interstitial lung disease, gastrointestinal dysmotility, Raynaud's and myopathy.

New insights from other scleroderma-specific autoantibodies

Perosa *et al.* [11] used a phage-based assay to study a cohort of 84 Italian scleroderma patients, all of whom had antibodies against centromere proteins A and B (CENP-A and CENP-B), and noted heterogeneity of the targeted epitopes. The group then focused on a specific immunodominant epitope of the CENP-A protein located at the amino terminus (amino acids 1–17), and isolated antibodies targeting one of two shorter peptides within this epitope. Autoantibodies recognizing these two CENP-A epitopes corresponded to distinct clinical subgroups with different risk levels of pulmonary vascular disease [11]. Intriguingly, these clinical associations were opposite in direction, with one group statistically more likely to have a greater risk of higher systolic pulmonary artery pressure and lower diffusing capacity of the lungs for carbon monoxide, and the other antibody group statistically less likely to have these clinical features. It is unknown from this study whether the patients had co-existing antibodies. Further studies are warranted to understand whether these two different CENP-A epitopes truly account for the observed difference in clinical phenotype, or whether co-existing antibodies may also have a role.

Antibodies to components of the Th/To complex [human RNase mitochondrial RNA processing (MRP) complex] have been described by various groups over the last several years [12]. Ribonuclease P protein subunit p25 (Rpp25) is a 25 kDa protein subunit of RNAse P and has been recently shown by the Canadian Scleroderma Research Group (CSRG) to be the main antigen target in scleroderma patients who are anti-Th/To positive [12]. In this study, 53 patients who were anti-nuclear antibody-positive but negative for extractable-nuclear antigens were identified from a cohort of 873 scleroderma patients enrolled in the CSRG registry between 2004 and 2009. Within this group, 19/53 (36%) were positive for Th/To antibodies as assessed by RNA immunoprecipitation; that is 19/873 (2.2%) of the starting cohort had these antibodies. When this set of 19 anti-Th/To antibody-positive samples was tested using a chemiluminescence immunoassay to detect Rpp25 antibodies, 12/19 were positive, whereas 10 patients were positive by ELISA using Rpp25 antigen-coated wells. The authors point out that the commercially available line immunoassay (LIA) testing for Th/To antibodies is based on the hPOP1 antigen, and that in this study, the 19 patients positive for Th/To by immunoprecipitation were negative using the LIA, suggesting either low prevalence of anti-hPOP1 antibodies in their cohort, or lack of reactivity in the LIA. Further studies are warranted to validate readouts of the different assays, and to precisely define which components of the RNAse MRP complex are targets of the immune response in scleroderma. Knowing this will provide the information to associate disease phenotype with these specificities.

NEWLY DISCOVERED ANTIBODIES

Eukaryotic initiation factor 2B

Betteridge *et al.* [13] recently reported a novel autoantibody to eukaryotic initiation factor 2B (eIF2B) in a small subset of seronegative scleroderma patients in the United Kingdom. eIF2B is a cytoplasmic multimeric protein consisting of five subunits that plays a role in the initiation of protein synthesis, helping tRNA bind to ribosomes. The specificity was identified using immunoprecipitation followed by mass spectrometry. Of 548 scleroderma patients tested, 7 (~1%) were positive for this autoantibody, which was clinically associated with diffuse cutaneous disease and interstitial lung disease (ILD). More recently, the same group using the same methodology reported a higher prevalence (9/128, 7%) of these antibodies in a largely North American scleroderma cohort. Consistent with results from the UK cohort, the majority of North American patients with anti-eIF2B had diffuse skin disease and ILD.

RuvBL1 and RuvBL2 antibodies

First described in 2014 by Kaji *et al.* [14], antibodies to RuvBL1/2 were observed in both Japanese and North American cohorts with a prevalence of ~2%.

RuvBL1 and 2 are ATPase homologues with a variety of functions including regulation of transcription and DNA repair [15]. Clinical associations with this antibody include diffuse skin disease and skeletal muscle involvement, similar to scleroderma-myositis overlap with PM-Scl antibodies. However, in contrast to the PM-Scl phenotype, patients with this antibody specificity were more likely to have an older age at scleroderma onset, be male and have a higher frequency of diffuse disease. Pauling *et al.* [16] also described autoantibodies to RuvBL1/2 in scleroderma patients with a similar prevalence and clinical association with scleroderma-overlap syndromes.

Anti-bicaudal D homolog 2 antibodies

The CSRG recently reported on a novel autoantigen in scleroderma patients, anti-bicaudal D homolog 2 (anti-BICD2), an intracellular protein involved in dynein and microtubule processes [17]. Sera from 451 scleroderma patients were tested with a paramagnetic bead immunoassay using recombinant BICD2. One hundred and sixteen of 451 (26%) of sera were positive for this antibody. Of these 116, 22 (19%) were monospecific, whereas 94 (81%) had multiple antibody specificities, most commonly anti-CENP-A. The authors propose that given BICD2's function in facilitating intracellular protein movement and mitosis, a shared specificity with CENP places both in the category of 'microtubule-related autoantibody targets.' Patients with single-specificity anti-BCID2 were more likely to have ILD (52.4 vs. 29.0%, $P=0.024$) and inflammatory myositis (31.8 vs. 9.6%, $P=0.004$) compared with the anti-BCID2-negative patients. Notably, 25% of patients in the single-specificity group had co-existing anti-U1RNP antibodies.

Interferon-inducible protein 16-antibodies

Anti-IFI-16 antibodies were first reported in up to 25% of patients with scleroderma over a decade ago [18]. As well, these antibodies have been described in cohorts of patients with Sjogren's syndrome and systemic lupus erythematosus [19]. Recent studies have put a new focus on IFI-16 antibodies in scleroderma [20]. In this work, novel clinical associations in scleroderma patients with IFI-16 antibodies and vascular disease were reported. McMahan and colleagues found that IFI-16 antibodies are associated with digital gangrene, and that in patients with higher anti-IFI-16 antibody levels, the risk of developing gangrene was greater. Furthermore, autoantibody levels were highest within 6 months of a digital ischemic event. As IFI-16 is expressed in vascular endothelial cells [21], a potential mechanism could be proposed in which antibodies to IFI-16 damage endothelial cells, resulting in vascular injury. The same group subsequently reported that scleroderma patients, dual positive for both anticentromere and anti-IFI-16 antibodies had an increased risk (OR 3.5, $P=0.03$) of digital vascular events relative to patients with only anticentromere antibodies [22]. Further studies on additional cohorts are warranted to confirm whether antibodies against centromere proteins and IFI-16 may have clinical utility as disease biomarkers for stratifying the risk of vascular events in scleroderma.

ANTIBODIES WITH FUNCTIONAL CONSEQUENCES

The notion that functional antibodies can play a role in disease pathogenesis is not novel; Grave's disease and myasthenia gravis are examples where antibodies are intimately involved in the pathophysiology of their associated conditions. Recent work in scleroderma has examined antireceptor antibodies and their potential for functional consequences. As this topic has been recently reviewed [23▪], we have focused on advances made in the last few years.

Angiotensin II type I receptor and endothelin-1 type A receptor antibodies

Originally described in the early 2000s in the organ transplant and preeclampsia literature, autoantibodies to angiotensin II type I receptor (AT1R) and endothelin-1 type A receptor (ETAR) have been reported in scleroderma patients and have been associated with disease phenotype, most commonly vascular complications. In recent years, a number of studies have demonstrated in-vitro functional consequences of these autoantibodies, including increased production of TGF-beta, inflammatory cytokines and reactive oxidative species [24,25]. Furthermore, both cross-sectional and longitudinal studies have demonstrated that these antibodies are associated with vascular complications including pulmonary arterial hypertension (PAH) and ischemic digital lesions [26]. Most recently, Avouac *et al.* [27] studied a prospective cohort of 90 patients to evaluate the ability of these antibodies to predict the occurrence of digital ulcerations. Univariable analysis revealed elevated levels of anti-AT1R and anti-ETAR antibodies were predictive of ischemic digital ulcers (hazard ratio 2.85, 95% CI 1.19–6.84 and hazard ratio 3.39, 95% CI 1.35–8.50). Upon controlling for other clinical predictors, as well as several angiogenic biomarkers, anti-ETAR autoantibodies remained an independent predictor of new ischemic

digital ulceration (hazard ratio 9.59, 95% CI 1.75–52.64) together with the presence at baseline of active digital ulceration or history of digital ulceration.

It should be noted that whereas data with AT1R and ETAR antibodies has been compelling over the past several years, there have been some studies with conflicting findings regarding prevalence and clinical association. In a recent cross-sectional study of 93 patients, Ilgen *et al.* reported no difference in anti-AT1R levels between scleroderma patients and healthy controls. Furthermore, no disease phenotypes associated with elevated autoantibody levels including skin subtype, presence of digital ulcers, or lung involvement [28].

Muscarinic-3 receptor

Muscarinic-3 receptor (M3R) autoantibodies have long been of interest to researchers studying the autonomic nervous system and gastrointestinal dysmotility. Upon stimulating the M3 receptor, acetylcholine – the primary mediator of gastrointestinal motility – is produced. Thus, antagonist/blocking antibodies to this receptor would explain the high prevalence of gastrointestinal dysmotility amongst scleroderma patients [29]. Recently, Kumar *et al.* [30] tested the hypothesis that IgG from scleroderma patients leads to neuropathy via inhibition of M3R on the myenteric cholinergic neurons, which progresses to myopathy by subsequent inhibition of M3R on the gastrointestinal smooth muscle cells. Using sera from 10 individual scleroderma patients, they demonstrated binding of scleroderma IgG to the myenteric plexus and smooth muscle cells in rat colonic sections by immunofluorescence, and showed co-localization with M3R. Addition of scleroderma IgG inhibited contraction of colonic smooth muscle and decreased acetylcholine release. Interestingly, treatment with intravenous immunoglobulin attenuated many of these effects.

Platelet-derived growth factor receptor antibodies

Stimulation of the platelet-derived growth factor receptor (PDGFR) on fibroblasts and smooth muscle cells results in cell activation. Thus, over the years, it was hypothesized that agonist antibodies to this receptor may play a role in scleroderma pathogenesis. However, the significance (and even the presence) of antibodies to PDGFR has been controversial. Differences in methodology have resulted in disparate results regarding their detection and function. Most recently, in an effort to obtain direct evidence of agonist activity of anti-PDGFR antibodies, Luchetti and colleagues engineered samples isolated from skin biopsies of healthy donors, which were engrafted to SCID mice. The skin graft was then injected with anti-PDGFR monoclonal antibodies generated from B cells isolated from a scleroderma patient, including either agonistic collagen-inducing anti-PDGFR mAB or a nonagonistic one. The agonistic monoclonal antibody resulted in a scleroderma-like phenotype, which the authors argue demonstrates the profibrotic role of PDGFR antibodies [31]. This group has also reported on the ability of agonistic anti-PDGFR antibodies to induce vascular smooth muscle cell proliferation *in vitro* in human pulmonary smooth muscle cells [32].

CONCLUSION

The study of autoantibodies in scleroderma continues to provide new insights that inform our understanding of the pathogenesis of this disease. As well, the ability to better phenotype patients based on antibody profile will ultimately enable more precise disease diagnosis, selection of the most appropriate therapy and real-time monitoring of the effectiveness of treatment in each patient.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

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

1. Allanore Y, Simms R, Distler O, *et al.* Systemic sclerosis. Nat Rev Dis Primers 2015; 1:15002.
2. Shah AA, Rosen A, Hummers L, *et al.* Close temporal relationship between onset of cancer and scleroderma in patients with RNA polymerase I/III antibodies. Arthritis Rheum 2010; 62:2787–2795.
3. Shah AA, Casciola-Rosen L, Rosen A. Review: cancer-induced autoimmunity in the rheumatic diseases. Arthritis Rheumatol 2015; 67:317–326.
4. ■■ Joseph CG, Darrah E, Shah AA, *et al.* Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science 2014; 343:152–157.

In this seminal article, evidence for a model of cancer-induced autoimmunity is presented. The authors demonstrate somatic mutation and/or loss of heterozygosity frequently in the gene encoding the RNApol3 large subunit in cancers from scleroderma patients with antibodies against this protein. Data presented indicates that the initial immune response may be directed against the mutation-associated neoepitope in the cancer, subsequently spreading to the wild type antigen.

5. Lazzaroni M-G, Cavazzana I, Colombo E, *et al.,* EUSTAR co-authors. Malignancies in patients with anti-RNA polymerase III antibodies and systemic sclerosis: analysis of the EULAR Scleroderma Trials and Research Cohort and possible recommendations for screening. J Rheumatol 2017; 44:639–647.
6. Airo' P, Ceribelli A, Cavazzana I, *et al.* Malignancies in Italian patients with systemic sclerosis positive for anti-RNA polymerase III antibodies. J Rheumatol 2011; 38:1329–1334.
7. Moinzadeh P, Fonseca C, Hellmich M, *et al.* Association of anti-RNA polymerase III autoantibodies and cancer in scleroderma. Arthritis Res Ther 2014; 16:R53.
8. ■ Igusa T, Hummers LK, Visvanathan K, *et al.* Autoantibodies and scleroderma phenotype define subgroups at high-risk and low-risk for cancer. Ann Rheum Dis 2018; 77:1179–1186.

This article reports the quantified risk of different cancer types in specific autoantibody strata of scleroderma patients.

9. Xu GJ, Shah AA, Li MZ, *et al.* Systematic autoantigen analysis identifies a distinct subtype of scleroderma with coincident cancer. Proc Natl Acad Sci 2016; 113:E7526–E7534.
10. Shah AA, Xu G, Rosen A, *et al.* Brief report: anti-RNPC-3 Antibodies as a marker of cancer-associated scleroderma. Arthritis Rheumatol 2017; 69:1306–1312.
11. Perosa F, Favoino E, Favia IE, *et al.* Subspecificities of anticentromeric protein A antibodies identify systemic sclerosis patients at higher risk of pulmonary vascular disease. Medicine (Baltimore) 2016; 95:e3931.
12. Mahler M, Fritzler MJ, Satoh M. Autoantibodies to the mitochondrial RNA processing (MRP) complex also known as Th/To autoantigen. Autoimmun Rev 2015; 14:254–257.
13. Betteridge ZE, Woodhead F, Lu H, *et al.* Brief report: anti-eukaryotic initiation factor 2B autoantibodies are associated with interstitial lung disease in patients with systemic sclerosis. Arthritis Rheumatol 2016; 68:2778–2783.
14. Kaji K, Fertig N, Medsger TA, *et al.* Autoantibodies to RuvBL1 and RuvBL2: a novel systemic sclerosis-related antibody associated with diffuse cutaneous and skeletal muscle involvement. Arthritis Care Res (Hoboken) 2014; 66:575–584.
15. Gorynia S, Bandeiras TM, Pinho FG, *et al.* Structural and functional insights into a dodecameric molecular machine - the RuvBL1/RuvBL2 complex. J Struct Biol 2011; 176:279–291.
16. Pauling JD, Salazar G, Lu H, *et al.* Presence of antieukaryotic initiation factor-2B, anti-RuvBL1/2 and antisynthetase antibodies in patients with antinuclear antibody negative systemic sclerosis. Rheumatology (Oxford) 2018; 57:712–717.
17. Fritzler MJ, Hudson M, Choi MY, *et al.* Bicaudal D2 is a novel autoantibody target in systemic sclerosis that shares a key epitope with CENP-A but has a distinct clinical phenotype. Autoimmun Rev 2018; 17:267–275.
18. Costa S, Mondini M, Caneparo V, *et al.* Detection of anti-IFI16 antibodies by ELISA: clinical and serological associations in systemic sclerosis. Rheumatology (Oxford) 2011; 50:674–681.
19. Baer AN, Petri M, Sohn J, *et al.* Association of antibodies to interferon-inducible protein-16 with markers of more severe disease in primary Sjögren's syndrome. Arthritis Care Res (Hoboken) 2016; 68:254–260.
20. McMahan ZH, Shah AA, Vaidya D, *et al.* Anti-interferon-inducible protein 16 antibodies associate with digital gangrene in patients with scleroderma. Arthritis Rheumatol 2016; 68:1262–1271.
21. Gariglio M, Azzimonti B, Pagano M, *et al.* Immunohistochemical expression analysis of the human interferon-inducible gene IFI16, a member of the HIN200 family, not restricted to hematopoietic cells. J Interferon Cytokine Res 2002; 22:815–821.
22. McMahan ZH, Wigley FM, Casciola-Rosen L. Risk of digital vascular events in scleroderma patients who have both anticentromere and anti-interferon-inducible protein 16 antibodies. Arthritis Care Res (Hoboken) 2017; 69: 922–926.
23. ■ Berger M, Steen VD. Role of antireceptor autoantibodies in pathophysiology of scleroderma. Autoimmun Rev 2017; 16:1029–1035.

This article provides a thorough overview of antireceptor antibodies in scleroderma, highlighting both clinical associations as well as evidence of their role in the pathophysiology of the disease.

24. Kill A, Tabeling C, Undeutsch R, *et al.* Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. Arthritis Res Ther 2014; 16:R29.
25. Riemekasten G, Cabral-Marques O. Antibodies against angiotensin II type 1 receptor (AT1R) and endothelin receptor type A (ETAR) in systemic sclerosis (SSc)-response. Autoimmun Rev 2016; 15:935.
26. Becker MO, Kill A, Kutsche M, *et al.* Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. Am J Respir Crit Care Med 2014; 190:808–817.
27. Avouac J, Riemekasten G, Meune C, *et al.* Autoantibodies against endothelin 1 type a receptor are strong predictors of digital ulcers in systemic sclerosis. J Rheumatol 2015; 42:1801–1807.
28. İlgen U, Yayla ME, Düzgün N. Antiangiotensin II type 1 receptor autoantibodies (AT1R-AAs) in patients with systemic sclerosis: lack of association with disease manifestations. Rheumatol Int 2017; 37:593–598.
29. Moroncini G, Svegliati Baroni S, Gabrielli A. Agonistic antibodies in systemic sclerosis. Immunol Lett 2018; 195:83–87.
30. Kumar S, Singh J, Kedika R, *et al.* Role of muscarinic-3 receptor antibody in systemic sclerosis: correlation with disease duration and effects of IVIG. Am J Physiol Gastrointest Liver Physiol 2016; 310:G1052–G1060.
31. Luchetti MM, Moroncini G, Jose Escamez M, *et al.* Induction of scleroderma fibrosis in skin-humanized mice by administration of anti-platelet-derived growth factor receptor agonistic autoantibodies. Arthritis Rheumatol 2016; 68:2263–2273.
32. Svegliati S, Amico D, Spadoni T, *et al.* Agonistic anti-PDGF receptor autoantibodies from patients with systemic sclerosis impact human pulmonary artery smooth muscle cells function in vitro. Front Immunol 2017; 8:75.

Calcinosis in scleroderma

Antonia Valenzuela[a], *Paula Song*[b], *and Lorinda Chung*[c]

Purpose of review
To provide an update on the available literature regarding the epidemiology, pathophysiology, diagnosis, and treatment of calcinosis cutis in patients with systemic sclerosis (SSc).

Recent findings
We identified observational studies that describe the frequency of calcinosis in SSc and associated clinical features; molecular studies exploring potential pathogenic mechanisms; and case reports and case series describing new diagnostic approaches and treatments.

Summary
Calcinosis cutis is the deposition of insoluble calcium in the skin and subcutaneous tissues. It represents a major clinical problem in patients with SSc affecting at least one quarter of patients. It is associated with longer disease duration, digital ulcers, acro-osteolysis, positive anticentromere antibody, and positive anti-PM/Scl antibody. Although pathogenesis is unknown, there is evidence supporting local trauma, chronic inflammation, vascular hypoxia, and dysregulation of bone matrix proteins as potential mechanisms. Diagnosis can be made clinically or with plain radiography. Several pharmacologic therapies have been tried for calcinosis with variable and modest results, but surgical excision of calcium deposits remains the mainstay of treatment.

Keywords
calcifications, calcinosis cutis, systemic sclerosis

INTRODUCTION

Calcinosis cutis is the deposition of insoluble calcium in the skin and subcutaneous tissues [1] (Fig. 1). There are five subtypes of calcinosis: dystrophic, metastatic, iatrogenic, idiopathic, and calciphylaxis [2]. Dystrophic calcinosis is the subtype associated with autoimmune connective tissue diseases (ACTD) [3] such as systemic sclerosis (SSc) [4]. Although the detailed pathophysiology of calcinosis cutis remains poorly understood, the general mechanism for dystrophic calcinosis is the deposition of calcified material in damaged tissue in the setting of normal serum calcium and phosphate levels [5]. We will review the most current literature regarding associated clinical factors, pathogenesis, diagnostic approach, and treatment options for dystrophic calcinosis as seen in SSc.

EPIDEMIOLOGY AND ASSOCIATED CLINICAL FACTORS

The prevalence of calcinosis in SSc ranges from 18 to 49%. This large range is likely attributable to variable definitions based on clinical and/or radiographic assessments, and differences in patient populations [6▪,7▪▪,8–10]. The prevalence increases in people who have had SSc for a prolonged duration, as calcinosis typically occurs more than 10 years after diagnosis [4,11]. Small single-center studies showed that factors associated with calcinosis include male sex, digital ulcers, digital pitting scars, acro-osteolysis, telangiectasias, anticentromere antibody (ACA), and anti-PM/Scl antibody [8,10,12–15]. An international cohort study of 5218 patients later confirmed the association of calcinosis with digital ulcers, telangiectasias, and ACA along with discovering a novel association with osteoporosis [16].

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KEY POINTS

- Calcinosis is a common and potentially debilitating manifestation in patients with SSc, most frequently affecting the hands, and particularly the fingers.
- Clinical features found to be associated with calcinosis in SSc in observational studies are longer disease duration, digital ulcers, acro-osteolysis, positive ACA, and positive anti-PM/Scl antibody.
- Diagnosis can be made clinically or with plain radiography. There is also growing evidence for the use of ultrasonography in evaluating calcinosis in SSc.
- The detailed pathophysiology of calcinosis cutis remains unclear, but there is evidence supporting chronic inflammation, vascular hypoxia, local trauma, and dysregulation of bone matrix proteins as potential mechanisms.
- Several medications have been described with variable results, but surgery remains the mainstay for the treatment of calcinosis.

Recent studies have shown different clinical associations with calcinosis based on the population studied. In a single-center study of 215 SSc patients in the United States, calcinosis was associated with limited cutaneous SSc (lcSSc) [17▪▪]. In Mexican and Malaysian cohorts, calcinosis was associated with diffuse cutaneous SSc (dcSSc) [7▪▪,18]. Antinucleolar and anti-Scl-70 antibodies were more prevalent and ACA was less prevalent in Mexican patients with calcinosis [7▪▪]. Similarly, dcSSc and antitopoisomerase (Scl-70) antibody were predictors of calcinosis in a cohort of 1305 SSc patients from the Canadian Scleroderma Research Group (CSRG) registry, which includes patients from Canada and Mexico [10]. However, in a multicenter study of 1009 African Americans with SSc, no associations were seen with cutaneous subtype, male sex, or ACA [6▪].

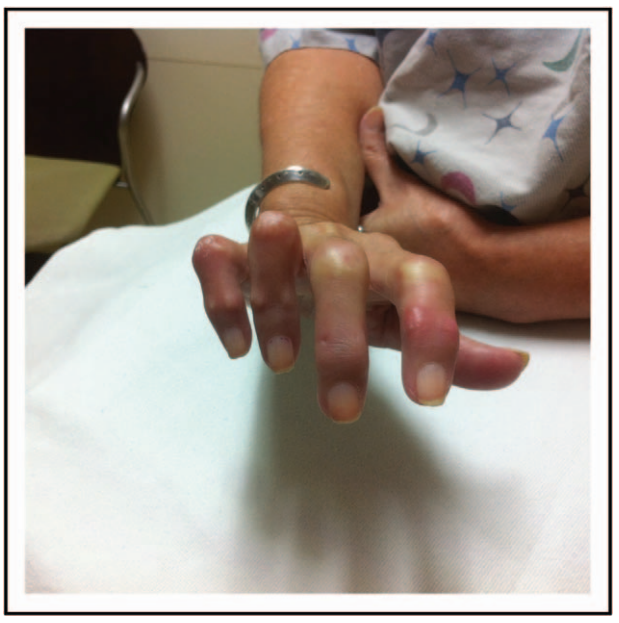

FIGURE 1. Calcinosis of the hand, overlying contractured joints that have frequently been traumatized.

CLINICAL PRESENTATION

Calcinosis in SSc presents as subcutaneous nodules in digits or in pressure point areas such as elbows, knees, or ischial tuberosities. Calcinosis occurs most frequently in the hands (65–83%), proximal upper extremity (27%), knee or proximal lower extremity (10–22%), and hip (6.7%) [7▪▪,19]. Calcinosis can also affect the trunk, chest, and buttocks, and more obscure locations such as the maxillary sinuses, spine, and paraspinal tissues [7▪▪,20]. Calcinosis may be painful and accompanied by soft tissue swelling, ulcers with superimposed infection, or deformities particularly in the hands leading to functional limitations [2,20,21]. Patients may also complain of a 'toothpaste-like' material extruding from the skin, which can be a point of entry for infections.

DIAGNOSIS

Although calcinosis is often clearly palpable or visible on physical examination, imaging can help confirm the diagnosis of subclinical deposits. Plain radiography is sensitive in detecting calcinosis and is the first-line imaging modality for evaluation in patients with ACTD [22] (Figs. 2 and 3). A radiographic scoring system for hand calcinosis was recently developed and validated that may standardize the measurement of calcium deposits for clinical and research purposes [24]. This scoring system takes into account the body area covered, density, number, and anatomic location of calcinosis lesions to provide an estimate of calcinosis burden, with excellent inter-rater and intra-rater reliability. Another method of categorizing calcinosis has also been proposed based on clinical and radiographic shapes and patterns of the lesions [19]. This approach divides calcinosis into four subtypes: mousse, stone, net, and plate. The authors showed that the net form took the longest time to heal (mean 140 ± 22 days), whereas stone calcinosis took the shortest (30 ± 12 days), suggesting that this categorization has clinical significance in the management and prognosis of calcinosis.

There is also growing evidence for the use of ultrasound in evaluating calcinosis in SSc. Freire *et al.* showed that ultrasound has a sensitivity of 89% in detecting calcinosis [25], with no significant

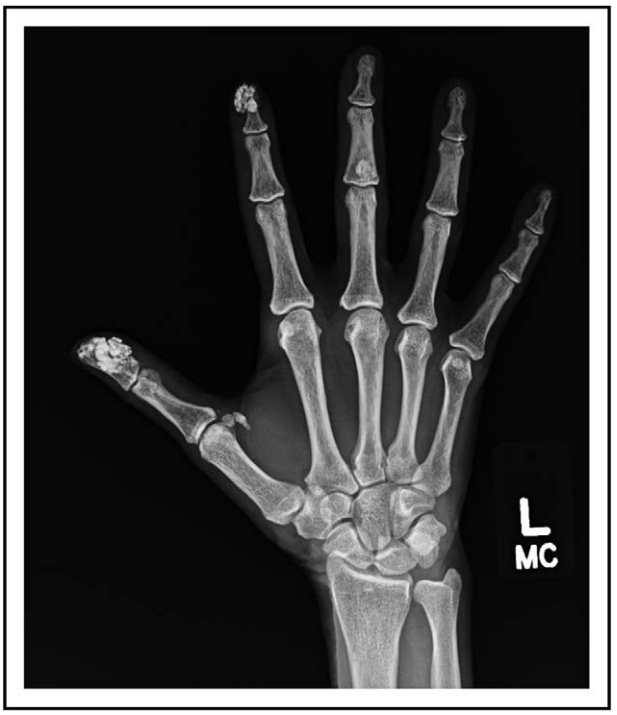

FIGURE 2. 'Wet cotton-wool appearance' of calcinosis on radiography of the hands. Reproduced with permission from [23].

difference in calcinosis detection when compared to radiography [26]. A recent study of 10 SSc patients proposed the use of Power Doppler to detect inflammation around calcium deposits, thus identifying lesions that may be targeted with anti-inflammatory medications [27]. In addition, an ultrasound technology used to detect micro-calcifications in breast tissue has been reported to differentiate hydroxyapatite deposits, as seen in SSc, from other types of calcifications [28].

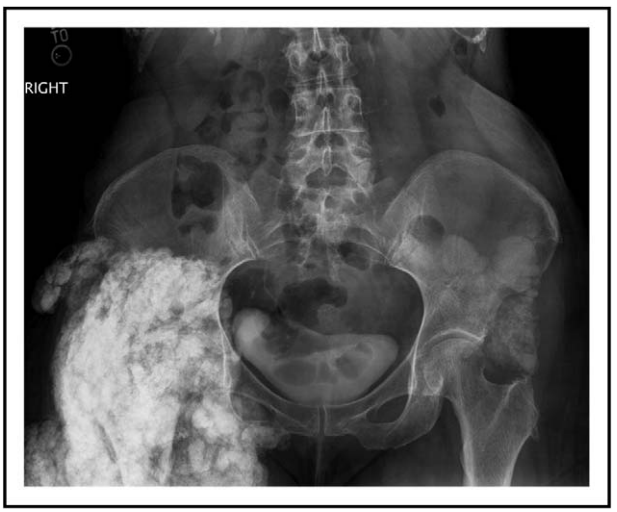

FIGURE 3. Frontal radiograph of the pelvis in a 57-year-old female with systemic sclerosis demonstrates severe calcinosis around the right hip and surrounding soft tissues. Calcium deposition is also seen affecting the left hip and left hemipelvis.

Other experimental modalities include multidetector computed tomography (MDCT), dual-energy computed tomography (DECT), and MRI. MDCT may better assess the extent of calcinotic lesions as a result of improved resolution and 3D capabilities compared to traditional CT [29]. A study of DECT in 16 SSc patients demonstrated successful detection of calcinosis in the subcutaneous tissue, tendon sheaths, carpal tunnel, and adjacent to muscles [30]. MRI can detect calcinosis deposits as well, providing better visualization of edema or inflammation of surrounding tissue that may indicate calcinosis development [31].

PATHOPHYSIOLOGY

The pathophysiology behind dystrophic calcification is incompletely understood. However, several mechanisms have been proposed, including chronic inflammation, vascular hypoxia, recurrent trauma, and abnormalities in bone matrix proteins. Elevated levels of serum interleukin-1, interleukin-6, interleukin-1β, and tumor necrosis factor (TNF)-α have been found in juvenile dermatomyositis (JDM) calcinosis, supporting the role of inflammation in calcinosis development [32].

Evidence suggests that vascular ischemia also contributes to calcinosis development. Davies *et al.* demonstrated increased expression of the hypoxia-associated glucose transporter molecule (GLUT-1) in skin biopsies of SSc patients with calcinosis [33]. Additionally, several studies [16] have shown that the presence or history of digital ulcers [10,34] and/or acro-osteolysis [12–14] predicts calcinosis development. Another study showed that upregulation of VEGF, a potent angiogenic factor induced by hypoxia, was associated with increased osteoclast activity in SSc patients with acro-osteolysis, 73% of whom had calcinois [35▪]. This hypoxia-induced osteoclast activity in SSc may also be involved in development of calcinosis, possibly explaining the association between calcinosis and osteoporosis [16,17▪▪,36].

Calcinosis preferentially affects the dominant hand and other acral sites, which suggests that repetitive trauma can contribute to calcinosis development [37▪▪]. These areas of recurrent trauma may be more susceptible due to the impaired compensatory angiogenesis that characterizes the proliferative obliterative vasculopathy seen in SSc [38,39▪▪].

Davies *et al.* also found increased expression of bone matrix proteins, such as osteonectin and

matrix gamma-carboxyglutamic acid protein (MGP), in calcinotic skin of SSc patients [40]. These proteins are involved in ectopic calcification via upregulation of osteonectin, an activator of calcification, in the setting of suppressed levels of the inhibitor protein MGP [41,42]. MGP must also be in its gamma-carboxylated form and bind to bone morphogenic protein-2 to inhibit calcification. This carboxylated form is vitamin K dependent [43]. Wallin *et al.* have proposed mechanisms in which oxidative stress, which is critical to microvascular injury in SSc, may inhibit vitamin K, resulting in under-gamma-carboxylated and inactive MGP, and thus dysregulated calcification [44].

Further studies are necessary to better define these mechanisms and elucidate others that contribute to the development of calcinosis in SSc.

TREATMENT

General measures and supportive therapies

Calcinosis remains a therapeutic challenge in patients with SSc. General measures include improving blood flow to the extremities by avoiding trauma, stress, and cold exposure. If there is suspicion for superinfection of calcinotic lesions, antibiotics covering streptococci and staphylocci should be prescribed. Acetaminophen, nonsteroidal anti-inflammatory agents, and even opioids [45] may be used to alleviate pain. If calcinosis becomes ulcerated, standard wound care with hydrocolloid membrane products may be useful [46].

Medical therapies

Several medications have been described in the management of calcinosis, with variable results [47]. The evidence supporting these mainly comes from small retrospective studies, case series, and case reports (Table 1).

Calcium channel blockers

Calcium channel blockers (CCBs) reduce the intracellular calcium influx in the affected tissues, altering the formation and crystallization of the calcium nidus. Diltiazem is the most used and studied CCBs for treating calcinosis. Early case reports showed encouraging results with 240 to 480 mg/day of diltiazem for calcinosis in SSc patients [48–50], which was confirmed in a retrospective cohort of 78 patients with ACTD from the Mayo Clinic showing that diltiazem was effective in 9 of 17 patients as first-line therapy [51]. However, a larger retrospective study did not show a beneficial effect on radiographs from 12 SSc patients treated with 180 mg/day of diltiazem for 1 to 15 years [8]. An observational study using 5-year follow-up data from CSRG showed no significant associations between the use of CCBs and calcinosis [odds ratios (OR) 0.9, 95% confidence interval (CI) 0.73–1.05], except in patients with disease duration less than 5 years, in whom the use of CCBs was associated with decreased risk of calcinosis (OR 0.62, 95% CI 0.45–0.86) [52▪].

Bisphosphonates

Bisphosphonates may be useful in reversing the calcification process by inhibiting macrophage proinflammatory cytokine production and reducing bone resorption [53]. Anecdotal reports describe improvement of calcinosis with intravenous pamidronate in patients with JDM [54–56], and adult dermatomyositis [57]. Similarly, a case series showed partial reduction of calcinosis with alendronate therapy in six of nine JDM patients [58]. A case report describes resolution of calcinosis in a patient with lcSSc after 6 months of risedronate therapy for glucocorticoid-induced osteoporosis [59]. Given the paucity of evidence, the efficacy of bisphosphonates in calcinosis treatment remains unclear.

Warfarin

Low-dose warfarin has been used for treatment of ACTD-calcinosis, based on the rationale that it reduces the levels of MGP by preventing carboxylation of glutamic acid [20]. A case series of three SSc patients treated with warfarin, described resolution of calcinosis in two patients after 1 year [60]. In the Mayo Clinic study, four of 19 patients with calcinosis and ACTD taking warfarin for other indications had no changes in calcinosis compared with the group that did not receive warfarin [51]. In another study of six patients with extensive calcinosis (one with SSc) treated with warfarin for 14.6 months, five had worsening of calcinosis [61]. Indeed, there is some concern that warfarin can promote calcification through under-carboxylated MGP [20,62].

Sodium thiosulfate

Topical, intralesional and intravenous sodium thiosulfate (STS) have been studied as treatments for calcinosis [63]. A report describes two cases of ulcerative dystrophic calcinosis refractory to topical treatments that had excellent responses to topical 25% STS compounded in zinc oxide [64]. Four patients with calcinosis (one with SSc and two with dermatomyositis) showed significant decrease in size, erythema, and pain with topical 25% sodium metabisulfite (SM), a metabolite of STS. The authors hypothesized that topical SM may dissolve calcium

Table 1. Pharmacological treatments of calcinosis in systemic sclerosis

Treatment	Rationale/biology	Drug/dosage	Number of responses/ numbers of patients treated	Authors recommendations	References
Calcium channel blockers	Reduce intracellular calcium influx in the affected tissues and local macrophages	Diltiazem 240–480 mg/day × 1–12 years	4/4	Moderate	Palmieri *et al.* [48]
		120 mg/day × 2 years	1/1		Dolan *et al.* [49]
		240 mg /day × 5 years	1/1		Farah *et al.* [50]
Bisphosphonates	Inhibit macrophage proinflammatory cytokine production and reduce calcium turnover	Risedronate × 6 months	1/1	Low	Fujii *et al.* [59]
Warfarin	Inhibits the production of gamma-carboxyglutamic acid, which has calcium-binding properties	1 mg/day × 1 year 1 mg/day × 7–28 months	2/3 0/1	Low	Cukierman *et al.* [60] Lassoued *et al.* [61]
Sodium thiosulfate (STS)	Potent antioxidant and vasodilator that also chelates and dissolves calcium deposits	Topical: STS 25% compounded in zinc oxide	2/2	Moderate	Bair *et al.* [64]
		25% sodium metabisulfite	1/1		Barrio-Diaz *et al.* [65]
		Intralesional: STS 1–3 g × 1 year	2/2		Goosens *et al.* [66]
		STS 12.5–150 mg × 1–4 times	5/5		Baumgartner-Nielsen *et al.* [67]
		Intravenous: 20 g/day, 5 days/month, at least six cycles	0/1		Mageau *et al.* [69]
Anti-TNF	Potential role of inflammation and TNF-alpha in calcinosis	Infliximab 3 mg/kg intravenous at 0, 2, and 6 weeks, and every 8 weeks × 7 months	1/1	Low	Tosonidouu *et al.* [70]
Rituximab	Chimeric anti-CD20 antibody that depletes B lymphocytes	375 mg/m^2 intravenous weekly × 4	1/1	Low	De Paula *et al.* [71]
		375 mg/m$_2$ intravenous weekly × 4	3/6		Giugglioli *et al.* [72]
		1 gram intravenous × 2 at 2 week-interval and then every 6 months	0/1		Hurabielle *et al.* [73]
		1 gram intravenous × 2 at 2 week-interval and then every year 375 mg/m^2 intravenous weekly × 4	0/1 0/1		Dubos *et al.* [74] Dubos *et al.* [74]
Minocycline	Tetracycline antibiotic with anti-inflammatory and calcium-binding properties	50–100 mg/day for 3.5 years	8/9	High	Robertson *et al.* [75]
Ceftriaxone	Third-generation cephalosporin able to bind calcium ions, and form insoluble calcium complexes	2 grams intravenous × 20 days	1/1	Low	Reiter *et al.* [76]
Aluminum hydroxide	Decreases serum phosphate levels by decreasing intestinal absorption thus reducing the calcification process	30 ml orally 4 times per day	1/1	Low	Hudson *et al.* [77]
Triamcinolone acetonide injection	Anti-inflammatory effect	Intralesional 20 mg/ml every 4–8 weeks × 6 months	1/1	Moderate	Hazen *et al.* [78]
Colchicine	Anti-inflammatory effect by disrupting leukocyte chemotaxis and phagocytosis through inhibiting microtubule polymerization	1 mg daily × 2 months	1/1	Moderate	Fuchs *et al.* [79]
		1 mg daily × 4 months	1/1		Vereecken *et al.* [80]
Intravenous immunoglobulin	Effect based on anti-inflammatory properties, possibly related to suppression of activated macrophages	2 g/day in a 4-day protocol once a month × 5 cycles	1/1	Moderate	Schanz *et al.* [81]

deposits and promote local vasodilation and wound healing [65]. Goossens *et al.* reported two cases of weekly intralesional injections of 1–3 g STS leading to pain relief, functional improvement, and 59% size reduction after 12 months [66]. A larger series describes the treatment of eight lesions in six patients (five with SSc and one with nephrogenic systemic fibrosis) with injections of 12.5–275 mg STS 150 mg/ml for up to 4 weeks. By weeks 4 and 12, the lesions decreased in size by 67% and 90%, respectively, and all patients reported improved pain and disability [67]. A report of three patients with ACTD-associated calcinosis treated with intravenous STS, after failing multiple prior therapies, did not show any notable clinical improvement of calcinosis [68]. However, another series of four patients (lcSSc, dermatomyositis, JDM, SLE) demonstrated improvement in calcinosis after 6 cycles of intravenous STS except in the patient with lcSSc [69]. More research is needed to evaluate STS as an option for calcinosis treatment.

Biologic agents

A patient with SSc-myositis overlap and refractory calcinosis treated with infliximab 3 mg/kg infused at 0, 2 and 6 weeks, and every 8 weeks thereafter, showed reduction in size of calcifications and no new deposits at 41 months in serial CT imaging [70].

Most literature on biologics for calcinosis has focused on rituximab (RTX). A case report showed RTX given in four weekly infusions (375 mg/m^2) improved or completely resolved calcinotic lesions [71]. Additionally, a series of 10 SSc patients treated with RTX for ILD, skin fibrosis and/or arthritis, reported improvement in three of six patients who had calcinosis [72]. However, a flare of calcinosis in a patient with dcSSc who received RTX to treat underlying ILD and arthritis was recently reported [73]. Similarly, two patients treated with RTX had progression of calcinosis 6 and 12 months after treatment. At present, RTX cannot be recommended for this indication in the absence of successful controlled trials [74].

Others

In a case series of nine lcSSc patients with calcinosis, 50 or 100 mg/day of minocycline for a mean of 3.5 years resulted in reduced ulceration and inflammation, with a modest decrease in the size of deposits in eight patients [75]. Only scattered case reports have documented the efficacy of ceftriaxone [76], aluminum hydroxide [77], triamcinolone acetonide injection [78], colchicine [51,79,80], and intravenous immunoglobulins [81] in treating SSc-calcinosis. A new potential treatment comes from preliminary observations from the Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma (PHAROS) registry wherein two patients with SSc-pulmonary arterial hypertension (PAH) and calcinosis treated with subcutaneous treprostinil for PAH experienced radiographic improvement in their calcinosis lesions after 6 months (Shapiro *et al.*, unpublished data). Oral treprostinil is currently being studied in a clinical trial of SSc patients with calcinosis of the hands (NCT02663895).

Procedures

A prospective study of nine patients (three with SSc) with calcinosis found that three hextracorporeal shock wave lithotripsy (ESWL) sessions at 3-week intervals reduced the size and pain from calcinosis at 6 months [82]. A recent 12-week study of three weekly sessions of ESWL on calcinosis lesions in four SSc patients found a reduction in lesion size in three patients and pain improvement in two patients [83].

The carbon dioxide (CO) laser-tissue vaporization procedure allows excellent visualization and vaporization of calcium deposits and has been shown to improve pain and function in selected patients with SSc-calcinosis [84,85].

Patients with large, localized, and symptomatic lesions especially located over tendons, blood vessels, and nerves should be referred for surgery [86,87▪▪]. In the Mayo Clinic experience, all 11 patients who underwent surgical excision alone and 16 out of 17 patients who received medical and surgical therapy responded to treatment. In contrast, only seven of 19 patients treated with medical therapy alone had any response [51]. Specific techniques such as curettage of calcinosis [88] or debulking with a high-speed micro-burr [89] effectively reduce pain and disability scores, but lesions can recur.

CONCLUSION

Calcinosis is a common problem in patients with SSc. It most frequently affects the hands, particularly the fingers. Although pathogenesis is unclear, there is evidence supporting chronic inflammation, vascular hypoxia, local trauma, and dysregulation of bone matrix proteins as potential mechanisms. There are no universally effective medical treatments for calcinosis in patients with SSc; however, limited data have been published supporting the use of several pharmacological therapies including CCBs, bisphosphonates, warfarin, and STS. Surgical excision of calcinosis remains the mainstay for treatment. Clinical trials using novel outcome measures

are necessary to determine the efficacy of current and emerging treatments.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Chander S, Gordon P. Soft tissue and subcutaneous calcification in connective tissue diseases. Curr Opin Rheumatol 2012; 24:158–164.
2. Boulman N, Slobodin G, Rozenbaum M, Rosner I. Calcinosis in rheumatic diseases. Semin Arthritis Rheum 2005; 34:805–812.
3. Walsh JS, Fairley JA. Calcifying disorders of the skin. J Am AcadDermatol 1995; 33(5 Pt 1):693–706.
4. Gutierrez A Jr, Wetter DA. Calcinosis cutis in autoimmune connective tissue diseases. Dermatol Ther 2012; 25:195–206.
5. Reiter N, El-Shabrawi L, Leinweber B, *et al.* Calcinosis cutis: part I. Diagnostic pathway. J Am Acad Dermatol 2011; 65:1–12.
6. ■ Morgan ND, Shah AA, Mayes MD, *et al.* Clinical and serological features of systemic sclerosis in a multicenter African American cohort: analysis of the genome research in African American scleroderma patients clinical database. Medicine 2017; 96:e8980.

This is a comprehensive retrospective and prospective clinical database of 1009 African-American patients with SSc from 18 academic centers throughout the United States, confirming association of calcinosis with longer disease duration in this subset of patients.

7. ■■ Cruz-Dominguez MP, Garcia-Collinot G, Saavedra MA, *et al.* Clinical, biochemical, and radiological characterization of the calcinosis in a cohort of Mexican patients with systemic sclerosis. Clin Rheumatol 2017; 36: 111–117.

This study identifies and quantifies the distribution of calcinosis in a cohort of 109 Mexican patients with SSc and its association with clinical features and autoantibodies.

8. Vayssairat M, Hidouche D, Abdoucheli-Baudot N, Gaitz JP. Clinical significance of subcutaneous calcinosis in patients with systemic sclerosis. Does diltiazem induce its regression? Ann Rheum Dis 1998; 57:252–254.
9. Belloli L, Ughi N, Massarotti M, *et al.* Role of fetuin-A in systemic sclerosis-associated calcinosis. J Rheumatol 2010; 37:2638–2639.
10. Baron M, Pope J, Robinson D, *et al.* Calcinosis is associated with digital ischaemia in systemic sclerosis-a longitudinal study. Rheumatology (Oxford) 2016; 55:2148–2155.
11. Galluccio F, Allanore Y, Czirjak L, *et al.* Points to consider for skin ulcers in systemic sclerosis. Rheumatology (Oxford) 2017; 56(suppl_5):v67–v71.
12. Avouac J, Mogavero G, Guerini H, *et al.* Predictive factors of hand radiographic lesions in systemic sclerosis: a prospective study. Ann Rheum Dis 2011; 70:630–633.
13. Johnstone EM, Hutchinson CE, Vail A, *et al.* Acro-osteolysis in systemic sclerosis is associated with digital ischaemia and severe calcinosis. Rheumatology (Oxford) 2012; 51:2234–2238.
14. Koutaissoff S, Vanthuyne M, Smith V, *et al.* Hand radiological damage in systemic sclerosis: comparison with a control group and clinical and functional correlations. Semin Arthritis Rheum 2011; 40:455–460.
15. Tolosa-Vilella C, Morera-Morales ML, Simeon-Aznar CP, *et al.* Digital ulcers and cutaneous subsets of systemic sclerosis: clinical, immunological, nailfold capillaroscopy, and survival differences in the Spanish RESCLE Registry. Semin Arthritis Rheum 2016; 46:200–208.
16. Valenzuela A, Baron M, Herrick AL, *et al.* Calcinosis is associated with digital ulcers and osteoporosis in patients with systemic sclerosis: a scleroderma clinical trials consortium study. Semin Arthritis Rheum 2016; 46:344–349.
17. ■■ Pai S, Hsu V. Are there risk factors for scleroderma-related calcinosis? Mod Rheumatol 2018; 28:518–522.

This cross-sectional study was performed to better understand the prevalence and clinical features associated with calcinosis in a single-center cohort of 215 SSc outpatients. Longer disease duration and osteoporosis were independently associated with calcinosis.

18. Sujau I, Ng CT, Sthaneshwar P, *et al.* Clinical and autoantibody profile in systemic sclerosis: baseline characteristics from a west Malaysian cohort. Int J Rheum Dis 2015; 18:459–465.
19. Bartoli F, Fiori G, Braschi F, *et al.* Calcinosis in systemic sclerosis: subsets, distribution and complications. Rheumatology (Oxford) 2016; 55:1610–1614.
20. Herrick AL, Gallas A. Systemic sclerosis-related calcinosis. J Scleroderma Relat Disord 2016; 1:194–203.
21. Vitiello M, Abuchar A, Santana N, *et al.* An update on the treatment of the cutaneous manifestations of systemic sclerosis: the dermatologist's point of view. J Clin Aesthet Dermatol 2012; 5:33–43.
22. Shahi V, Wetter DA, Howe BM, *et al.* Plain radiography is effective for the detection of calcinosis cutis occurring in association with autoimmune connective tissue disease. Br J Dermatol 2014; 170:1073–1079.
23. Valenzuela A, Chung L. Calcinosis. In: Varga J, Denton C, Wigley F, *et al.*, editors. Scleroderma. Cham: Springer; 2017.
24. Chung L, Valenzuela A, Fiorentino D, *et al.* Validation of a novel radiographic scoring system for calcinosis affecting the hands of patients with systemic sclerosis. Arthritis Care Res (Hoboken) 2015; 67:425–430.
25. Freire V, Bazeli R, Elhai M, *et al.* Hand and wrist involvement in systemic sclerosis: US features. Radiology 2013; 269:824–830.
26. Elhai M, Guerini H, Bazeli R, *et al.* Ultrasonographic hand features in systemic sclerosis and correlates with clinical, biologic, and radiographic findings. Arthritis Care Res (Hoboken) 2012; 64:1244–1249.
27. Suliman YA, Kafaja S, Fitzgerald J, *et al.* Ultrasound characterization of cutaneous ulcers in systemic sclerosis. Clin Rheumatol 2018; 37: 1555–1561.
28. Pracon G, Plaza M, Walentowska-Janowicz M, Sudol-Szopinska I. The value of ultrasound in the diagnosis of limited scleroderma – a case report. J Ultrason 2015; 15:326–331.
29. Freire V, Becce F, Feydy A, *et al.* MDCT imaging of calcinosis in systemic sclerosis. Clin Radiol 2013; 68:302–309.
30. Hsu V, Bramwit M, Schlesinger N. Use of dual-energy computed tomography for the evaluation of calcinosis in patients with systemic sclerosis. Clin Radiol 2015; 34:1557–1561.
31. Kimball AB, Summers RM, Turner M, *et al.* Magnetic resonance imaging detection of occult skin and subcutaneous abnormalities in juvenile dermatomyositis. Implications for diagnosis and therapy. Arthritis Rheum 2000; 43:1866–1873.
32. Shimizu M, Ueno K, Ishikawa S, *et al.* Role of activated macrophage and inflammatory cytokines in the development of calcinosis in juvenile dermatomyositis. Rheumatology (Oxford) 2014; 53:766–767.
33. Davies CA, Jeziorska M, Freemont AJ, Herrick AL. The differential expression of VEGF, VEGFR-2, and GLUT-1 proteins in disease subtypes of systemic sclerosis. Hum Pathol 2006; 37:190–197.
34. Luders S, Friedrich S, Ohrndorf S, *et al.* Detection of severe digital vasculopathy in systemic sclerosis by colour Doppler sonography is associated with digital ulcers. Rheumatology (Oxford) 2017; 56:1865–1873.
35. ■ Park JK, Fava A, Carrino J, *et al.* Association of acroosteolysis with enhanced osteoclastogenesis and higher blood levels of vascular endothelial growth factor in systemic sclerosis. Arthritis Rheumatol 2016; 68:201–209.

This study analyzed peripheral blood mononuclear cells (PBMCs) from 26 SSc patients (11 with acro-osteolysis and 15 without acro-osteolysis) and 14 healthy controls, and found increased osteoclast formation and higher VEGF levels in SSc patients with acro-osteolysis as compared with SSc patients without acro-osteolysis and healthy controls. The majority of SSc patients with acro-osteolysis also had calcinosis.

36. Omair MA, Pagnoux C, McDonald-Blumer H, Johnson SR. Low bone density in systemic sclerosis. A systematic review. J Rheumatol 2013; 40: 1881–1890.
37. ■■ Gauhar R, Wilkinson J, Harris J, *et al.* Calcinosis preferentially affects the thumb compared to other fingers in patients with systemic sclerosis. Scand J Rheumatol 2016; 45:317–320.

This study shows hand radiographs of 68 SSc patients demonstrating that the thumbs, followed by the index finger, are more commonly affected by calcinosis than other digits, providing insight into the role of repetitive trauma in the pathogenesis of SSc-related calcinosis.

38. Asano Y, Sato S. Vasculopathy in scleroderma. Semin Immunopathol 2015; 37:489–500.
39. ■■ Cantatore FP, Maruotti N, Corrado A, Ribatti D. Angiogenesis dysregulation in the pathogenesis of systemic sclerosis. Biomed Res Int 2017; 2017: 5345673.

This review article is focused on current knowledge on the mechanisms responsible for angiogenesis dysregulation in systemic sclerosis.

40. Davies CA, Jeziorska M, Freemont AJ, Herrick AL. Expression of osteonectin and matrix GLA protein in scleroderma patients with and without calcinosis. Rheumatology (Oxford) 2006; 45:1349–1355.
41. Dhore CR, Cleutjens JP, Lutgens E, *et al.* Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2001; 21:1998–2003.
42. Zebboudj AF, Shin V, Bostrom K. Matrix GLA protein and BMP-2 regulate osteoinduction in calcifying vascular cells. J Cell Biochem 2003; 90: 756–765.

43. Spronk HM, Soute BA, Schurgers LJ, *et al.* Matrix GLA protein accumulates at the border of regions of calcification and normal tissue in the media of the arterial vessel wall. Biochem Biophys Res Commun 2001; 289:485–490.

44. Wallin R, Wajih N, Greenwood GT, Sane DC. Arterial calcification: a review of mechanisms, animal models, and the prospects for therapy. Med Res Rev 2001; 21:274–301.

45. Ughi N, Crotti C, Ingegnoli F. Effectiveness and safety of oxycodone/naloxone in the management of chronic pain in patients with systemic sclerosis with recurrent digital ulcers: two case reports. Clin Interv Aging 2016; 11:307–311.

46. Netsch D. Calcinosis cutis: WOC nurse management. J Wound Ostomy Continence Nurs 2018; 45:83–86.

47. Daoussis D, Antonopoulos I, Liossis SN, *et al.* Treatment of systemic sclerosis-associated calcinosis: a case report of rituximab-induced regression of CREST-related calcinosis and review of the literature. Semin Arthritis Rheum 2012; 41:822–829.

48. Palmieri GM, Sebes JI, Aelion JA, *et al.* Treatment of calcinosis with diltiazem. Arthritis Rheum 1995; 38:1646–1654.

49. Dolan AL, Kassimos D, Gibson T, Kingsley GH. Diltiazem induces remission of calcinosis in scleroderma. Br J Rheumatol 1995; 34:576–578.

50. Farah MJ, Palmieri GM, Sebes JI, *et al.* The effect of diltiazem on calcinosis in a patient with the CREST syndrome. Arthritis Rheum 1990; 33:1287–1293.

51. Balin SJ, Wetter DA, Andersen LK, Davis MD. Calcinosis cutis occurring in association with autoimmune connective tissue disease: the Mayo Clinic experience with 78 patients, 1996–2009. Arch Dermatol 2012; 148: 455–462.

52. ▪ Li G, Adachi JD, Cheng J, *et al.* Relationship between calcium channel blockers and skin fibrosis in patients with systemic sclerosis. Clin Exp Rheumatol 2017; 106:56–60.

Study of 5-year follow-up data from the Canadian Scleroderma Research Group registry to assess the relationship between use of calcium channel blockers and clinical features of SSc, including calcinosis.

53. Dima A, Balanescu P, Baicus C. Pharmacological treatment in calcinosis cutis associated with connective-tissue diseases. Rom J Intern Med 2014; 52:55–67.

54. Marco Puche A, Calvo Penades I, Lopez Montesinos B. Effectiveness of the treatment with intravenous pamidronate in calcinosis in juvenile dermatomyositis. Rom J Intern Med 2010; 28:135–140.

55. Slimani S, Abdessemed A, Haddouche A, Ladjouze-Rezig A. Complete resolution of universal calcinosis in a patient with juvenile dermatomyositis using pamidronate. Joint Bone Spine 2010; 77:70–72.

56. Palaniappan P, Lionel AP, Kumar S. Successful treatment of calcinosis cutis in juvenile dermatomyositis with pamidronate. J Clin Rheumatol 2014; 20:454–455.

57. Canas CA, Bonilla-Abadia F, Mejia M, Tobon GJ. Recovery of severe muscular and fascial calcinosis after treatment with bisphosphonates in a child with juvenile dermatomyositis. J Clin Rheumatol 2015; 21:267–269.

58. Saini I, Kalaivani M, Kabra SK. Calcinosis in juvenile dermatomyositis: frequency, risk factors and outcome. Rheumatol Int 2016; 36:961–965.

59. Fujii N, Hamano T, Isaka Y, *et al.* Risedronate: a possible treatment for extraosseous calcification. Clin Calcium 2005; 15(Suppl 1):75–78.

60. Cukierman T, Elinav E, Korem M, Chajek-Shaul T. Low dose warfarin treatment for calcinosis in patients with systemic sclerosis. Ann Rheum Dis 2004; 63:1341–1343.

61. Lassoued K, Saiag P, Anglade MC, *et al.* Failure of warfarin in treatment of calcinosis universalis. Am J Med 1988; 84:795–796.

62. Palaniswamy C, Sekhri A, Aronow WS, *et al.* Association of warfarin use with valvular and vascular calcification: a review. Clin Cardiol 2011; 34:74–81.

63. Nigwekar SU, Kroshinsky D, Nazarian RM, *et al.* Calciphylaxis: risk factors, diagnosis, and treatment. Am J Kidney Dis 2015; 66:133–146.

64. Bair B, Fivenson D. A novel treatment for ulcerative calcinosis cutis. J Drugs Dermatol 2011; 10:1042–1044.

65. Barrio-Diaz P, Moll-Manzur C, Àlvarez-Veliz S, Vera-Kellet C. Topical sodium metabisulfite for the treatment of calcinosis cutis: a promising new therapy. Br J Dermatol 2016; 175:608–611.

66. Goossens J, Courbebaisse M, Caudron E, *et al.* Efficacy of intralesional sodium thiosulfate injections for disabling tumoral calcinosis: two cases. Semin Arthritis Rheum 2017; 47:451–455.

67. Baumgartner-Nielsen J, Olesen AB. Treatment of skin calcifications with intralesional injection of sodium thiosulphate: a case series. Acta Derm Venereol 2016; 96:257–258.

68. Song P, Fett NM, Lin J, *et al.* Lack of response to intravenous sodium thiosulfate in three cases of extensive connective tissue disease-associated calcinosis cutis. Br J Dermatol 2017; 176:1412–1415.

69. Mageau A, Guigonis V, Ratzimbasafy V, *et al.* Intravenous sodium thiosulfate for treating tumoral calcinosis associated with systemic disorders: report of four cases. Joint Bone Spine 2017; 84:341–344.

70. Tosounidou S, MacDonald H, Situnayake D. Successful treatment of calcinosis with infliximab in a patient with systemic sclerosis/myositis overlap syndrome. Rheumatology (Oxford) 2014; 53:960–961.

71. de Paula DR, Klem FB, Lorencetti PG, *et al.* Rituximab-induced regression of CREST-related calcinosis. Clin Rheumatol 2013; 32:281–283.

72. Giuggioli D, Lumetti F, Colaci M, *et al.* Rituximab in the treatment of patients with systemic sclerosis. Our experience and review of the literature. Autoimmun Rev 2015; 14:1072–1078.

73. Hurabielle C, Allanore Y, Kahan A, Avouac J. Flare of calcinosis despite rituximab therapy. Semin Arthritis Rheum 2014; 44:e5–e6.

74. Dubos M, Ly K, Martel C, Fauchais AL. Is rituximab an effective treatment of refractory calcinosis? BMJ Case Rep 2016; pii:bcr2015213179.

75. Robertson LP, Marshall RW, Hickling P. Treatment of cutaneous calcinosis in limited systemic sclerosis with minocycline. Ann Rheum Dis 2003; 62:267–269.

76. Reiter N, El-Shabrawi L, Leinweber B, Aberer E. Subcutaneous morphea with dystrophic calcification with response to ceftriaxone treatment. Ann Rheum Dis 2010; 63:e53–e55.

77. Hudson PM, Jones PE, Robinson TW, Dent CE. Extensive calcinosis with minimal scleroderma: treatment of ectopic calcification with aluminum hydroxide. Proc R Soc Med 1974; 67:1166–1168.

78. Hazen PG, Walker AE, Carney JF, Stewart JJ. Cutaneous calcinosis of scleroderma. Successful treatment with intralesional adrenal steroids. Arch Dermatol 1982; 118:366–367.

79. Fuchs D, Fruchter L, Fishel B, *et al.* Colchicine suppression of local inflammation due to calcinosis in dermatomyositis and progressive systemic sclerosis. Clin Rheumatol 1986; 5:527–530.

80. Vereecken P, Stallenberg B, Tas S, *et al.* Ulcerated dystrophic calcinosis cutis secondary to localised linear scleroderma. Int J Clin Pract 1998; 52:593–594.

81. Schanz S, Ulmer A, Fierlbeck G. Response of dystrophic calcification to intravenous immunoglobulin. Arch Dermatol 2008; 144:585–587.

82. Sultan-Bichat N, Menard J, Perceau G, *et al.* Treatment of calcinosis cutis by extracorporeal shock-wave lithotripsy. J Am Acad Dermatol 2012; 66: 424–429.

83. Blumhardt S, Frey DP, Toniolo M, *et al.* Safety and efficacy of extracorporeal shock wave therapy (ESWT) in calcinosis cutis associated with systemic sclerosis. Clin Exp Rheumatol 2016; 100:177–180.

84. Bottomley WW, Goodfield MJ, Sheehan-Dare RA. Digital calcification in systemic sclerosis: effective treatment with good tissue preservation using the carbon dioxide laser. Br J Dermatol 1996; 135:302–304.

85. Chamberlain AJ, Walker NP. Successful palliation and significant remission of cutaneous calcinosis in CREST syndrome with carbon dioxide laser. Dermatol Surg 2003; 29:968–970.

86. Merlino G, Germano S, Carlucci S. Surgical management of digital calcinosis in CREST syndrome. Aesthetic Plast Surg 2013; 37:1214–1219.

87. ▪▪ Fujimoto M, Asano Y, Ishii T, *et al.* The wound/burn guidelines - 4: guidelines for the management of skin ulcers associated with connective tissue disease/vasculitis. J Dermatol 2016; 43:729–757.

Japanese Dermatological Association guidelines on the treatment of skin ulcers and calcinosis associated with connective tissue diseases, providing a good review of available diagnostic and therapeutic approaches.

88. Saddic N, Miller JJ, Miller OF 3rd, Clarke JT. Surgical debridement of painful fingertip calcinosis cutis in CREST syndrome. Arch Dermatol 2009; 145:212–213.

89. Lapner MA, Goetz TJ. High-speed burr debulking of digital calcinosis cutis in scleroderma patients. J Hand Surg Am 2014; 39:503–510.

Lung transplantation in scleroderma: recent advances and lessons

Renea Jablonski, Jane Dematte, and Sangeeta Bhorade

Purpose of review
The purpose of this review is to highlight recent data regarding feasibility and outcomes following lung transplantation for patients with systemic sclerosis related pulmonary disease as well as to emphasize areas of uncertainly in need of further study. We include a description of our centre's approach to lung transplant evaluation and posttransplant management in this complex patient population.

Recent findings
Historical data have demonstrated that patients with scleroderma have an increased risk of complications following lung transplantation owing to the multisystem nature of disease, particularly concurrent gastrointestinal, cardiac and renal involvement. Emerging data support the safety of lung transplant in appropriately selected patients with scleroderma-related interstitial lung disease and pulmonary arterial hypertension.

Summary
Accumulating evidence validates that a diagnosis of scleroderma is not *a priori* a contraindication to lung transplant. In the carefully selected patient, both short-term and long-term outcomes following lung transplantation are comparable to counterparts with fibrotic lung disease or pulmonary arterial hypertension. However, further prospective study to detail how these patients should be evaluated and managed posttransplant is definitely needed. Cardiac disease is an emerging cause of morbidity and mortality in the scleroderma population and deserves particular attention during the pre and posttransplant period.

Keywords
lung transplantation, outcomes, scleroderma

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease characterized by vascular dysfunction and widespread fibrosis of the skin and visceral organs affecting 150–300 persons per million [1]. Historically, scleroderma renal crisis (SRC) was a major cause of mortality among patients with SSc [2], although the use of angiotensin-converting enzyme (ACE) inhibitors has significantly improved survival [3]. Recent data from the EUSTAR database reveal that cardiovascular, pulmonary and infectious diseases are the major source of mortality in SSc [4▪▪]. As many as 94% of patients with SSc have radiographic lung involvement [5] and 60–70% display physiologic impairment on pulmonary function testing [6]. SSc-pulmonary arterial hypertension (PAH) affects 3.6–32% of SSc patients [7] and carries a worse prognosis than idiopathic PAH [8]. Despite targeted therapy with immunosuppression or pulmonary vasodilators, a subset of patients progress to end-stage disease with severe functional impairment.

Lung transplant evaluation in connective tissue disease (CTD) usually commences when the disease progresses despite maximum medical therapy. However, early referral in SSc is desirable owing to the systemic nature of disease and need for multidisciplinary evaluation and management. Although formal guidelines for lung transplant referral and listing have been updated recently [9], whether or how the guidelines for idiopathic pulmonary fibrosis (IPF) or idiopathic PAH should be modified for SSc remains unclear given the different natural histories of disease. Here, we review the existing literature with regards to safety, feasibility and outcomes

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KEY POINTS

- Lung transplantation is an option for appropriately selected patients with SSc-related ILD or PAH that is refractory to medical therapy as outcomes after lung transplantation in SSc are comparable to patients with other fibrotic lung diseases and PAH.
- Early referral for lung transplantation is preferred due to the systemic nature of the disease and the need for multidisciplinary evaluation and management.
- Careful evaluation for cardiac disease, including assessment of diastolic function in patients with SSc, is necessary given the perioperative and postoperative haemodynamic changes that may occur.
- Gastrointestinal complications, specifically abnormal gastroesophageal motility, are common in SSc after lung transplantation, although there is significant variability among experienced transplant centres regarding this evaluation.

in patients with SSc undergoing lung transplant, discuss our approach to this unique population and highlight outstanding questions in this area.

CURRENT LITERATURE SUPPORTING LUNG TRANSPLANT FOR SCLERODERMA

According to the international registry report, SSc continues to be an uncommon reason for lung transplant with 228 patients (0.6% overall) receiving lung transplant for SSc-ILD and 176 patients (0.5% overall) for SSc-PAH [10]. Unfortunately, little data describe the number of SSc patients declined for lung transplant and the reason(s) underlying refusal. However, multiple primarily single-centre cohorts are providing reassurance that lung transplant for SSc can be performed safely with outcomes comparable to other patients with advanced lung disease.

Guidelines for referral/candidate selection

Current International Society of Heart and Lung Transplantation (ISHLT) guidelines regarding candidate selection for lung transplant in CTD suggest if 'there are no extrapulmonary contraindications to transplantation, it is reasonable to use similar guidelines to those proposed for idiopathic ILD' [9] and most extrapolate the recommendations for idiopathic ILD (Table 1) or PAH (Table 2) to this group. Although explicit exclusion criteria are not defined, the following conditions may be considered contraindications to lung transplant: uncontrolled gastroesophageal reflux (GER) or dysphagia despite maximal medical management, oesophageal aperistalsis, abnormal renal function or significant left ventricular dysfunction (systolic or diastolic). Multidisciplinary evaluation is essential to the longitudinal care of patients with SSc undergoing lung transplant to optimize nutrition and functional status as well as aggressively evaluate for and treat extrapulmonary manifestations of disease.

Table 1. Consensus guidelines for lung transplant candidate selection in interstitial lung disease

Interstitial lung disease candidate selection[a]
Recommendations for timing of lung transplant evaluation
Histopathologic or radiographic evidence of UIP or NSIP, regardless of pulmonary function
Abnormal lung function defined by FVC $<$ 80% predicted or DLCO $<$ 40% predicted
Dyspnoea or functional limitation due to pulmonary disease
Any oxygen requirement, including limited to exertion
Failure to improve dyspnoea, supplemental oxygen requirement or lung function following a clinically indicated trial of medical therapy for inflammatory ILD
Recommendations for timing of lung transplant listing
Decline in FVC $\geq$ 10% over 6-month period
Decline in DLCO $\geq$ 15% over 6-month period
Desaturation to $<$ 88% or distance $<$ 250 m during 6-min walk testing or $>$ 50 m decline in 6-min walk test distance over 6-month period
Evidence of pulmonary hypertension on right heart catheterization or echocardiogram
Hospitalization due to worsening pulmonary status, pneumothorax or acute exacerbation of ILD

DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; NSIP, nonspecific interstitial pneumonia; UIP, usual interstitial pneumonia.
[a]Adapted from 2014 ISHLT consensus document for the selection of lung transplant candidates [9].

The optimal timing of transplant evaluation and listing remains unclear given the heterogeneous nature of SSc progression. Use of physiologic data may identify patients with SSc-ILD at an increased risk of mortality, either through a scoring system combining ever-smoking status, age, and diffusing capacity for carbon monoxide (DLCO) [11▪] or longitudinal trends in forced vital capacity (FVC) in the presence of diffuse lung disease [12]. The development of elevated pulmonary artery pressures in patients with ILD, or progressive symptoms in PAH despite pulmonary vasodilator therapy, portends a poor prognosis and should prompt early referral for lung transplant evaluation. At our centre, we work closely with our ILD, PAH and rheumatology groups to encourage early referral to establish a longitudinal relationship with the patient and to expectantly manage complications that could limit lung transplant candidacy.

Table 2. Consensus guidelines for lung transplant candidate selection in pulmonary vascular disease

Pulmonary vascular disease candidate selection[a]
Recommendations for timing of lung transplant evaluation
NYHA class III or IV symptoms despite escalating vasodilator therapy
Rapidly progressive disease (if weight and functional status are not limiting concerns)
Use of parenteral pulmonary vasodilators despite NYHA functional class or symptoms
Known or suspected pulmonary veno-occlusive disease or pulmonary capillary hemangiomatosis
Recommendations for timing of lung transplant listing
NYHA class III or IV symptoms despite a trial of combination therapy, including prostanoids, lasting a minimum of 3 months
Cardiac index < 2 l/min/m^2
Mean right atrial pressure > 15 mmHg
6-min walk test distance < 250 m
Development of significant haemoptysis, pericardial effusion and/or signs of progressive right heart failure (recurrent ascites, rising bilirubin, increasing BNP or renal insufficiency)

NYHA, New York Heart Association.
[a]Adapted from 2014 ISHLT consensus document for the selection of lung transplant candidates [9].

Choice of procedure

Although single lung transplant (SLT) may maximize organ distribution, existing data support longer survival for patients with both IPF [13] and chronic obstructive pulmonary disease [14] who receive bilateral lung transplant (BLT). Similarly, although both SLT and BLT have been performed for PAH, mortality appears to be improved with BLT [15], likely due to better postoperative right ventricular function and ventilation-perfusion matching. Although 291 of 341 (85%) lung transplants performed over the past decade for SSc were bilateral (personal communication, ISHLT), it remains unclear whether SLT or BLT is the procedure of choice in patients with SSc, as there are no data comparing the two approaches. Notably, BLT in the younger SSc population may provide a superior reserve to compensate for any decline in lung function due to chronic lung allograft dysfunction (CLAD), which remains the leading cause of mortality among all lung transplant recipients.

Whether combined heart and lung transplant (HLT) is advisable for a subset of patients with SSc-PAH and significant cardiac dysfunction depends on the presence of left ventricular involvement and the ability of the right ventricle (RV) to recover systolic function postoperatively. We routinely obtain cardiac MRI and right/left heart catheterizations and engage our PAH and cardiology colleagues to as part of the pre-lung transplant evaluation to help make this distinction. Notably, the increasing recognition that the RV responds rapidly to the normalization of pulmonary artery (PA) pressures following BLT has led to a sharp decrease in HLT in recent years.

Perioperative management

Haemodynamic changes in patients with elevated pulmonary artery pressures occur following implantation of the donor lungs with rapid normalization of pulmonary vascular resistance. Longstanding PAH can result in right ventricular hypertrophy, favouring a postoperative hyperdynamic state that predisposes to development of pulmonary oedema. SSc is frequently associated with occult left ventricular diastolic dysfunction, which is uncovered when a chronically underfilled left ventricle (LV) is unable to accommodate to the rapid increase in preload following lung transplant, leading to worsening diastolic dysfunction and elevated left atrial pressures, pulmonary venous hypertension and capillary leak. Use of positive inotropes for blood pressure support may further worsen forward flow by increasing outflow obstruction.

Venovenous ECMO (VV-ECMO) is increasingly used preoperatively to support oxygenation in patients with progressive hypoxemic respiratory failure or to offload a failing RV. Contemporary data indicate comparable post-lung transplant outcomes when highly selected patients are supported via VV-ECMO [16▪▪,17], although this benefit may be restricted to high-volume centres [18]. Intraoperative ECMO offers multiple benefits including the ability to gradually increase flow through the pulmonary vascular bed or improve haemodynamic stability in a veno-arterial (VA) configuration or to support gas exchange in a venovenous deployment. Postoperatively, VA-ECMO or VV-ECMO may be utilized in the setting of primary graft dysfunction or to minimize ventilator-induced lung injury or toxic concentrations of inhaled oxygen. Unfortunately, no data exist to describe the use of ECMO specifically in the SSc population.

Preservation of renal function in SSc patients involves maintenance of an appropriate volume status and avoidance of potentially nephrotoxic medications. Sympathomimetic amines should be avoided, as they carry an increased risk of kidney injury [19▪] and may promote digital ischemia. Owing to the possible association between cyclosporine A and SRC [20], we favour the combination of tacrolimus, mycophenolate mofetil and prednisone for maintenance immunosuppression.

Short and long-term outcomes

Experienced centres are transplanting patients with SSc-ILD and SSc-PAH with comparable outcomes to patients with idiopathic ILD or PAH. Although granular data on early complications following lung transplant are limited, the incidence of PGD appears similar comparing SSc with non-SSc ILD [21,22▪▪], although patients with SSc are at a high risk of early issues, including need for prolonged mechanical ventilation, pleural space complications and infections [23▪▪]. A classic SSc-specific contraindication to lung transplant was thought to be increased autoimmunity, though data to support this hypothesis are lacking. Although there is a suggestion that acute rejection is more common following lung transplant for SSc [21,24], this is not consistent across published cohorts [22▪▪,23▪▪]. Notably, SSc-specific complications post-lung transplant are limited to reports of digital ulceration [25,26] and SRC [21,26,27▪▪] so the pre-lung transplant evaluation should assess risk factors for these specific manifestations of SSc.

Contemporary data replicated across centres demonstrate that patients undergoing lung transplantation for SSc-ILD or SSc-PAH have short-term and long-term mortality comparable to a group composed of predominantly IPF patients [21,22▪▪,23▪▪,28]. One nationwide analysis of UNOS data suggests that the 1-year mortality of SSc patients falls between IPF and PAH [29], although this group had a higher percentage of SSc-PAH than the single centre, primarily SSc-ILD cohorts. Long-term outcomes in carefully selected patients also demonstrate similar 3 and 5-year mortality [22▪▪] as well as comparable [21,24] or even improved [23▪▪] freedom from CLAD despite frequent oesophageal dysfunction (Table 3).

CONSIDERATIONS SPECIFIC TO PATIENTS WITH SCLERODERMA

Although the primary reason leading to consideration of lung transplant is limiting dyspnoea, unravelling the driver of this symptom in patients with SSc can be difficult. Owing to the systemic nature of involvement, patients with SSc may have functional limitations due to fatigue, arthralgias or myalgias increasing the work of locomotion, muscle inflammation, deconditioning, cardiac or pulmonary disease.

In addition to the established guidelines for lung transplant in fibrotic ILD and PAH (Tables 1, 2), certain considerations deserve mention when considering lung transplant for SSc (Table 4).

General

Given the comorbidities and multiple organ systems that may be involved in SSc, we urge caution when

Table 3. Outcomes following lung transplant for SSc

Ref. (Dates of inclusion)	Number of patients	Survival		Chronic lung allograft dysfunction (CLAD)	
		SSc	Comparison group	SSc	Comparison group
Saggar *et al.* [21] (2003–2007)	15 SSc, 38 IPF	1 year: 93%	1 year: 87%	ND	
Sottile *et al.* [24] (1998–2010)	23 SSc, 46 non CTD-ILD	1 year: 83% 3 years: 83% 5 years: 76%	1 year: 91% 3 years: 77% 5 years: 64%	ND	
Bernstein *et al.* [29] (2005–2012)	229 SSc, 201 PAH, 3333 ILD	30 days: 97% 1 year: 81% 3 years: 72%	30 days: 91% PAH, 96% ILD 1 year: 84% PAH, 84% ILD 3 years: 68% PAH, 70% ILD	NR	
Miele *et al.* [22▪▪] (2000–2012)	35 SSc, 264 diffuse fibrotic lung disease	1 year: 94% 3 years: 77% 5 years: 70%	1 year: 88% 3 years: 68% 5 years: 54%	ND	
Crespo et al. [23▪▪] (2005–2013)	72 SSc, 311 pulmonary fibrosis	30 days: 100% 1 year: 81% 5 years: 66%	30 days: 96% 1 year: 79% 5 years: 56%	Improved BOS1+ free survival in SSc*	
Chan *et al.* [28] (2008–2014)	26 SSc, 155 restrictive lung disease	30 days: 88% 1 year: 73% 3 years: 69% 5 years: 65%	30 days: 95% 1 year: 80% 3 years: 70% 5 years: 67%	NR	NR

ND, no difference; NR, not reported; SSc, systemic sclerosis.
*$P < 0.05$ vs. comparison group.

Table 4. Organ-specific manifestations of scleroderma and suggested testing

Organ system	SSc-specific involvement	Clinical diagnostic tests and evaluation	Potential contraindications for LT
Gastrointestinal (GI)	Oropharyngeal dysphagia Oesophageal dysmotility Gastroesophageal reflux disease (GERD) Delayed gastric emptying Small intestinal bacterial overgrowth (SIBO) Malnutrition Intestinal pseudo-obstruction Delayed colonic transit	Upper GI barium swallow Oesophageal manometry Combined multichannel intraluminal impedance and pH testing Esophagoduodenoscopy (EGD) with oesophageal biopsies Gastric emptying study	Persistent aspiration despite speech therapy 'Wide-open' oesophagus Symptomatic reflux despite multiple maximal therapy High-grade Barret's oesophagus
Cardiac	Pulmonary arterial hypertension (PAH) Atherosclerosis Left ventricular (LV) systolic or diastolic dysfunction Pericardial effusion Constrictive pericarditis Myocardial fibrosis Arrythmias Conduction defects	Transthoracic echocardiogram (TTE) Right heart catheterization with measurement of left ventricular end-diastolic pressure (LVEDP) Left heart catheterization TTE Cardiac MRI	Irreversible right heart dysfunction Coronary artery disease requiring bypass Depressed LV function
Renal	Scleroderma renal crisis (SRC)	History Laboratory assessment of renal function Evaluation for RNA polymerase III antibodies	Estimated GFR $\leq$ 50 Rapidly progressive skin disease
Peripheral vascular	Raynaud's phenomena	History Physical examination	Expectant management (avoidance of vasopressors, radial artery catheters, cold temperatures)
Skin	Digital ulcers Calcinosis	History Physical examination	Not a contraindication Expectant management (close monitoring for skin breakdown, aggressive treatment for possible infections)
Neuromuscular	Myositis Diaphragm muscle weakness	Laboratory assessment of CPK, aldolase Physical examination Upright/supine spirometry Diaphragm electromyography (EMG)	Myositis despite immunosuppression Active diaphragm involvement

LT, lung transplant.

considering patients over the age of 65 years for lung transplant. Similarly, meticulous evaluation of extrapulmonary issues including cardiac and gastrointestinal disease should be undertaken during the transplant evaluation as highlighted below. Given the increased risk of SRC and progressive skin disease early in the disease course, careful assessment of these conditions should be performed when lung transplant is being considered within the first 5 years following the onset of SSc.

Gastrointestinal involvement

Both aspiration and GER have been associated with the subsequent development of CLAD [30]. As such, oesophageal dysfunction historically represented the primary extrapulmonary limitation to lung transplant in SSc. In addition to SSc-related involvement, lung transplant may further impair oesophageal motility and gastric emptying due to vagal nerve injury and/or medication effects [31]. Notably, SSc patients can have a range of gastrointestinal manifestations, which require detailed and frequent gastrointestinal evaluation [32]. In our experience, small bowel dysmotility due to autonomic neuropathy and fibrosis leading to impaired tolerance of enteral feeding has been a critical and underreported symptom in this population.

No consensus guidelines exist detailing the optimal pre-lung transplant evaluation for gastrointestinal involvement in the SSc population and significant variability between centres is described. At our centre, we undertake rigorous pretransplant assessment, which includes barium oesophagram, esophagogastroduodenoscopy with biopsies and manometry with 24-h impedance studies. We employ high-dose proton pump inhibitor therapy with strict enteral feeding for 3 months postoperatively, although other experienced centres perform postpyloric tube feeding only until formal swallow evaluation can be completed. Due to abnormal small bowel motility and fibrosis, we commonly add prokinetic agents to improve

tolerance of feeding. Although Nissen fundoplication is associated with increased freedom from CLAD [33] and may result in improved forced expiratory volume in 1 second in some patients [34], the utility of this therapy in patients with concurrent GER and oesophageal dysmotility following lung transplant remains unclear and some centres consider alternative surgical procedures including Roux-en-Y gastric bypass [35].

Cardiac

The prevalence of primary cardiac disease in SSc is difficult to estimate due to the diversity of cardiac manifestations that can occur. Autopsy data indicate that pathologic cardiac involvement is present in over half of SSc patients [36], although clinically significant disease occurs in approximately 10% of patients [37]. The increasing recognition of cardiovascular mortality as improved therapies for SRC and pulmonary disease have lengthened lifespan [4▪▪] and frequency of pathologic cardiac involvement in SSc patients undergoing lung transplant [27▪▪] suggests that aggressive pretransplant evaluation is critical. Apart from PAH, SSc can result in right or left ventricular dysfunction, pericarditis, arrhythmias and coronary artery disease [38]. Left ventricular diastolic dysfunction and pulmonary venous hypertension are common in SSc [39] and associated with poor outcomes including PGD [40]. Although cardiac involvement occurs in both limited and diffuse cutaneous SSc [41], there may be a particular association between the presence of rapid progression of skin thickness [42] or myopathy [43] and heightened risk of cardiac death.

Pre-lung transplant cardiac evaluation in patients with SSc begins with a focused history and physical examination followed by directed cardiac testing. Mandatory testing includes transthoracic echocardiography with Doppler and right/left heart catheterization with LVEDP measurement. If the history is concerning for arrhythmia, 24-h Holter monitoring is preferred over less sensitive ECG. Cardiac MRI may be a useful and sensitive tool for the detection of subtle cardiac involvement in SSc, including assessment of underlying inflammation, fibrosis or microvascular perfusion defects [44].

Renal

The incidence of kidney dysfunction following lung transplant is variably reported, although one recent single-centre analysis revealed abnormal kidney function in 59% of patients following lung transplant [19▪]. SRC is a concern in patients undergoing lung transplant evaluation, particularly in light of its association with high-dose corticosteroid use [45]. Classic risk factors associated with SRC include the presence of RNA polymerase III antibodies, rapidly progressive or diffuse skin involvement, and duration of disease less than 3 years [46,47] although how this should be integrated into the pre-lung transplant assessment is unclear.

Neuromuscular

Neurologic complications following lung transplant are common [48] and may be exacerbated by the vascular abnormalities associated with SSc. As a result, the frequency of neurological issues due to calcineurin inhibitors as well as other vascular catastrophes occurring in the perioperative and postoperative periods may be heightened in the SSc population.

Peripheral and autonomic neuropathies are the most frequent neurologic complication in SSc [49]. The presence of mononeuritis multiplex or mononeuropathy can be a manifestation of vasculitis and should raise concern for a concurrent process such as systemic lupus erythematous or cryoglobulinemia [50]. Although direct central nervous system (CNS) involvement in SSc remains a matter of debate, the same vasculopathy that causes Raynaud's phenomenon can result in a CNS vasculitis that responds to immunosuppressive therapy [51].

Musculoskeletal involvement in SSc is common, affecting 4–37% of patients in the EUSTAR database [41] and up to 80% in other series [52]. Proximal muscle weakness is most commonly seen, with a smaller subset of patients displaying muscle atrophy or muscle enzyme abnormalities. Muscle weakness can include the diaphragm, which would represent an absolute contraindication to lung transplant. Interestingly, elevations in aldolase better predicted subsequent development of myopathy compared with CPK [53] and may be a useful screening tool. As weakness and deconditioning are common in all patients referred for lung transplant, all patients, including those with SSc, should routinely participate in pulmonary rehabilitation during the lung transplant evaluation [54].

Peripheral vascular

Vascular involvement, including Raynaud's phenomenon, is a common feature of SSc. Half of SSc patients experience digital ulcers at some point in their disease course, which can involve the fingertip pulp, areas of prior calcinosis or bony prominences around the joints. Digital ulcers occur early in the course of disease, 43% within the first year following the initial non-Raynaud's phenomenon

manifestation and in 73% of patients within the first 5 years [55]. Younger age at disease onset and higher Rodnan skin scores have been associated with earlier digital ulcer recurrence, as it has shorter duration between the initial and second digital ulcer episode [55]; these historical features should be queried during the pre-lung transplant evaluation.

Cutaneous

Prospective evaluation of lung transplant candidates includes careful attention to cutaneous manifestations of SSc particularly active and healing digital ulcers, which may increase the risk of critical digital ischemia or infection in the perioperative period. Postoperative management should include avoidance of radial artery catheters and minimization of vasopressor exposure. Although progression of cutaneous disease has not been reported post-lung transplant, treatment with mycophenolate mofetil, often used maintenance immunosuppression, may improve skin thickness [56].

CONCLUSION

Taken together, existing data support the safety of lung transplant as a therapy of last resort in patients with SSc-ILD and SSc-PAH. Reproducible data demonstrate that in 'appropriately selected candidates', both short and long-term outcomes, including mortality and freedom from CLAD, are comparable to non-SSc recipients. However, critical questions remain including what should serve as exclusionary criteria for lung transplant, best practices for evaluation and treatment of gastrointestinal dysfunction, management of immunosuppression in patients at a high risk for infectious complications and whether the presence of autoimmunity impacts outcomes and management. Multicentre and prospectively managed cohorts are needed given the small number of patients with SSc undergoing evaluation for lung transplant and surgery, inviting a new area of collaboration among experienced centres to move this growing field forward into the future.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Barnes J, Mayes MD. Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. Curr Opin Rheumatol 2012; 24:165–170.
2. Traub YM, Shapiro AP, Rodnan GP, *et al.* Hypertension and renal failure (scleroderma renal crisis) in progressive systemic sclerosis. Review of a 25-year experience with 68 cases. Medicine 1983; 62:335–352.
3. Steen VD, Costantino JP, Shapiro AP, Medsger TA Jr. Outcome of renal crisis in systemic sclerosis: relation to availability of angiotensin converting enzyme (ACE) inhibitors. Ann Intern Med 1990; 113:352–357.
4. ■■ Elhai M, Meune C, Boubaya M, *et al.* Mapping and predicting mortality from systemic sclerosis. Ann Rheum Dis 2017; 76:1897–1905.

Provocative assessment in European SSc cohort detailing excess mortality due to cardiovascular, pulmonary and infectious diseases and highlighting increase in rate of deaths due to SSc.

5. Salaffi F, Carotti M, Di Donato E, *et al.* Computer-aided tomographic analysis of interstitial lung disease (ILD) in patients with systemic sclerosis (SSc). Correlation with pulmonary physiologic tests and patient-centred measures of perceived dyspnea and functional disability. PLoS One 2016; 11:e0149240.
6. Steen VD, Owens GR, Fino GJ, *et al.* Pulmonary involvement in systemic sclerosis (scleroderma). Arthritis Rheum 1985; 28:759–767.
7. Yang X, Mardekian J, Sanders KN, *et al.* Prevalence of pulmonary arterial hypertension in patients with connective tissue diseases: a systematic review of the literature. Clin Rheumatol 2013; 32:1519–1531.
8. Chung L, Liu J, Parsons L, *et al.* Characterization of connective tissue disease-associated pulmonary arterial hypertension from REVEAL: identifying systemic sclerosis as a unique phenotype. Chest 2010; 138:1383–1394.
9. Weill D, Benden C, Corris PA, *et al.* A consensus document for the selection of lung transplant candidates: 2014–an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015; 34:1–15.
10. Yusen RD, Edwards LB, Dipchand AI, *et al.*, International Society for H. The Registry of the International Society for Heart and Lung Transplantation: thirty-third adult lung and heart-lung transplant report-2016; focus theme: primary diagnostic indications for transplant. J Heart Lung Transplant 2016; 35:1170–1184.
11. ■ Morisset J, Vittinghoff E, Elicker BM, *et al.* Mortality risk prediction in scleroderma-related interstitial lung disease: the SADL model. Chest 2017; 152:999–1007.

Derivation of a mortality risk prediction system using three variables (ever smoking history, age, DLCO) that can be easily applied to the evaluation of patients with SSc-ILD in a clinical setting.

12. Goh NS, Hoyles RK, Denton CP, *et al.* Short-term pulmonary function trends are predictive of mortality in interstitial lung disease associated with systemic sclerosis. Arthritis Rheumatol 2017; 69:1670–1678.
13. Schaffer JM, Singh SK, Reitz BA, *et al.* Single- vs double-lung transplantation in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis since the implementation of lung allocation based on medical need. JAMA 2015; 313:936–948.
14. Thabut G, Christie JD, Ravaud P, *et al.* Survival after bilateral versus single lung transplantation for patients with chronic obstructive pulmonary disease: a retrospective analysis of registry data. Lancet 2008; 371:744–751.
15. Bando K, Armitage JM, Paradis IL, *et al.* Indications for and results of single, bilateral, and heart-lung transplantation for pulmonary hypertension. J Thorac Cardiovasc Surg 1994; 108:1056–1065.
16. ■■ Biscotti M, Gannon WD, Agerstrand C, *et al.* Awake extracorporeal membrane oxygenation as bridge to lung transplantation: a 9-year experience. Ann Thorac Surg 2017; 104:412–419.

A large series from highly experienced centre demonstrating that awake ECMO can be used as a successful bridge to lung transplantation and allow for daily physical therapy, particularly when employed before respiratory failure leads to severe physical deconditioning.

17. Ius F, Natanov R, Salman J, *et al.* Extracorporeal membrane oxygenation as a bridge to lung transplantation may not impact overall mortality risk after transplantation: results from a 7-year single-centre experience. Eur J Cardiothorac Surg 2018; 54:334–340.
18. Hayes D Jr, Tobias JD, Tumin D. Center volume and extracorporeal membrane oxygenation support at lung transplantation in the lung allocation score era. Am J Respir Crit Care Med 2016; 194:317–326.
19. ■ Nguyen AP, Gabriel RA, Golts E, *et al.* Severity of acute kidney injury in the post-lung transplant patient is associated with higher healthcare resources and cost. J Cardiothorac Vasc Anesth 2017; 31:1361–1369.

A single-centre retrospective study detailing correlation with use of cardiopulmonary bypass, prolonged surgical time and intraoperative vasopressor use with postoperative acute kidney injury as well as increased costs and hospitalizations associated with this complication post-lung transplantation.

20. Denton CP, Sweny P, Abdulla A, Black CM. Acute renal failure occurring in scleroderma treated with cyclosporin A: a report of three cases. Br J Rheumatol 1994; 33:90–92.

21. Saggar R, Khanna D, Furst DE, *et al.* Systemic sclerosis and bilateral lung transplantation: a single centre experience. Eur Respir J 2010; 36:893–900.

22. ■■ Miele CH, Schwab K, Saggar R, *et al.* Lung transplant outcomes in systemic sclerosis with significant esophageal dysfunction. A comprehensive single-center experience. Ann Am Thorac Soc 2016; 13:793–802.

A single-centre experience describing equivalent outcomes that is notable for discussing reasons to refuse transplant in patients with SSc with a minority of denials due to oesophageal dysfunction.

23. ■■ Crespo MM, Bermudez CA, Dew MA, *et al.* Lung transplant in patients with scleroderma compared with pulmonary fibrosis. Short- and long-term outcomes. Ann Am Thorac Soc 2016; 13:784–792.

Largest single-centre series of SSc patients undergoing transplant, including 60% with severe oesophageal dysfunction, demonstrating comparable short and long-term outcomes.

24. Sottile PD, Iturbe D, Katsumoto TR, *et al.* Outcomes in systemic sclerosis-related lung disease after lung transplantation. Transplantation 2013; 95:975–980.

25. Fernandez-Codina A, Berastegui C, Pinal-Fernandez I, *et al.* Lung transplantation in systemic sclerosis: a single center cohort study. Joint Bone Spine 2018; 85:79–84.

26. Launay D, Savale L, Berezne A, *et al.,* Working Group on Heart/Lung transplantation in systemic sclerosis of the French Network on Pulmonary H. Lung and heart-lung transplantation for systemic sclerosis patients. A monocentric experience of 13 patients, review of the literature and position paper of a multidisciplinary Working Group. Presse Med 2014; 43:e345–e363.

27. ■■ Pradere P, Tudorache I, Magnusson J, *et al.,* Working Group on Heart/Lung Transplantation in Systemic Sclerosis. Lung transplantation for scleroderma lung disease: an international, multicenter, observational cohort study. J Heart Lung Transplant 2018; 37:903–911.

A large retrospective, international European cohort of 90 SSc patients who underwent lung transplantation or HLT notable for the universal presence of pathologic cardiac involvement on explanted hearts and high incidence of postlung transplantation SRC despite a mean duration of 10 years since SSc diagnosis.

28. Chan EY, Goodarzi A, Sinha N, *et al.* Long-term survival in bilateral lung transplantation for scleroderma-related lung disease. Ann Thorac Surg 2018; 105:893–900.

29. Bernstein EJ, Peterson ER, Sell JL, *et al.* Survival of adults with systemic sclerosis following lung transplantation: a nationwide cohort study. Arthritis Rheumatol 2015; 67:1314–1322.

30. D'Ovidio F, Mura M, Tsang M, *et al.* Bile acid aspiration and the development of bronchiolitis obliterans after lung transplantation. J Thorac Cardiovasc Surg 2005; 129:1144–1152.

31. Davis CS, Shankaran V, Kovacs EJ, *et al.* Gastroesophageal reflux disease after lung transplantation: pathophysiology and implications for treatment. Surgery 2010; 148:737–744; discussion 744–745.

32. Emmanuel A. Current management of the gastrointestinal complications of systemic sclerosis. Nat Rev Gastroenterol Hepatol 2016; 13:461–472.

33. Cantu E 3rd, Appel JZ 3rd, Hartwig MG, *et al.* Maxwell Chamberlain Memorial Paper. Early fundoplication prevents chronic allograft dysfunction in patients with gastroesophageal reflux disease. Ann Thorac Surg 2004; 78: 1142–1151; discussion 1142–1151.

34. Lau CL, Palmer SM, Howell DN, *et al.* Laparoscopic antireflux surgery in the lung transplant population. Surg Endosc 2002; 16:1674–1678.

35. Kent MS, Luketich JD, Irshad K, *et al.* Comparison of surgical approaches to recalcitrant gastroesophageal reflux disease in the patient with scleroderma. Ann Thorac Surg 2007; 84:1710–1715; discussion 1715–1716.

36. Follansbee WP, Miller TR, Curtiss EI, *et al.* A controlled clinicopathologic study of myocardial fibrosis in systemic sclerosis (scleroderma). J Rheumatol 1990; 17:656–662.

37. Ioannidis JP, Vlachoyiannopoulos PG, Haidich AB, *et al.* Mortality in systemic sclerosis: an international meta-analysis of individual patient data. Am J Med 2005; 118:2–10.

38. Lambova S. Cardiac manifestations in systemic sclerosis. World J Cardiol 2014; 6:993–1005.

39. Fox BD, Shimony A, Langleben D, *et al.* High prevalence of occult left heart disease in scleroderma-pulmonary hypertension. Eur Respir J 2013; 42:1083–1091.

40. Porteous MK, Ky B, Kirkpatrick JN, *et al.* Diastolic dysfunction increases the risk of primary graft dysfunction after lung transplant. Am J Respir Crit Care Med 2016; 193:1392–1400.

41. Walker UA, Tyndall A, Czirjak L, *et al.* Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group database. Ann Rheum Dis 2007; 66:754–763.

42. Perera A, Fertig N, Lucas M, *et al.* Clinical subsets, skin thickness progression rate, and serum antibody levels in systemic sclerosis patients with antitopoisomerase I antibody. Arthritis Rheum 2007; 56:2740–2746.

43. Follansbee WP, Zerbe TR, Medsger TA Jr. Cardiac and skeletal muscle disease in systemic sclerosis (scleroderma): a high risk association. Am Heart J 1993; 125:194–203.

44. Hachulla AL, Launay D, Gaxotte V, *et al.* Cardiac magnetic resonance imaging in systemic sclerosis: a cross-sectional observational study of 52 patients. Ann Rheum Dis 2009; 68:1878–1884.

45. Steen VD, Medsger TA Jr. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. Arthritis Rheum 1998; 41:1613–1619.

46. Penn H, Howie AJ, Kingdon EJ, *et al.* Scleroderma renal crisis: patient characteristics and long-term outcomes. QJM 2007; 100:485–494.

47. DeMarco PJ, Weisman MH, Seibold JR, *et al.* Predictors and outcomes of scleroderma renal crisis: the high-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis trial. Arthritis Rheum 2002; 46: 2983–2989.

48. Mateen FJ, Dierkhising RA, Rabinstein AA, *et al.* Neurological complications following adult lung transplantation. Am J Transplant 2010; 10:908–914.

49. Amaral TN, Peres FA, Lapa AT, *et al.* Neurologic involvement in scleroderma: a systematic review. Semin Arthritis Rheum 2013; 43:335–347.

50. Hurst RL, Berianu F, Ginsburg WW, *et al.* Cryoglobulinemic vasculitis in a patient with CREST syndrome. J Clin Neurosci 2014; 21:1821–1823.

51. Abers MS, Iluonakhamhe EK, Goldsmith CE, Kass JS. Central nervous system vasculitis secondary to systemic sclerosis. J Clin Neurosci 2013; 20:1168–1170.

52. Medsger TA Jr, Rodnan GP, Moossy J, Vester JW. Skeletal muscle involvement in progressive systemic sclerosis (scleroderma). Arthritis Rheum 1968; 11:554–568.

53. Toledano C, Gain M, Kettaneh A, *et al.* Aldolase predicts subsequent myopathy occurrence in systemic sclerosis. Arthritis Res Ther 2012; 14:R152.

54. Li M, Mathur S, Chowdhury NA, *et al.* Pulmonary rehabilitation in lung transplant candidates. J Heart Lung Transplant 2013; 32:626–632.

55. Hachulla E, Clerson P, Launay D, *et al.* Natural history of ischemic digital ulcers in systemic sclerosis: single-center retrospective longitudinal study. J Rheumatol 2007; 34:2423–2430.

56. Namas R, Tashkin DP, Furst DE, *et al.,* Participants in the Scleroderma Lung Study I, members of the Scleroderma Lung Study IIRG. Efficacy of mycophenolate mofetil and oral cyclophosphamide on skin thickness: post hoc analyses from two randomized placebo-controlled trials. Arthritis Care Res (Hoboken) 2018; 70:439–444.

Recent progress in systemic sclerosis-interstitial lung disease

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Purpose of review
Systemic sclerosis is a systemic autoimmune disorder wherein interstitial lung disease (ILD) is the major source or morbidity and mortality. Research into mechanisms of fibrosis and its intersection with autoimmunity, specifically lung fibrosis, has accelerated and been applied to autoimmune disorders such as scleroderma. This review highlights important emerging insights and treatment trials.

Recent findings
The important elements of this review focus on the challenges faced in identifying patients not only who develop lung disease but who are at a higher risk for progression given the heterogeneous natural history of ILD in scleroderma. Risk assessment scoring models using radiographic and physiologic parameters are highlighted and recent and ongoing clinical trials in scleroderma ILD are discussed.

Summary
The implications of much of this ongoing work is a potential paradigm shift in our ability to identify those patients at risk for progression, and to offer novel therapies that can limit the progression of inflammatory and fibrotic lung disease in this challenging group of patients.

Keywords
anti fibrotic therapy, interstitial lung disease, pulmonary fibrosis, scleroderma

INTRODUCTION

Interstitial lung disease (ILD) is the leading cause of mortality in systemic sclerosis (SSc). Although ILD is more common in patients with diffuse cutaneous disease, it can occur in those with limited cutaneous disease as well. The predominant pathologic process in SSc is nonspecific interstitial pneumonia (NSIP), though usual interstitial pneumonia (UIP) and other forms of ILD may occur. Patients with ILD may also have concomitant or undetected pulmonary vascular disease, which can confound the clinical significance of ILD and increase mortality.

Importantly, the natural history of those with ILD may be difficult to predict and thus some patients with mild ILD may not progress, while others may have a progressive or even rapidly progressive course that requires timely intervention. Some patients may present with incomplete phenotypic forms with ILD that suggest SSc, or ILD precedes typical features of SSc, and in such patients the lack of early recognition of disease may delay therapeutic intervention. Finally, scleroderma patients with dyspnoea or respiratory failure can have a variety of comorbidities such as aspiration leading to pneumonia, concomitant emphysema, myocardial disease, diastolic dysfunction and lung toxicity due to drugs.

The results of Scleroderma Lung Study I and II (SLS I, SLS II) have not only offered valuable insights into treatment responses to immunosuppressive agents but also to those physiologic and radiographic features, which help assess prognosis and potential response to treatment. Understanding the risk factors both for ILD and ILD progression in SSc is essential so that those deemed at highest risk can be considered for available therapeutic interventions and consideration for ongoing clinical trials.

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KEY POINTS

- Predictive models for ILD in scleroderma are emerging that may better allow clinicians to identify those patients at highest risk for progressive ILD.
- Recent clinical trials utilizing novel agents in idiopathic pulmonary fibrosis are now being applied to autoimmune disorders including scleroderma.
- Autologous stem cell transplant may offer an additional therapeutic approach in select scleroderma patients with interstitial lung disease.

PREVALENCE OF INTERSTITIAL LUNG DISEASE IN SCLERODERMA AND STRATEGIES TO SCREEN FOR INTERSTITIAL LUNG DISEASE

Prevalence of ILD in scleroderma varies among studies, though occurs more commonly in patients with diffuse SSc (up to 50%) than in those with limited SSc. Given the high prevalence of ILD in SSc, routine screening with high-resolution computed tomography (HRCT) of the chest is recommended. HRCT may be more sensitive in detecting ILD than pulmonary function tests (PFTs) alone and a HRCT Chest that shows no fibrosis at baseline portends a very low risk for the development of subsequent ILD [1,2].

Once ILD is identified, decisions regarding prognosis and predictors of progression can be challenging, given that some patients may have subclinical disease on HRCT that may not progress. Goh *et al.* [3] developed an algorithm that assesses extent of fibrosis (>20%) on HRCT, or lesser degrees of fibrosis combined with declines in forced vital capacity (FVC) (< 70%), can predict mortality and offer guidance regarding treatment considerations. This approach to assess risk has been replicated in subsequent studies [4,5].

Baseline and serial assessment of FVC, total lung capacity and diffusion capacity offer important information regarding prognosis of ILD, response to treatment and can also help assess the possibility of underlying pulmonary hypertension. Six-minute walk testing, while convenient to perform in assessing for oxygenation and exaggerated heart rate responses that may be seen in pulmonary vascular disease, may not be reliable in SSc due to Raynaud's and other comorbidities. Although FVC remains the single most reproducible physiologic assessment of parenchymal lung disease, the thresholds designated for FVC in clinical trials in SSc have been extrapolated from idiopathic pulmonary fibrosis (IPF), which may not reflect the natural history of decline of SSc. Recent data looking at PFTs at 1 and 2 years compared with 15-year survival data suggest that 1-year trends in PFT decline predict survival in patients with extensive lung disease. In those with lesser degrees of fibrosis, composite indices that combine smaller changes in FVC (<10%) with diffusing capacity of the lung for carbon monoxide (DLCO) changes of more than 15% at 2 years of evaluation are more predictive of mortality [6,7]. An emerging concept known as the MCID (minimally clinical important difference) in FVC suggests that even smaller changes in FVC (3–5.3% changes) can correlate with patient reported outcomes and findings on HRCT [7]. The DLCO may correlate with extent of fibrosis and, thus may be of prognostic value though may be confounded if there is an unknown concomitant pulmonary vascular disease [8].

Several predictive models using readily available demographic and clinical data have been developed to assess prognosis in patients with connective tissue disease and ILD. The GAP (sex, age and lung physiology) ILD model was originally developed to assess 1, 3 and 5-year mortality in IPF but has been applied and validated in patients with connective tissue disease, including scleroderma [9]. This model involves four parameters, including age, gender, type of ILD and FVC. Another model, SADL (smoking history, age, DLCO), has been validated specifically in scleroderma patients and incorporates age, smoking history and DLCO to predict all-cause mortality in SSc ILD [10▪]. Finally, the SPAR (SpO_2, arthritis) model utilizes optimal cutoffs for desaturation of oxygen with 6-min walk testing in combination with the presence of arthritis, which were both identified as independent predictors for ILD progression in a derivation and validated cohort [11▪].

CURRENT THERAPIES IN SYSTEMIC SCLEROSIS-INTERSTITIAL LUNG DISEASE

Scleroderma Lung Study I and II

There are currently no approved targeted therapies for the treatment of scleroderma. Treatment strategies have focused on traditional immunosuppressive therapies that have proven efficacy in other rheumatic conditions. The two best studied medications in the treatment of SSc, and specifically SSc-ILD, have been cyclophosphamide and mycophenolate mofetil (MMF), and both have been studied in randomized controlled trials (RCTs) (Table 1).

SLS I, a multicentre, double-blind RCT was designed to assess change in FVC as the primary endpoint comparing treatment with 1 year of oral

Table 1. Current treatments for systemic sclerosis-interstitial lung disease

Drug	Mechanism of action	Evidence for use
Cyclophosphamide [12,14]	Alkylating agent, cross links DNA, decreasing DNA synthesis and preventing cell division	SLS I: improvement in FVC, dyspnoea scores, chest imaging; RCT with trend towards improvement in FVC
Mycophenolate mofetil [13]	Inhibits inosine monophosphate dehydrogenase, inhibits de-novo guanosine nucleotide synthesis, prevents T and B proliferation	SLS II: stabilization in FVC or DLCO

FVC, forced vital capacity; RCT, randomized controlled trial; SSc, systemic sclerosis.

cyclophosphamide versus placebo [12]. The results showed a modest benefit in terms of decline of FVC in patients treated with cyclophosphamide compared with placebo, though that difference was no longer evident after 2 years follow-up.

SLS II compared the efficacy of MMF for 24 versus 12 months of oral cyclophosphamide, and showed similar but modest improvement in FVC between the two therapies [13]. A third study examined the use of intravenous cyclophosphamide for 6 months with transition to azathioprine, though no statistically significant difference in FVC between treatment and placebo group was noted [14]. Furthermore, cross comparison of MMF patients in SLS II compared with placebo in SLS I showed greater improvement in FVC, DLCO and skin scores in the MMF-treated group [15]. Given the systemic toxicity associated with cyclophosphamide, there has been a general shift towards MMF in SSc ILD based on available clinical trials.

ADJUNCTIVE TREATMENTS FOR SYSTEMIC SCLEROSIS-INTERSTITIAL LUNG DISEASE

Oesophageal dysfunction leading to reflux and aspiration may play a role in initiating or exacerbating ILD in patients with scleroderma [16]. Assessment of oesophageal diameter measured on HRCT has been associated with worse fibrosis scores, lower pulmonary function and mortality [17]. The use of 24 or 48-h oesophageal manometry with pH assessment can be useful to determine the degree of acid and nonacid reflux in patients with or without clinical symptoms or radiographic evidence of oesophageal dysmotility. In general, patients are treated with high doses of proton pump inhibitors, and in severe cases, surgical intervention may be considered, though there are no prospective trials available regarding treatment of oesophageal dysfunction in scleroderma-related ILD.

Vaccination for pneumococcal pneumonia, influenza and varicella should be considered in all SSc patients on immunomodulating therapy. Supplemental oxygen in appropriate patients and pulmonary rehabilitation are all important adjunctive therapies in SSc patients with ILD.

NEW CLINICAL TRIALS IN SYSTEMIC SCLEROSIS-INTERSTITIAL LUNG DISEASE

Although traditional immunosuppressive therapies remain the mainstay of treatment, improvement in FVC with these medications may not be sustained, as demonstrated in SLS I. As a result, there is significant interest to develop targeted therapies for SSc-ILD (Table 2) [18–28,29▪▪].

Antifibrotic therapies

Two oral antifibrotic medications, pirfenidone and nintedanib (an oral tyrosine kinase inhibitor), were recently approved for use in IPF [18,19]. Both medications slowed disease progression, as measured by FVC. An open-label phase II trial of pirfenidone in SSc-ILD (LOTUSS) was completed and demonstrated acceptable tolerability, even with over 60% of patients concurrently taking MMF [20]. The Scleroderma Lung Study III, comparing oral MMF and placebo, with oral MMF and oral pirfenidone for 18 months is currently underway and recruiting individuals (ClinicalTrials.gov Identifier NCT03221257).

A phase III double-blind, RCT of nintendanib in SSc-ILD recently completed recruitment and allowed for the concurrent use of MMF and Prednisone (< 10 mg daily) (ClinicalTrials.gov Identifier: NCT02597933). The primary endpoint of this trial was a change in FVC annually over 52 weeks and secondary endpoints included absolute change in the modified Rodnan skin score (mRSS) from baseline and St. George's Respiratory Questionnaire at 52 weeks [30].

Tocilizumab

Tocilizumab, a mAb against the interleukin (IL)-6 receptor, is currently approved for use in rheumatoid

Table 2. Investigational treatments for systemic sclerosis-interstitial lung disease

Drug	Mechanism of action	Evidence for use
Pirfenidone [19,20]	Antifibrotic and anti-inflammatory effects	ASCEND (IPF RCT), improved FVC and progression free survival in IPF; LOTUSS study for SSc-ILD
Nintedanib [18]	Inhibits receptor tyrosine kinases and nonreceptor tyrosine kinases	INPULSIS (IPF RCT), reduction in FVC decline; Phase III trial in progress in SSc-ILD
Tocilizumab [21]	mAb to IL-6 receptor	Phase II trial, trend towards improvement in mRSS at 48 weeks, trend towards slower decline in FVC
Rituximab [22,23]	mAb against CD20 on B-lymphocytes	Small randomized trials, less decline or improvement in FVC
Lysophosphatidic acid receptor antagonist, SAR100842 [24]	LPA_1 receptor antagonist	Phase II trial, excellent safety profile
Autotaxin inhibitor (GLPG1690) [25]	Autotaxin inhibitor	Phase IIa trial in IPF, well tolerated
Fresolimumab [26]	mAb to TGF-β	Small open-label trial, improvement in mRSS and skin biomarkers
Haematopoietic stem cell transplant [27,28,29▪▪]	Lymphocyte ablation	ASSIST, improvement in FVC ASTIS, improved event-free and overall survival SCOT, improved event-free survival and less transplant-related mortality

FVC, forced vital capacity; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; SSc, systemic sclerosis.

arthritis (RA) and has been evaluated in SSc. On the basis of preliminary data suggesting that IL-6 levels may be associated with DLCO and FVC decline in SSc-ILD, and IL-6 levels are elevated in the SSc serum, a phase II trial of tocilizumab in SSc was conducted [21,31,32]. The primary endpoint was a reduction in mRSS at week 24. Although this endpoint was not met, there was a trend towards improvement in skin scores in the tocilizumab group compared with the placebo group at week 48. In addition, there was a trend towards a slower decline in FVC in the tocilizumab group, although the study was not specifically designed to examine this endpoint. Results from the open-label period of the trial showed skin score improvements and FVC stabilization in the placebo-treated patients who were transitioned to tocilizumab [33]. A phase III trial evaluating the efficacy and safety of Tocilizumab in SSc has completed enrolment with results expected soon.

Rituximab

Rituximab, a mAb against CD20 used in the treatment of RA and ANCA-associated vasculitis, is of interest in the treatment of SSc-ILD. In a small nested case–control study, SSc patients treated with rituximab versus matched controls (not treated with rituximab) showed improvement in mRSS, and in a small subset of patients with ILD, there was no further decline in FVC [22]. On the basis of this study and other smaller studies, there is hope that rituximab may be beneficial in SSc-ILD [23]. Plans are currently underway to evaluate rituximab versus cyclophosphamide in the treatment of connective tissue disease-associated ILD (RECITAL) [34]. The trial will compare 1 g of intravenous Rituximab given twice at a 2-week interval with intravenous cyclophosphamide 600 mg/m^2 administered monthly. The primary endpoint will be a change in FVC at 24 weeks and secondary endpoints include change in FVC at 48 weeks, change in oxygen requirement and total 48-week corticosteroid exposure.

Lysophosphatidic acid and autotaxin

Lysophosphatidic acid (LPA), a small bioactive lipid mediator, with important roles in the development of both pulmonary and dermal fibrosis in mouse models [35,36]. It has been evaluated in an early phase clinical trial in SSc. A phase II trial of an LPA_1 receptor antagonist SAR100842 demonstrated an excellent safety profile and potential for clinical efficacy with reduction in mRSS [24]. Patients with SSc-ILD were not specifically examined, and further studies are needed to determine whether targeting the LPA pathway may provide benefit in patients with SSc-ILD.

Meanwhile, the LPA-producing enzyme autotaxin (ATX) has been studied in SSc and IPF and found to have an important role in mediating dermal fibrosis in diffuse SSc patients and in models of IPF [37,38]. A Phase IIa study of the autotaxin inhibitor, GLPG1690, in patients with IPF was recently

conducted and the drug was well tolerated [25]. A Phase III trial of GLPG1690 in IPF is currently slated to begin in late 2018, and there is much interest in developing this inhibitor further for SSc-ILD.

Fresolimumab/transforming growth factor-β pathway

One of the key mediators of fibrosis is the pleotropic cytokine, transforming growth factor-beta (TGF-β). Given the important role of TGF-β in fibrosis, it is a natural target for therapy in SSc. Fresolimumab, a mAb to TGF-β that neutralizes all isoforms of the molecule, has been studied in a recent small open-label trial [26]. Two different doses of fresolimumab were compared in patients with early (< 2 years) dcSSc with the primary outcomes being a change in mRSS as well as a change in mRNA levels of two skin biomarkers, cartilage oligomeric protein (COMP) and thrombospondin-1 (THBS1) at 24 weeks. Both treatment groups showed a relative rapid decrease in mRSS and THBS1, suggesting that selective targeting of the TGF-β pathway may be beneficial in the treatment of SSc patients. Larger studies are needed to determine the long-term effectiveness of this drug and whether it will be useful in SSc-ILD.

Autologous stem cell transplant

Given the relatively poor prognosis of SSc-ILD and the lack of targeted therapies for the disease, autologous haematopoietic stem cell transplant (HSCT) presents a potentially attractive therapeutic option. Three major trials have compared HSCT to cyclophosphamide in SSc patients with internal organ involvement. ASSIST (Autologous Stem Cell Systemic Sclerosis Immune Suppression Trial), a single-centre study, found that patients with dcSSc and internal organ involvement treated with HSCT compared with IV cyclophosphamide for 6 months had an improvement in mRSS and FVC at 12 months [27]. The ASTIS (Autologous Stem cell Transplantation International Scleroderma) trial, a multicentre study in Europe and Canada, found that dcSSc patients treated with HSCT had improved event-free and overall survival despite a 10% treatment-related mortality in the HSCT group, when compared with 1 year of IV cyclophosphamide [28]. The SCOT (Scleroderma: Cyclophosphamide Or Transplantation) trial, a multicentre study in North America, compared IV cyclophosphamide for 1 year with HSCT, and found improved event-free and overall survival with HSCT. Rates of treatment-related death and posttransplantation use of disease-modifying therapy was also lower than other transplant studies [29▪▪]. HSCT may be considered for patients with severe disease who have been refractory to other treatment options.

CHALLENGES IN SCLERODERMA TREATMENT

There are many challenges to developing new and effective treatments for SSc. The disease is heterogeneous, with some patients having severe skin involvement only and others having internal organ involvement such as ILD, pulmonary hypertension or gastrointestinal disease. This heterogeneity poses challenges when trying to identify a singular treatment approach, and additionally affects enrolment of patients in clinical trials. The trajectory of the disease is also variable, with rapid and slow progressors. Predicting which patients may have more rapid progression of disease remains a challenge. To date, clinical outcomes in trials have focused on the mRSS (a validated measure of skin thickness in 17 locations, graded on a scale of 0–3) and the FVC. New efforts are underway to develop a more comprehensive outcome measure that would incorporate multiple variables. The Composite Responder Index in diffuse cutaneous SSc (CRISS) is one such measure, and is awaiting validation in a clinical trial [39]. This index incorporates both mRSS and FVC, as well as the physician global assessment, the patient global assessment and the Health Assessment Questionnaire-Disability Index (HAQ-DI).

CONCLUSION

There has been significant progress in understanding the pathogenesis and risk factors of scleroderma and as a result novel targeted therapies are in development for patients with SSc-ILD. Although traditional immunosuppressive drugs such as cyclophosphamide and MMF are currently the mainstay of treatment, new clinical trials are incorporating these immunosuppressives as part of the trial protocols. With further advances in understanding the disease, and development of new composite indexes and models in assessing treatment responses, effective therapies for scleroderma lung fibrosis will emerge in the next decade.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Suliman YA, Dobrota R, Huscher D, *et al.* Brief report: pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. Arthritis Rheumatol 2015; 67:3256–3261.
2. Hoffmann-Vold AM, Aalokken TM, Lund MB, *et al.* Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis Rheumatol 2015; 67:2205–2212.
3. Goh NS, Desai SR, Veeraraghavan S, *et al.* Interstitial lung disease in systemic sclerosis: a simple staging system. Am J Respir Crit Care Med 2008; 177:1248–1254.
4. Hax V, Bredemeier M, Didonet Moro AL, *et al.* Clinical algorithms for the diagnosis and prognosis of interstitial lung disease in systemic sclerosis. Semin Arthritis Rheum 2017; 47:228–234.
5. Moore OA, Goh N, Corte T, *et al.* Extent of disease on high-resolution computed tomography lung is a predictor of decline and mortality in systemic sclerosis-related interstitial lung disease. Rheumatology (Oxford) 2013; 52:155–160.
6. Goh NS, Hoyles RK, Denton CP, *et al.* Short-term pulmonary function trends are predictive of mortality in interstitial lung disease associated with systemic sclerosis. Arthritis Rheumatol 2017; 69:1670–1678.
7. Kafaja S, Clements PJ, Wilhalme H, *et al.* Reliability and minimal clinically important differences of forced vital capacity: results from the Scleroderma Lung Studies (SLS-I and SLS-II). Am J Respir Crit Care Med 2017. [Epub ahead of print]
8. Tashkin DP, Volkmann ER, Tseng CH, *et al.* Relationship between quantitative radiographic assessments of interstitial lung disease and physiological and clinical features of systemic sclerosis. Ann Rheum Dis 2016; 75:374–381.
9. Ryerson CJ, Vittinghoff E, Ley B, *et al.* Predicting survival across chronic interstitial lung disease: the ILD-GAP model. Chest 2014; 145:723–728.
10. ■ Morisset J, Vittinghoff E, Elicker BM, *et al.* Mortality risk prediction in scleroderma-related interstitial lung disease: the SADL model. Chest 2017; 152:999–1007.

This study looked at a prediction model for scleroderma ILD using a derivation and validation cohort to produce a practical tool to predict all-cause mortality in scleroderma-related ILD.

11. ■ Wu W, Jordan S, Becker MO, *et al.* Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model. Ann Rheum Dis 2018; 77:1326–1332.

This study examined two cohorts of scleroderma patients with mild ILD and followed them prospectively to create a model for risk for progression of ILD that may be useful in clinical practice.

12. Tashkin DP, Elashoff R, Clements PJ, *et al.* Cyclophosphamide versus placebo in scleroderma lung disease. N Engl J Med 2006; 354:2655–2666.
13. Tashkin DP, Roth MD, Clements PJ, *et al.* Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. Lancet Respir Med 2016; 4:708–719.
14. Hoyles RK, Ellis RW, Wellsbury J, *et al.* A multicenter, prospective, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral azathioprine for the treatment of pulmonary fibrosis in scleroderma. Arthritis Rheum 2006; 54:3962–3970.
15. Volkmann ER, Tashkin DP, Li N, *et al.* Mycophenolate mofetil versus placebo for systemic sclerosis-related interstitial lung disease: an analysis of scleroderma lung studies I and II. Arthritis Rheumatol 2017; 69:1451–1460.
16. Savarino E, Bazzica M, Zentilin P, *et al.* Gastroesophageal reflux and pulmonary fibrosis in scleroderma: a study using pH-impedance monitoring. Am J Respir Crit Care Med 2009; 179:408–413.
17. Winstone TA, Hague CJ, Soon J, *et al.* Oesophageal diameter is associated with severity but not progression of systemic sclerosis-associated interstitial lung disease. Respirology 2018. [Epub ahead of print]
18. Richeldi L, du Bois RM, Raghu G, *et al.* Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med 2014; 370:2071–2082.
19. King TE Jr, Bradford WZ, Castro-Bernardini S, *et al.* A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med 2014; 370:2083–2092.
20. Khanna D, Albera C, Fischer A, *et al.* An open-label, phase II study of the safety and tolerability of pirfenidone in patients with scleroderma-associated interstitial lung disease: the LOTUSS trial. J Rheumatol 2016; 43:1672–1679.
21. Khanna D, Denton CP, Jahreis A, *et al.* Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. Lancet 2016; 387:2630–2640.
22. Jordan S, Distler JH, Maurer B, *et al.* Distler O, group ERs. Effects and safety of rituximab in systemic sclerosis: an analysis from the European Scleroderma Trial and Research (EUSTAR) group. Ann Rheum Dis 2015; 74:1188–1194.
23. Daoussis D, Liossis SN, Tsamandas AC, *et al.* Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. Rheumatology (Oxford) 2010; 49:271–280.
24. Allanore Y, Distler O, Jagerschmidt A, *et al.* Double-blind, randomized, 8-week placebo-controlled followed by a 16-week open label extension study, with the LPA1 receptor antagonist SAR100842 for patients with diffuse cutaneous systemic sclerosis. Arthritis Rheumatol 2018. [Epub ahead of print]
25. Maher TM, van der Aar EM, Van de Steen O, *et al.* Safety, tolerability, pharmacokinetics, and pharmacodynamics of GLPG1690, a novel autotaxin inhibitor, to treat idiopathic pulmonary fibrosis (FLORA): a phase 2a randomised placebo-controlled trial. Lancet Respir Med 2018; 6:627–635.
26. Rice LM, Padilla CM, McLaughlin SR, *et al.* Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. J Clin Invest 2015; 125:2795–2807.
27. Burt RK, Shah SJ, Dill K, *et al.* Autologous nonmyeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised phase 2 trial. Lancet 2011; 378:498–506.
28. van Laar JM, Farge D, Sont JK, *et al.* Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. JAMA 2014; 311: 2490–2498.
29. ■■ Sullivan KM, Goldmuntz EA, Keyes-Elstein L, *et al.* Myeloablative autologous stem-cell transplantation for severe scleroderma. N Engl J Med 2018; 378:35–47.

This prospective trial utilized autologous stem cell transplant in scleroderma patients, including patients with ILD, and compared with cyclophosphamide showed improved event-free survival.

30. Distler O, Brown KK, Distler JHW, *et al.* Design of a randomised, placebo-controlled clinical trial of nintedanib in patients with systemic sclerosis-associated interstitial lung disease (SENSCIS). Clin Exp Rheumatol 2017; 35(Suppl 106):75–81.
31. De Lauretis A, Sestini P, Pantelidis P, *et al.* Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. J Rheumatol 2013; 40:435–446.
32. Khan K, Xu S, Nihtyanova S, *et al.* Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. Ann Rheum Dis 2012; 71:1235–1242.
33. Khanna D, Denton CP, Lin CJF, *et al.* Safety and efficacy of subcutaneous tocilizumab in systemic sclerosis: results from the open-label period of a phase II randomised controlled trial (faSScinate). Ann Rheum Dis 2018; 77:212–220.
34. Saunders P, Tsipouri V, Keir GJ, *et al.* Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease (RECITAL): study protocol for a randomised controlled trial. Trials 2017; 18:275.
35. Tager AM, Lacamera P, Shea BS, *et al.* The lysophosphatidic acid receptor LPA(1) links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med 2008; 14:45–54.
36. Castelino FV, Seiders J, Bain G, *et al.* Amelioration of dermal fibrosis by genetic deletion or pharmacologic antagonism of lysophosphatidic acid receptor 1 in a mouse model of scleroderma. Arthritis Rheum 2011; 63:1405–1415.
37. Castelino FV, Bain G, Pace VA, *et al.* An autotaxin/lysophosphatidic acid/interleukin-6 amplification loop drives scleroderma fibrosis. Arthritis Rheumatol 2016; 68:2964–2974.
38. Black KE, Berdyshev E, Bain G, *et al.* Autotaxin activity increases locally following lung injury, but is not required for pulmonary lysophosphatidic acid production or fibrosis. FASEB J 2016; 30:2435–2450.
39. Khanna D, Berrocal VJ, Giannini EH, *et al.* The American College of Rheumatology provisional composite response index for clinical trials in early diffuse cutaneous systemic sclerosis. Arthritis Rheumatol 2016; 68: 299–311.

Muscle disease in scleroderma

Julie J. Paik

Purpose of review
This review discusses the most updated literature of muscle disease in scleroderma in the past year.

Recent findings
In the past year, two studies have highlighted that fibrosis is a prevalent histopathologic feature in muscle biopsies of patients with scleroderma muscle disease. In addition, microangiopathy was a common co-feature on muscle biopsies. A fibrosing myopathy, or fibrosis predominance on muscle histopathology, is associated with a unique clinical phenotype in patients with scleroderma. When compared with those with an inflammatory myopathy, patients with a fibrosing myopathy tend to have diffuse scleroderma, lower muscle enzymes, nonirritable myopathy, and elevated cardiac enzymes. These patients are also reported to have a higher risk of cardiopulmonary complications and cardiac death when compared with those with an inflammatory myopathy.

Summary
Although there are clear cases of overlap myositis, it is clear that muscle disease in scleroderma is being redefined, and it is crucial to start recognizing that the muscle is an organ that can directly be affected by scleroderma. Fibrosis can occur early in scleroderma muscle disease, and a unique histologic subtype of muscle disease, fibrosing myopathy, is associated with a higher risk of mortality.

Keywords
fibrosis, myopathy, scleroderma

INTRODUCTION

Muscle disease, or myopathy, in scleroderma has been thought to be a relative bystander in comparison with other organ disease manifestations. Although it is clear that a subset of patients has overlap myositis or other reasons for a myopathy such as malnutrition, disuse, or other neurologic diseases, studies in the past year have demonstrated that fibrosis in scleroderma muscle disease can occur early in the onset of muscle weakness and is not a late manifestation of end-stage muscle disease typical of polymyositis or immune-mediated necrotizing myopathy.

EPIDEMIOLOGY OF MUSCLE DISEASE IN SCLERODERMA

Due to a lack of classification criteria to parse out the heterogeneity of muscle disease in scleroderma, the prevalence of muscle disease in scleroderma varies widely. In earlier studies, the definition of muscle disease was simply based on the presence of muscle weakness or a combination of weakness and abnormal muscle enzymes, abnormal electromyography, or muscle biopsy [1–4]. In more recent studies, the prevalence of muscle weakness in a large scleroderma cohort has been reported to be approximately 25% [5], whereas a meta-analysis in 2013 reported that the prevalence of proximal muscle weakness was 16%, and myositis was 13% [6].

Although the prevalence continues to be variable depending on the definition of myopathy used for each study, it is becoming more apparent that systemic sclerosis (SSc) patients with concomitant muscle disease have poorer outcomes including disability and death [5,7,8]. At this point in time, however, there is no targeted treatment because we do not yet fully understand the mechanisms and pathogenesis of muscle disease in scleroderma. Prior studies have reported predominant inflammation or necrosis, which is responsive to immunosuppression, however, the decision to treat with steroids requires careful consideration because of the risk of renal crisis.

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KEY POINTS

- Muscle fibrosis can be an early, not end stage marker of muscle disease in scleroderma.
- Vascular involvement on muscle histology is more common in scleroderma muscle disease than inflammatory myopathies.
- A fibrosing myopathy is a unique histopathologic subset of scleroderma muscle disease that may portend a more severe prognosis.

FIBROSIS IS THE PREDOMINANT HISTOPATHOLOGIC FEATURE IN SCLERODERMA MUSCLE DISEASE

Prior studies in muscle histopathology of weak scleroderma patients have demonstrated that it is heterogeneous. In one study of 42 scleroderma patients with muscle biopsies, histopathologic diagnoses ranged from overlap polymyositis, dermatomyositis, necrotizing myopathy, and fibrosis alone [9]. Other studies have primarily described that the predominant features on muscle biopsies of weak scleroderma patients were necrosis and inflammation [3,10] supporting the finding that in the spectrum of overlap myositis, SSc is the most common connective tissue disease associated with idiopathic inflammatory myopathies, accounting for 42.6% of overlap myositis patients [11–13].

In the past year, two studies have highlighted that fibrosis is a prevalent feature on muscle histopathology. Previously, fibrosis was primarily reported in autopsy cases when it was first described in scleroderma muscle disease [1]. In contrast, in the past year, these two studies demonstrated that fibrosis may indeed be an earlier marker of muscle disease in scleroderma and may be more prevalent than previously reported. The clinical relevance of fibrosis predominance on muscle biopsy in scleroderma muscle disease was explored in the first study.

First, we conducted a retrospective, cross-sectional study to characterize the clinical phenotype of patients with a 'fibrosing myopathy' or those patients with biopsies with predominant fibrosis without inflammation/necrosis [14▪]. The study population consisted of 37 scleroderma patients with muscle biopsies, of which 8 had a fibrosing myopathy and 29 with an inflammatory myopathy. The patients who had a fibrosing myopathy on both hematoxylin and eosin stain (H&E) and Gomori trichome showed evidence of endomysial and perimysial fibrosis with myopathic features of internalized nuclei and increased variability of myofibers. The patients with an 'inflammatory myopathy' were scleroderma patients who had met the European Neuromuscular Center (ENMC) criteria for either polymyositis, nonspecific myositis, dermatomyositis, or a necrotizing myopathy [15]. To get a sense of the clinical phenotype of those with a fibrosing myopathy, the clinical characteristics of the fibrosing group were compared with the inflammatory group.

Compared with those with an inflammatory myopathy, patients with a fibrosing myopathy were more likely to have diffuse skin disease (87 versus 62%, $P = 0.18$), African American race (62.5 versus 37.9%, $P = 0.20$), lower mean creatine kinase values (516 ± 391 versus 2477 ± 3511 IU/l; $P = 0.007$) and lower aldolase values (13.8 ± 4.7 versus 27.3 ± 4.7, $P = 0.01$). Interestingly, autoantibody testing against Scl-70 and U3 RNP were detected more frequently in the fibrosing group compared with the inflammatory group ($P = 0.031$ and $P = 0.0004$). The most striking finding of this study was that patients with a fibrosing myopathy also had a significant higher morality (5 of 8 (62.5%) versus 4 of 29 (14.3%); $P = 0.0005$). The reason for the increased mortality in those with a fibrosing myopathy were carefully reviewed and of the five patients who were deceased, three of five (60%) likely had cardiac disease that primarily contributed to death.

Despite the severe myopathic clinical phenotype of these patients with a fibrosing myopathy, they tended to have lower creatine kinase levels and less frequent findings of membrane instability (i.e. spontaneous activity) on electromyography (EMG). This highlights the importance of considering a muscle biopsy in those scleroderma patients who have prominent weakness even without markedly elevated creatine kinase levels. This may identify the etiology causing the weakness and may have therapeutic implications. As shown in Table 1, to date, there are distinct histopathologic subsets that have been described in scleroderma muscle disease. The optimal treatment for a fibrosing myopathy is unknown. However, if such a biopsy is found in a patient with scleroderma, it would be important to adopt an aggressive treatment strategy to prevent the predicted steep decline in clinical trajectory.

Lastly, this study also demonstrated that patients with a fibrosing myopathy had a higher risk for cardiac involvement when compared with scleroderma patients with an inflammatory myopathy. Thus, early cardiac work-up with cardiac biomarkers such as troponin I, cardiac MRI, and EKG for conduction defects may be warranted. Overall, although this study was cross-sectional, it is the first study to report that fibrosing myopathy is a unique

Table 1. Key diagnostic, clinical features, and treatment of systemic sclerosis-associated myopathy

Histopathologic diagnoses in SSc-associated myopathy	Diagnostic hallmarks	Clinical phenotype	Potential treatment
Polymyositis or nonspecific myositis	Primary inflammation on muscle biopsy Elevated creatine kinase, aldolase (typically at least two to three times ULN) Irritable myopathy on EMG Prominent muscle edema on MRI	Diffuse or limited scleroderma	Can consider steroids acutely if very weak (absolute careful monitoring for renal crisis); if no response to MMF, would consider IVIG and/or rituximab
Dermatomyositis	Perifasicular atrophy, perivascular inflammation on biopsy Elevated creatine kinase, aldolase (up to 2–3 ULN) Irritable myopathy on EMG Muscle edema and fasciits on MRI	Diffuse or limited scleroderma; may have clear overlap skin findings of dermatomyositis (Gottrons, heliotrope)	Same as above
Immune-mediated necrotizing myopathy	Predominant necrosis with paucity of inflammatory cells Elevated creatine kinase and aldolase (up to five times ULN) Irritable myopathy on EMG Prominent muscle edema on muscle MRI	Diffuse or limited scleroderma; patients can develop early fatty replacement on muscle MRI	Same as above
Fibrosing myopathy	Predominant muscle fibrosis without necrosis or inflammation Mildly elevated creatine kinase or normal creatine kinase/aldolase Nonirritable myopathy Fasciitis > intramuscular edema on MRI	Diffuse>>limited High risk for cardiac involvement with myocardial fibrosis	Unknown, given rapid clinical deterioration would consider treatment with immunosuppression such as MMF with or without IVIG

EMG, electromyography; IVIG, intravenous immunoglobulin; MMF, mycophenalate mofetil; SSc, systemic sclerosis; ULN, upper limit of normal.

histologic subtype of muscle disease in scleroderma with a distinct clinical phenotype.

The second study focused on the histopathologic features of scleroderma muscle biopsies in 35 patients at the University of Siena in Italy [16▪▪]. This study was a comprehensive pathologic study to determine the individual histopathologic features that were unique to scleroderma muscle tissue. Thirty-five patients underwent a vastus lateralis biopsy if they presented with clinical or EMG features of muscle involvement. In addition to routine staining, vascular involvement was assessed by endothelial markers (cluster of differentiation 31, CD31), pro-angiogenic vascular endothelial growth factor A (VEGF-A). Muscle fibrosis was assessed by the analysis of type I collagen (Coll-I) and transforming growth factor B (TGF-B). Furthermore, all histopathologic features were compared with those who either had idiopathic inflammatory myopathies (IIM) or noninflammatory myopathies (NIM).

Similar to the first study, the results demonstrated that the histology of the 35 patients showed myopathic features with multifocal endomysial fibrosis without prominent necrosis or regeneration. When comparing individual histologic features, fibrosis (81%) was much higher compared with IIM (32%, $P < 0.05$) or NIM (18%. $P < 0.05$). In particular, Coll-I and TGF-B revealed strong expression in activated endomysial and perimysial myofibroblasts of the SSc myopathy group. Quantitative analysis demonstrated that Coll-I and TGF-B are statistically higher in the SSc-myopathy group compared with IIM ($P < 0.05$, $P < 0.01$) and NIM ($P < 0.01$, $P < 0.01$) groups.

Vascular involvement, represented by VEGF-A, was also statistically much higher in scleroderma myopathy when compared with the IIM or NIM. Membranolytic attack complex (MAC) was increased compared with IIM, and there was consistent upregulation of MHC-I without any relevant infiltrates or myonecrosis. Lastly, electron microscope also demonstrated that vascular alterations consisting of thickening and lamination of the basement membrane was coupled to endomysial and perimysial fibrosis.

Overall, this study had a wealth of histopathologic data including ultrastructural analysis from electron microscopy, and these patients had a muscle biopsy at the onset of muscle involvement and early in their scleroderma disease process (approximately 4 ± 3 years). The importance of these patients having a muscle biopsy early in the

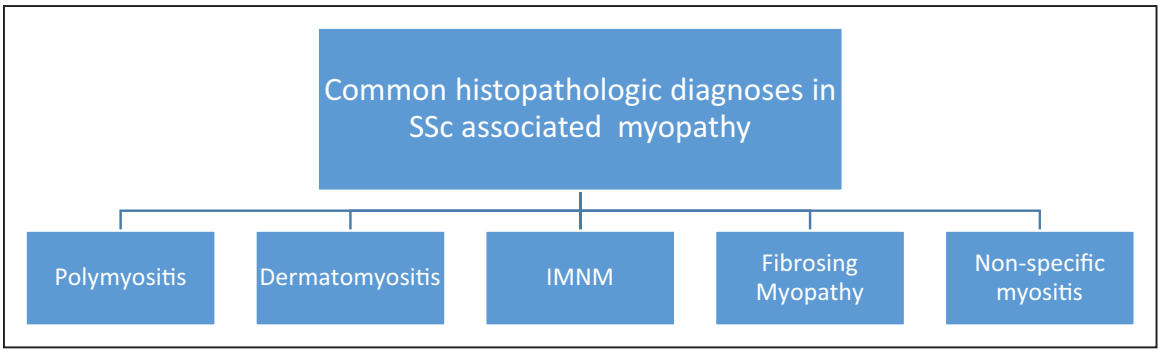

FIGURE 1. Common histopathologic diagnoses in scleroderma-associated myopathy. Illustration of the most commonly reported hisopathologic diagnoses to date in scleroderma-associated myopathy. IMNM, immune-mediated necrotizing myopathy.

onset of muscle involvement is key because similar to the first study (scleroderma disease duration approximately 4 ± 3 years), it is capturing the biological profile of these patients when the muscle disease is clinically relevant and not at the end stage of their scleroderma disease process or at autopsy. Both these studies are highlighting that fibrosis can indeed occur early in the muscle disease of scleroderma without necrosis or inflammation and that histology may be an important distinguishing method of deciding on specific targeted therapies. Until there are other noninvasive methods to distinguish fibrosis from inflammation in scleroderma muscle disease, histology will be an important guide in the treatment of muscle disease. For example, if there is prominent necrosis or inflammation diagnostic of a polymyositis or an immune-mediated necrotizing myopathy, corticosteroids (lowest possible dose) would have to be considered even in patients with scleroderma. It is to be determined if the classification of scleroderma muscle disease by histopathology may be predictive of outcomes, but it is certainly apparent that there are clear histopathologic subsets of myopathy in scleroderma (see paradigm of scleroderma muscle disease, Fig. 1).

PM-SCL AUTOANTIBODY: AN UPDATE IN THE CLINICAL PHENOTYPE OF PATIENTS WITH MYOSITIS AND OVERLAP SCLERODERMA FEATURES

In the context of scleroderma, autoantibodies frequently characterize distinct phenotypic subsets of disease. It is well known that in those patients with scleroderma, anti-PM-Scl antibody when compared with those who are seronegative, tended to have a significantly higher survival rates [17]. However, an in-depth investigation to the myopathic phenotype of anti-PM-Scl-positive patients in myositis has not been conducted. The objective of this study was to define the clinical features of myositis patients with anti-PM/Scl 75 and/or anti-PM-Scl 100 autoantibodies at disease onset and during the course of disease and compare them to patients with other forms of myositis. In this longitudinal cohort study, 41 anti-PM-Scl-positive patients were compared with 132 patients with antisynthetase syndrome, 178 patients with dermatomyositis, and 135 patients with immune-mediated necrotizing myopathy. Although muscle weakness was a presenting feature in just 37% of anti-PM-Scl-positive patients, 93% eventually developed weakness. Unlike other groups, anti-PM-Scl-positive patients had more severe weakness in arm abductors than hip flexors. No differences were found between patients with only anti-PM-Scl 100 or only anti-PM-Scl 75 autoantibodies.

Not surprisingly, compared with the other groups, anti-PM-Scl-positive patients were more likely to have extramuscular manifestations including Raynaud's phenomenon, sclerodactyly, telangiectasias, gastroesophageal reflux disease, puffy hands, and calcinosis. However, only 30% met the 2013 American College of Rheumatology/European League Against Rheumatism classification criteria for systemic sclerosis [18].

Overall, this study demonstrated that anti-PM-Scl-positive patients with myositis had a distinct clinical phenotype characterized by a unique pattern of weakness in which arm abductors were weaker than hip flexors. Furthermore, similar to antisynthetase syndrome and dermatomyositis patients, patients with anti-PM-Scl autoantibodies shared certain features such as frequent heliotrope rash and Gottrons papules, ILD and mechanics hands. Therefore, when evaluating a patient with myositis with Raynaud's, mechanics hands, or ILD, testing for anti-PM-Scl antibody should be considered in addition to antisynthetases. Conversely, when evaluating a patient with scleroderma with PM-Scl positivity, particular attention should be paid to the pattern of weakness and the development of ILD.

CONCLUSION

Muscle involvement in scleroderma is heterogeneous, but new studies are emerging that demonstrate that fibrosis can be an early not end-stage marker of muscle involvement in scleroderma. A subset of patients with predominant fibrosis also have a distinct clinical phenotype and may portend a more severe prognosis with cardiac death. More than ever, the importance of early detection of muscle disease in scleroderma is crucial in order to prevent poor outcomes. Although there have been no newly discovered autoantibodies in scleroderma muscle disease, anti-PM-Scl antibody continues to be an important autoantibody at the intersection of scleroderma and myositis with a distinct pattern of weakness. Future classification schema either by histology alone or a combination of muscle features needs to be developed in order to properly study and understand this previously underappreciated manifestation of scleroderma.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Medsger TA, Rodnan GP, Moossy J, Vester JW. Skeletal muscle involvement in progressive systemic sclerosis (scleroderma). Arthritis Rheum 1968; 11:554–568.
2. Clements PJ, Furst DE, Campion DS, *et al.* Muscle disease in progressive systemic sclerosis: diagnostic and therapeutic considerations. Arthritis Rheum 1978; 21:62–71.
3. Ranque B, Authier F-J, Le-Guern V, *et al.* A descriptive and prognostic study of systemic sclerosis-associated myopathies. Ann Rheum Dis 2009; 68:1474–1477.
4. Averbuch-Heller L, Steiner I, Abramsky O. Neurologic manifestations of progressive systemic sclerosis. Arch Neurol 1992; 49:1292–1295.
5. Paik JJ, Wigley FM, Mejia AF, Hummers LK. Severity of muscle weakness independently associates with disability as measured by the Health Assessment Questionnaire-Disability Index (HAQ-DI) in scleroderma. Arthritis Care Res (Hoboken) 2016; 68:1695–1703.
6. Muangchan C, Canadian Scleroderma Research Group. Baron M, Pope J, *et al.* The 15% rule in scleroderma: the frequency of severe organ complications in systemic sclerosis. A systematic review. J Rheumatol 2013; 40:1545–1556.
7. Bhansing KJ, van Riel PLCM, van Engelen BG, *et al.* Patients with Systemic Sclerosis/polymyositis Overlap Have a Worse Survival Rate Than Patients Without It. J Rheumatol 2016; 43:1838–1843.
8. Jung M, Bonner A, Hudson M, *et al.* on behalf of the Canadian Scleroder. Myopathy is a poor prognostic feature in systemic sclerosis: results from the Canadian Scleroderma Research Group (CSRG) cohort. Scand J Rheumatol 2014; 43:217–220.
9. Paik JJ, Wigley FM, Lloyd TE, *et al.* Spectrum of muscle histopathologic findings in forty-two scleroderma patients with weakness. Arthritis Care Res (Hoboken) 2015; 67:1416–1425.
10. Bhansing KJ, Lammens M, Knaapen HKA, *et al.* Scleroderma-polymyositis overlap syndrome versus idiopathic polymyositis and systemic sclerosis: a descriptive study on clinical features and myopathology. Arthritis Res Ther 2014; 16:R111.
11. Aguila LA, Lopes MRU, Pretti FZ, *et al.* Clinical and laboratory features of overlap syndromes of idiopathic inflammatory myopathies associated with systemic lupus erythematosus, systemic sclerosis, or rheumatoid arthritis. Clin Rheumatol 2014; 33:1093–1098.
12. Pakozdi A, Nihtyanova S, Moinzadeh P, *et al.* Clinical and serological hallmarks of systemic sclerosis overlap syndromes. J Rheumatol 2011; 38:2406–2409.
13. Troyanov Y, Targoff IN, Tremblay J-L, *et al.* Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine (Baltimore) 2005; 84:231–249.
14. ■ Paik JJ, Wigley FM, Shah AA, *et al.* Association of fibrosing myopathy in systemic sclerosis and higher mortality. Arthritis Care Res 2017; 69: 1764–1770.

This study describes a distinct clinical phenotype of scleroderma patients with a fibrosing myopathy and highlights the high-risk association with cardiac disease.

15. Hoogendijk JE, Amato AA, Lecky BR, *et al.* 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10-12 October 2003, Naarden, The Netherlands. Neuromuscul Disord NMD 2004; 14:337–345.
16. ■■ Corallo C, Cutolo M, Volpi N, *et al.* Histopathological findings in systemic sclerosis-related myopathy: fibrosis and microangiopathy with lack of cellular inflammation. Ther Adv Musculoskelet Dis 2017; 9:3–10.

This study highlights that fibrosis is the predominant histopathologic feature with microangiopathy using quantitative analysis on immunohistochemistry and electron microscopy.

17. Koschik RW, Fertig N, Lucas MR, *et al.* Anti-PM-Scl antibody in patients with systemic sclerosis. Clin Exp Rheumatol 2012; 30:S12–S16.
18. van den Hoogen F, Khanna D, Fransen J. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2013; 65: 2737–2747.

Insights into myofibroblasts and their activation in scleroderma: opportunities for therapy?

Dafni A. Gyftaki-Venieri, David J. Abraham, and Markella Ponticos

Purpose of review
The persistence of myofibroblasts is a key feature of fibrosis and in fibrotic diseases including scleroderma. This review evaluates the emerging concepts of the origins and cell populations that contribute to myofibroblasts and the molecular mechanisms that govern phenotypic conversion and that highlight opportunities for new interventional treatments in scleroderma.

Recent findings
Studies have defined heterogeneity in fibroblast-like cells that can develop into myofibroblast in normal wound healing, scarring and fibrosis. Characterizing these distinct cell populations and their behaviour has been a key focus. In addition, the overarching impact of epigenetic regulation of genes associated with inflammatory responses, cell signalling and cell communication and the extracellular matrix (ECM) has provided important insights into the formation of myofibroblast and their function. Important new studies include investigations into the relationship between inflammation and myofibroblast production and further evidence has been gathered that reveal the importance of ECM microenvironment, biomechanical sensing and mechanotransduction.

Summary
This review highlights our current understanding and outlines the increasing complexity of the biological processes that leads to the appearance of the myofibroblast in normal functions and in diseased tissues. We also focus on areas of special interest in particular, studies that have therapeutic potential in fibrosis and scleroderma.

Keywords
epigenetic, fibroblast heterogeneity, fibrosis, myofibroblasts, scleroderma

INTRODUCTION

The myofibroblast is an activated mesenchymal cell-type associated with normal tissue repair as well as scarring and fibrosis and is characterized by collagen secretion and the expression of α-smooth muscle actin (αSMA). αSMA form prominent filament bundles known as stress fibres, which reinforce the cell cytoskeleton and promote contractile force generation [1].

Persistent activation to myofibroblast is one of the key aspects of pathological fibrosis that distinguishes it from controlled wound healing during tissue repair after acute injury. The persistent response of myofibroblasts to soluble factors, abnormal extracellular matrix (ECM) cues and dysregulated cellular communication, can lead to further matrix deposition and fibrosis [2▪▪]. Many have argued that inhibiting myofibroblast formation may represent an effective way to attenuate the fibrotic process or even halt or reverse established fibrosis. Therefore, targeting the formation, function and survival of myofibroblast has been the objective of many studies in fibrotic diseases such as scleroderma [3▪]. Scleroderma is an inflammatory, autoimmune, fibrotic connective tissue disease that has long been associated with persistent activation of fibroblasts and other progenitor cell types towards the myofibroblasts phenotype via the action of transforming growth factor β (TGFβ) and excessive deposition of ECM such as collagen and fibronectin [4].

A complex biological network of processes and factors is known to affect myofibroblast formation

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KEY POINTS

- Recent studies strongly suggest that the origins of myofibroblast may reside in diverse cell populations and progenitors cell types.
- Resident dermal fibroblasts are highly heterogeneous cell populations with distinct development origins and exhibiting different intrinsic transcriptional programmes and functional properties in tissue maintenance, repair and fibrosis.
- Exciting data highlight the role of several epigenetic regulatory pathways in governing the extent, tempo and severity of tissue fibrosis.
- ECM stiffness and biomechanical cues are critical drivers and regulators of fibrosis and there are specific cell signalling pathways that are involved in sensing the extracellular microenvironment.
- New studies focus on distinct, selective and innovative treatment modalities for fibrosis centred around evidence-based pathobiology targeting aspects of inflammation, cell signalling pathways, epigenetics regulators/modulators and critical interactions with the stromal environment.

and myofibroblast persistence within tissues (Fig. 1). In the past, the best described pathways to myofibroblast production were centred on activation by TGFβ and/or platelet-derived growth factor (PDGF). In recent years, it has emerged that other processes, such epigenetic regulation, inflammation, inter and intracellular communication/miscommunication and alteration in biomechanics and the ECM, are critical in normal and disease-associated myofibroblast differentiation. These factors form intricate and elaborate pathways that underpin regulatory control. Epigenetics influences act on the ECM and the altered stiffness and associated forces it presents, is exerted on resident fibroblasts, as well as on inflammatory signals and their effect on fibroblast signalling and cellular functions such as differentiation, proliferation, migration and survival. An additional facet of an already complex picture is the identification of fibroblast heterogeneity. Resident fibroblast subpopulations with varying functionality are likely to have a profound influence on the morphological and functional heterogeneity of myofibroblasts in both normal and diseased tissues. In this review, we highlight some of the more recent findings and new concepts under investigation, as

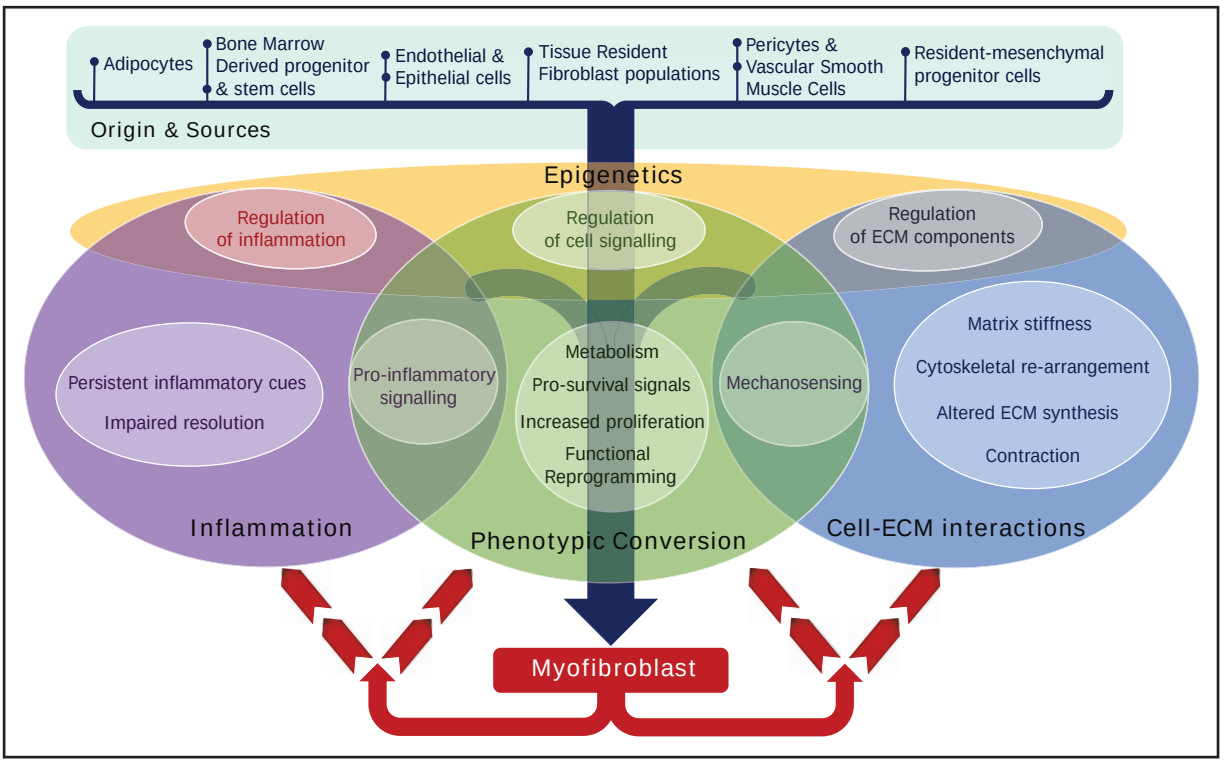

FIGURE 1. Myofibroblast activation. Key factors affecting the establishment of myofibroblasts include the origin of subpopulations within tissues, epigenetic regulation of critical cellular processes involved in tissue remodelling such as inflammatory responses and cell interactions with ECM and its biomechanical properties. Myofibroblasts modify the ECM and can themselves induce alterations in these processes, which result in persistent and sustained activation (red arrows).

well as discuss the opportunities for novel therapies that arise from these studies.

FIBROBLAST ORIGINS AND HETEROGENEITY

The notion of fibroblast heterogeneity and the presence of subpopulation in tissues is not new. Fibroblast diversity has preoccupied researchers of wound healing and various fibrotic diseases from as early as the 1980s where work by Bayreuther *et al.* [5] identified 11 fibroblast-like cell types in human skin. Advances in cell phenotyping and genetic markers for lineage tracking has greater increased our appreciation of fibroblast subsets. In addition, in order to understand the fibroblast/myofibroblast relationship and functionality, researchers have looked to the origins of these cells in tissues [6]. Potential sources of myofibroblast cell types include the resident tissue fibroblasts, epithelial cells, bone marrow derived progenitor stem cells, endothelial cells, pericytes, smooth muscle cells, resident mesenchymal progenitors and adipocytes [1,7,8].

In 2013, Driskell *et al.* [9] presented a comprehensive mouse skin study, which supported the idea that resident tissue fibroblasts may be a heterogeneous group of cells with specialized functions that regulate the ECM and coordinate neighbouring cell function. These authors demonstrated that spatial distribution in the upper and lower dermis correlates with distinct lineages. Elegant cell tracking studies using the developmental homeobox transcription factor engrailed 1 also identified an intrinsic fibroblast lineage, exhibiting surface expression of CD26 (DPP4; dipeptidyl peptidase-4), and responsible for healing and fibrosis, when explored in in-vivo models [10]. There is now good evidence suggesting that DPP4 (CD26) can regulate inflammatory responses, defines a pro-fibrotic fibroblast population and suggests that DPP4 (CD26) inhibition may be a potential treatment of fibrosis in the skin and other organs [11].

Recent studies have also identified distinct human fibroblast lineages and a variety of subpopulations that may result in profibrotic phenotypes. Using single-cell RNA sequencing on healthy skin fibroblasts, Tabib *et al.* [12▪] identified two major fibroblast populations defined by expression of SFRP2/DPP4 (CD26) and FMO1/LSP1 and five minor populations with different functional roles in inflammation and regulation of ECM deposition. A different study exploring human dermal fibroblasts, which identified at least four distinct subpopulations, was identified on the basis of of lin-CD90, CD39, RGS5 and DPP4 (CD26), each with distinct morphology and cellular function in terms of WNT signalling and interferon responsiveness [13].

Other recent studies have highlighted different sources of fibrosis-associated myofibroblasts. A perivascular mesenchymal stem cell-like population expressing the hedgehog transcriptional activator Gli1 is a myofibroblast source of solid organ and bone marrow fibrosis [14,15]. Inhibition of Gli1 directly or indirectly has therapeutic potential in targeting a particular profibrotic myofibroblast population in organ-based fibrosis [16].

INFLAMMATION AND THE MYOFIBROBLAST

Inflammation can trigger a range of signalling pathways, which ultimately result in tissue fibrosis. Both chronic inflammatory responses and the impairment of resolution of inflammatory cues can result in profibrotic signalling.

Interleukin-6 (IL-6) is critical in the activation of fibroblasts in scleroderma [17▪]. Taking advantage of the 'fasscinate' study in which scleroderma patients were treated with tocilizumab (an IL-6 receptor blocking antibody), Denton *et al.* [17▪] investigated the effects of IL-6 on fibroblast biology *in vivo*. They used explant dermal fibroblast cultures to demonstrate a profound effect of IL-6 on myofibroblast activation, highlighted the interactions between the TGFβ and IL-6 pathways, proposed IL-6 blockade as a way to regulate TGFβ-induced fibrotic pathways in addition to inflammatory pathways and raised the interest in IL-6 antagonism as a possible treatment for scleroderma [17▪].

Increased Toll-like receptor (TLR) activation has been demonstrated during injury and in patients with persistent tissue fibrosis. Subsequently, a range of studies have identified ways to therapeutically target TLR signalling. A novel function of the ubiquitin-editing cytosolic enzyme and major TLR negative regulator, A20, has been shown to attenuate TGFβ and lipopolysacharide-induced fibrotic responses. Thus, adiponectin, which upregulates A20 expression and reduces collagen expression, αSMA and other profibrotic gene expression by antagonising intracellular TGFβ/Smad signalling, has been proposed as a potential antifibrotic treatment [18]. TLR4 activation is also affected by the extracellular domain A (EDA) but not extracellular domain B splice variant of fibronectin, and a continuous exposure of resident fibroblasts to the EDA variant is thought to cause the persistent cutaneous fibrotic phenotype through a TLR4/TGFβ signalling cascade [19▪▪]. Similar to fibronectin, only one of the two splice variants of the glycoprotein tenascin-C has been positively correlated with scleroderma skin

biopsies and TLR4 activation after RNA-Seq [19▪▪]. Targeting specific splice variants of fibronectin and tenascin-C leads to a reduction in fibrotic responses, as T5342126, a compound that blocks association of TLR4 with its coreceptor MD2, was shown to prevent myofibroblast differentiation, collagen 1 stimulation and to reverse organ fibrosis in mice, highlighting the role of TLR4 in myofibroblast and fibrosis [20].

NLRP3 inflammasome-driven collagen production is another process that characterizes persistent skin fibrosis. IL-1 induced miRNA-155 has been shown to modulate fibrosis by upregulating NLRP3 inflammasome-driven collagen production. Potential inhibitors of IL-1 signalling (i.e. Rilonacept) have thus been proposed as potential scleroderma therapies [21].

EPIGENETIC EFFECTS ON FIBROBLAST ACTIVATION

Epigenetic regulation of several key wound healing processes and ECM components ensures tissue homeostasis [22]. However, epigenetic alterations in patients with organ fibrosis, thought to be triggered by persistent inflammation and profibrotic cytokine signalling, can promote myofibroblast phenotype and aid the initiation and establishment of fibrosis [23,24].

Various epigenetic modifications, such as DNA methylation, histone modifications and microRNAs, cause or enhance the fibrotic phenotype. Friend-leukaemia Virus Intergation 1 (FLI1) and Krüppel-like factor 5 (KLF5) are both epigenetically silenced due to their hypermethylation in scleroderma, while double heterozygous mice (FLI1+/-, KLF5+/-) spontaneously develop fibrotic hallmarks in the skin and oesophagus, such as impaired collagen fibril structure, and characteristic cytokine and chemokine expression profiles [25]. FLI1 downregulation has also been found to contribute to autoimmune response activation by activating autoimmune regulator (AIRE) expression in the thymus, connecting epigenetic modulation and the control of inflammatory processes during the fibrotic reaction [25]. Methyl-CpG-binding protein 2 (MeCP2) has been identified as an antifibrotic epigenetic regulator, as it can inhibit fibroblast transdifferentiation, migration and proliferation as well as modulate profibrotic genes such as collagen type 1 alpha 1 chain and α-SMA. Mediators such as nidogen-2, plasminogen activator urokinase and adenosine deactivase can be regulated to overexpress MeCP2 in scleroderma fibroblasts and thereby attenuate profibrotic responses [26▪]. Pharmacological or genetic inactivation of Jumonji domain-containing protein 3 (JMJD3) – a histone demethylase with an increased TGFβ-dependent expression in scleroderma – can also lead to antifibrotic effects on fibroblasts, mediated by the downregulation of the transcription factor FOS-related antigen 2 [27].

Poly(ADP-ribose) polymerase-1 (PARP-1) negatively regulates the canonical TGFβ signalling cascade in skin fibroblasts by inhibiting TGFβ/Smad3 signalling. In scleroderma, TGFβ signalling silences PARP-1 by hypermethylating its promoter region, leading to enhanced skin fibroblast activation and collagen production. The use of small molecule inhibitors for DNA methyltrasnferases has already been approved for clinical use (i.e. 5-azacytidine) and could also be applied to demethylate PARP-1 and facilitate blocking of TGFβ/Smad signalling [28].

Histone deacetylase (HDAC) inhibitors have also emerged as potential antifibrotic therapies. Scriptaid, a recently identified HDAC inhibitor, has shown antifibrotic properties through blocking TGFβ-induced fibrotic responses in fibroblasts, reducing ECM deposition, contractility and stiffness [29]. Furthermore, Bergmann *et al.* [23] have described the mitochondrial deacetylase sirtuin3 (SIRT3) as a target for fibroblast activation in scleroderma and other fibrotic diseases [30]. These authors demonstrated that SIRT3 has antifibrotic functions in skin and lung fibroblasts and these properties are impaired in scleroderma and using a novel SIRT3 agonist (hexafluoro) inhibits TGFβ- induced responses *in vitro* and *in vivo*.

CELL SIGNALLING AND MYOFIBROBLAST DIFFERENTIATION

TGFβ signalling has long been established as an important driver of both myofibroblast differentiation in fibrosis and in scleroderma. Apart from the canonical TGFβ-induced activation through SMADs and noncanonical activation involving MAPK, Rho, JNK, PI3K/AKT and Focal Adhesion Kinase (FAK) pathways, TGFβ orchestrates effects of other critical pathways such as the WNT/β-catenin and Hippo pathways [7,31] which have important roles in cell growth and survival metabolism, phenotypic modulation, epithelial-mesenchymal transition (EMT) and endo-mesenchymal transition (EndoMT).

Recently, Wei *et al.* [32] investigated the role of the nonneuronal cyclin-dependent kinase (CDK5) and its activator p35 (CDK5R1) in persistent myofibroblast production and fibrosis in scleroderma. They demonstrated that CDK5 is upregulated by TGFβ and expression is elevated in scleroderma dermal fibroblasts resulting in increased profibrotic gene expression. The specific CDK5 inhibitor

Roscovitine attenuated the myofibroblast phenotype and reduced collagen levels in scleroderma fibroblasts [32].

A new pathway involves the action of the protein tyrosine phosphatases PTP4A1, found to be overexpressed in scleroderma dermal fibroblasts, wherein PTP4A1 promotes TGFβ signalling by enhancing extracellular regulated kinase (ERK) activity and stimulating SMAD3. PTP4A1, which also interacts with and inhibits SRC kinase basal levels and activity, may be a selective therapeutic target for TGFβ-induced fibrosis [33]. Bosutinib, a third-generation Src kinase inhibitor, has also been tested *in vivo*, validating its antifibrotic effects as a result of Src/c-Abl kinase suppression and their inability to control TGFβ signalling, resulting in a dose-dependent decrease in skin fibrosis, collagen deposition, hydroxyproline levels, connective tissue growth factor (CTGF), ERK1 & 2 as well as other profibrotic genes [34].

Another novel signalling pathway was reported by Tomcik *et al.* [35] who demonstrated enhanced expression of the tribbles homologue 3 (TRB3) pseudokinase through TGFβ signalling to bind and activate Smad3 transcription factors in order to increase collagen secretion and induce a positive TGFβ/Smad3 signalling feedback loop to regulate fibroblast activation.

Aberrant metabolic processes that lead to the Warburg effect, mitochondrial dysfunction and oxidative stress, have been described in both fibrosis and scleroderma [36]. In a recent study, Goodwin *et al.* [37] demonstrated how hypoxia-induced glycolytic reprogramming promotes lung myofibroblast differentiation and suggested that targeting glycolysis may be a therapeutic alternative for patients with pulmonary fibrosis.

RESPONSES TO THE EXTRACELLULAR MATRIX

Extensive ECM deposition is another hallmark of fibrosis, which has long been thought of as just the consequence of a fibrotic state. However, one fundamental aspect of profibrotic environment is the regulation of fibroblast mechanoactivation towards the myofibroblastic phenotype, a process that is controlled largely by matrix stiffness [38]. Fibroblasts can probe, interrogate and 'sense' the ECM by interactions with their integrin-bound focal adhesion complex. Fibroblast can then integrate these mechanical cues into specific intracellular signalling pathways depending on ECM stiffness. In a stiff fibrotic environment, myofibroblasts persist, producing and crosslinking ECM components excessively, through multiple feed-forward loops and mechanotransduction pathways that promote the establishment of fibrosis [39].

Many studies aim to dissect the mechanotransduction pathways in organ fibrosis and how the ECM microarchitecture is involved, in order to identify targets to develop novel therapeutic strategies.

The HIPPO pathway appears the only identified mechanosensing pathway that negatively regulates fibrosis by blocking Yes Associated Protein (YAP) nuclear localization via its phosphorylation and degradation. Liang *et al.* [40▪] have recently shown that myofibroblast transdifferentiation occurs as a result of YAP activation in response to altered ECM stiffness, forming a feed-forward loop resulting in kidney fibrosis through the Hippo mechanosensing pathway. The use of verteporfin in mouse unilateral ureteral obstruction (UUO) models was also found to prevent UUO-induced matrix deposition and fibrosis through blocking YAP/TEAD association and consequently nuclear localization [40▪]. Other studies have found that bleomycin-induced skin fibrosis in mice was prevented with the use of dimethyl fumarate (DMF), wherein YAP/TAZ nuclear localization is blocked through the inhibitory phosphorylation of phosphatidylinositol 3 kinase/protein kinase B on the Akt/GSK3β/YAP/TAZ mechanotransduction pathway [41]. Downregulation of the Akt mechanosensing pathway has also been achieved through the use of selexipag and ACT-333679, to reduce expression and production of the ECM proteins collagen 1 and fibronectin, potentially interfering with the profibrotic myofibroblast activity in scleroderma [42].

Collagen bundle alignment is another important characteristic of the fibrotic matrix and has recently been correlated to the directed cell migration of fibroblasts towards the ECM and inhibited cell division via the upregulation of Arhgdib (Rho GDP-dissociation inhibitor 2) [43].

Notably, the use of KW6002, a selective antagonist of the Adenosine A2a receptor, which upon stimulation promotes the production of collagens 1 and 3, has been studied in the murine model of bleomycin-induced dermal fibrosis. KW6002 reduced skin thickness, myofibroblast accumulation and collagen bundle alignment, through crosstalk with the WNT/β-catenin signalling pathway [44].

Finally, stiff matrix cell stimulation has been shown to block myofibroblast apoptosis and enhance the fibrotic phenotype through the FAK/YAP/TAZ/BCLXL mechanotransduction pathway that affects the expression of the BCL-XL (B-cell lymphoma-extra large) gene [45▪▪]. As a result, it was shown that apoptosis pathways can be reactivated through interrupting mechanotransduction signals using navitoclax (ABT-263), a drug that

blocks BCL-XL action leading to a reduction of fibrosis in bleomycin mice [45▪▪].

CONCLUSION

Myofibroblasts are prevalent in most organs. They are metabolically active, contractile, regulate connective tissue homeostasis and are pivotal coordinators of tissue repair. The identification of mesenchymal progenitor populations and fibroblast heterogeneity suggests that myofibroblasts may arise from diverse developmental origins. In fibrosis, critical changes in myofibroblast phenotype and function, such as matrix production, growth factor secretion and increased survival and persistence, are regulated by aspects of inflammation, epigenetic modifiers, cell signalling and ECM composition and architecture alterations. Recent studies delineating the key regulatory pathways and networks involved have revealed potential new and innovative targets for improved and effective treatment of fibrosis.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Hinz B. The role of myofibroblasts in wound healing. Curr Res Transl Med 2016; 64:171–177.
2. ▪▪ Pakshir P, Hinz B. The big five in fibrosis: macrophages, myofibroblasts, matrix, mechanics, and miscommunication. Matrix Biol 2018; 68-69: 81–93.

A comprehensive review of myofibroblast behaviour and function. This review highlights the role of the inflammatory macrophage, biochemical signals and cell-to-cell/cell-to-matrix communication important in myofibroblast behaviour in tissue repair and fibrosis.

3. ▪ Denton CP, Khanna D. Systemic sclerosis. Lancet 2017; 390:1685–1699.

A comprehensive review of the scleroderma disease entity, covering advances in understanding autoimmunity, vasculopathy and fibrosis, and treatments for specific disease complications and future developments.

4. Lafyatis R. Transforming growth factor? At the centre of systemic sclerosis. Nat Rev Rheumatol 2014; 10:706–719.
5. Bayreuther K, Rodemann HP, Hommel R, *et al.* Human skin fibroblasts in vitro differentiate along a terminal cell lineage. Proc Natl Acad Sci U S A 1988; 85:5112–5116.
6. Garret SM, Frost DB, Feghali-Bostwick C. The mighty fibroblast and its utility in scleroderma research. J scleroderma Relat Disord 2017; 2:69–134.
7. Pardali E, Sanchez-Duffhues G, Gomez-Puerto MC, Ten Dijke P. TGF-β-induced endothelial-mesenchymal transition in fibrotic diseases. Int J Mol Sci [Internet] 2017; 18:pii: E2157.
8. Marangoni RG, Korman BD, Wei J, *et al.* Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. Arthritis Rheumatol 2015; 67:1062–1073.
9. Driskell RR, Lichtenberger BM, Hoste E, *et al.* Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature 2013; 504:277–281.
10. Rinkevich Y, Walmsley GG, Hu MS, *et al.* Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science 2015; 348: aaa2151.
11. Hu MS, Longaker MT. Dipeptidyl peptidase-4, wound healing, scarring, and fibrosis. Plast Reconstr Surg 2016; 138:1026–1031.
12. ▪ Tabib T, Morse C, Wang T, *et al.* SFRP2/DPP4 and FMO1/LSP1 define major fibroblast populations in human skin. J Invest Dermatol 2018; 138:802–810.

An in-depth study using single-cell RNA Seq to delineate and study dermal fibroblast populations and provides evidence to suggest there are distinct roles in ECM production, matrix deposition, inflammation, inflammation and differentiation.

13. Philippeos C, Telerman SB, Oulès B, *et al.* Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations. J Invest Dermatol 2018; 138:811–825.
14. Kramann R, Schneider RK, DiRocco DP, *et al.* Perivascular Gli1^{+} progenitors are key contributors to injury-induced organ fibrosis. Cell Stem Cell 2015; 16:51–66.
15. Schneider RK, Mullally A, Dugourd A, *et al.* Gli1 + mesenchymal stromal cells are a key driver of bone marrow fibrosis and an important cellular therapeutic target. Cell Stem Cell 2017; 20:785–800.e8.
16. Kramann R, Schneider RK. The identification of fibrosis-driving myofibroblast precursors reveals new therapeutic avenues in myelofibrosis. Blood 2018; 131:2111–2119.
17. ▪ Denton CP, Ong VH, Xu S, *et al.* Therapeutic interleukin-6 blockade reverses transforming growth factor-beta pathway activation in dermal fibroblasts: insights from the fasscinate clinical trial in systemic sclerosis. Ann Rheum Dis 2018. [Epub ahead of print]

Investigation of the effects of IL-6 and IL-6 antagonism (IL-6 receptor antibody; tocilizumab) on fibroblast biology *in vivo* as part of the 'fasscinate' study in which scleroderma patients were treated with tocilizumab. On the basis of these data, a phase 3 trial of tocilizumab is under way.

18. Bhattacharyya S, Wang W, Graham LVD, Varga J. A20 suppresses canonical Smad-dependent fibroblast activation: novel function for an endogenous inflammatory modulator. Arthritis Res Ther 2016; 18:216.
19. ▪▪ Bhattacharyya S, Midwood KS, Yin H, Varga J. Toll-like receptor-4 signaling drives persistent fibroblast activation and prevents fibrosis resolution in scleroderma. Adv Wound Care 2017; 6:356–369.

An excellent article following on from previous pioneering studies (in STM/Nat Comms) exploring the role of the TLRs and endogenous matrix ligands in scleroderma and defining TLR-4 signalling as a key driver of persistent fibroblast activation (myofibroblasts) and a failure to resolve chronic fibrosis in scleroderma.

20. Bhattacharyya S, Tamaki Z, Wang W, *et al.* Fibronectin EDA promotes chronic cutaneous fibrosis through toll-like receptor signaling. Sci Transl Med 2014; 6:232ra50.
21. Artlett CM, Sassi-Gaha S, Hope JL, *et al.* Mir-155 is overexpressed in systemic sclerosis fibroblasts and is required for NLRP3 inflammasome-mediated collagen synthesis during fibrosis. Arthritis Res Ther 2017; 19:144.
22. Piperigkou Z, Götte M, Theocharis AD, Karamanos NK. Insights into the key roles of epigenetics in matrix macromolecules-associated wound healing. Adv Drug Deliv Rev 2018; 129:16–36.
23. Bergmann C, Distler JH. Epigenetic factors as drivers of fibrosis in systemic sclerosis. Epigenomics [Internet] 2017; 9:463–477.
24. Yang J, Tian B, Brasier AR. Targeting chromatin remodeling in inflammation and fibrosis. Adv Protein Chem Struct Biol 2017; 107:1–36.
25. Takahashi T, Asano Y, Sugawara K, *et al.* Epithelial Fli1 deficiency drives systemic autoimmunity and fibrosis: possible roles in scleroderma. J Exp Med 2017; 214:1129–1151.
26. ▪ He Y, Tsou P-S, Khanna D, Sawalha AH. Methyl-CpG-binding protein 2 mediates antifibrotic effects in scleroderma fibroblasts. Ann Rheum Dis 2018; 77:1208–1218.

An informative article reporting evidence of epigenetic alteration in scleroderma and the dysregulation of histone demethylase modulating fibroblast activation by regulating the levels of histone methylation on transcription factor promoters. This study highlights modulation of histone demethylase as a potentially antifibrotic approach in scleroderma.

27. Bergmann C, Brandt A, Merlevede B, *et al.* The histone demethylase Jumonji domain-containing protein 3 (JMJD3) regulates fibroblast activation in systemic sclerosis. Ann Rheum Dis 2018; 77:150–158.
28. Zhang Y, Pötter S, Chen C-W, *et al.* Poly(ADP-ribose) polymerase-1 regulates fibroblast activation in systemic sclerosis. Ann Rheum Dis 2018; 77: 744–751.

29. Kim DJ, Dunleavey JM, Xiao L, *et al.* Suppression of TGFβ- mediated conversion of endothelial cells and fibroblasts into cancer associated (myo)fibroblasts via HDAC inhibition. Br J Cancer 2018; 118:1359–1368.

30. Akamata K, Wei J, Bhattacharyya M, *et al.* SIRT3 is attenuated in systemic sclerosis skin and lungs, and its pharmacologic activation mitigates organ fibrosis. Oncotarget 2016; 7:69321–69336.

31. Lecarpentier Y, Schussler O, Claes V, Vallée A. The myofibroblast: TGFβ-1, a conductor which plays a key role in fibrosis by regulating the balance between PPARγ and the canonical WNT pathway. Nucl Recept Res 2017; 4:101299.

32. Wei J, Marangoni RG, Fang F, *et al.* The nonneuronal cyclin- dependent kinase 5 is a fibrotic mediator potentially implicated in systemic sclerosis and a novel therapeutic target. Oncotarget 2018; 9:10294–10306.

33. Sacchetti C, Bai Y, Stanford SM, *et al.* PTP4A1 promotes TGFβ signaling and fibrosis in systemic sclerosis. Nat Commun 2017; 8:1060.

34. Wermuth PJ, Jimenez SA. Abrogation of transforming growth factor-β-induced tissue fibrosis in TBRIcaCol1a2Cre transgenic mice by the second generation tyrosine kinase inhibitor SKI-606 (Bosutinib). PLoS One 2018; 13:e0196559.

35. Tomcik M, Palumbo-Zerr K, Zerr P, *et al.* Tribbles homologue 3 stimulates canonical TGF-β signalling to regulate fibroblast activation and tissue fibrosis. Ann Rheum Dis 2016; 75:609–616.

36. Murgia F, Svegliati S, Poddighe S, *et al.* Metabolomic profile of systemic sclerosis patients. Sci Rep 2018; 8:7626.

37. Goodwin J, Choi H, Hsieh M, *et al.* Targeting hypoxia- inducible factor-1α/pyruvate dehydrogenase kinase 1 axis by dichloroacetate suppresses bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2018; 58:216–231.

38. Santos A, Lagares D. Matrix stiffness: the conductor of organ fibrosis. Curr Rheumatol Rep 2018; 20:2.

39. Schulz J-N, Plomann M, Sengle G, *et al.* New developments on skin fibrosis: essential signals emanating from the extracellular matrix for the control of myofibroblasts. Matrix Biol 2018; 68-69:522–532.

40. ■ Liang M, Yu M, Xia R, *et al.* Yap/Taz deletion in Gli+ cell-derived myofibroblasts attenuates fibrosis. J Am Soc Nephrol 2017; 28:3278–3290.
This article reports studies targeting components of the 'Hippo pathway' in scleroderma using a small molecule metabolite DMF. Investigations link the influence of DMF to blockade of TGFβ signalling and translocation of YAP/TAZ, and suggest a role for DMF in treating fibrosis is scleroderma. Interestingly, DMF also modulated proteasomal degradation and enhanced the antioxidant/anti-inflammatory response via activation of Nrf2.

41. Toyama T, Looney AP, Baker BM, *et al.* Therapeutic targeting of TAZ and YAP by dimethyl fumarate in systemic sclerosis fibrosis. J Invest Dermatol 2018; 138:78–88.

42. Cutolo M, Ruaro B, Montagna P, *et al.* Effects of selexipag and its active metabolite in contrasting the profibrotic myofibroblast activity in cultured scleroderma skin fibroblasts. Arthritis Res Ther 2018; 20:77.

43. Cao L, Lafyatis R, Burkly LC. Increased dermal collagen bundle alignment in systemic sclerosis is associated with a cell migration signature and role of Arhgdib in directed fibroblast migration on aligned ECMs. PLoS One 2017; 12:e0180751.

44. Zhang J, Corciulo C, Liu H, *et al.* Adenosine A 2a receptor blockade diminishes Wnt/β-catenin signaling in a murine model of bleomycin-induced dermal fibrosis. Am J Pathol 2017; 187:1935–1944.

45. ■■ Lagares D, Santos A, Grasberger PE, *et al.* Targeted apoptosis of myofibroblasts with the BH3 mimetic ABT-263 reverses established fibrosis. Sci Transl Med 2017; 9:eaal3765.
An important study focusing on myofibroblast programmed cell death and demonstrating that inhibition of apoptosis enhances the responses of myofibroblast to ECM and highlights the role of mechanosensing in persistent myofibroblast activation.

Mortality and survival in systemic sclerosis: a review of recent literature

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Purpose of review
Systemic sclerosis is a debilitating rheumatic disease with high morbidity and mortality. This review attempts to provide the most recent update on mortality and survival and their determinants in systemic sclerosis (SSc).

Recent findings
SSc remains an uncommon rheumatic disease with high mortality. There have been attempts to devise more comprehensive but simpler scoring systems to prognosticate survival in SSc, which will influence triaging of patients and guide the utilization of aggressive treatment strategies.

Summary
Updated literature review on mortality and survival in SSc has confirmed its high-case fatality but a slowly improving survival profile over time. It identifies some gaps in knowledge, especially in regards to ethnic differences.

Keywords
mortality, review, scleroderma, survival, systemic sclerosis

INTRODUCTION

Systemic sclerosis (SSc) is a rare chronic rheumatic autoimmune disorder with high mortality and reduced survival [1]. Although survival studies have shown improvement, by far, it carries the highest case-based morbidity and mortality among all rheumatic diseases [2], more specifically in patients with diffuse cutaneous disease. Progress in the development of disease-modifying agents and interventions has been slow [2–5]. Rapid progression, involvement of visceral organs and delayed detection have been major drivers of mortality and reduced survival. In this review, we summarize the latest evidence on mortality and survival of SSc, highlight novel predictors and determinants of the same. We also discuss prognosticating systems in this patient group.

HISTORICAL AND CHANGING PERSPECTIVES OF SYSTEMIC SCLEROSIS MORTALITY

Older studies have demonstrated a persistently high mortality rate among patients with SSc, between 1.05-fold and 7.2-fold [6,7]. In a recent meta-analysis by Rubio-Rivas *et al.* [8] in 2014, they found an overall standardized mortality ratio (SMR) of 2.72, ranging from 1.05 to 5.40 across studies. Even in more recent years, studies across the globe have consistently reported a higher SMR (range 1.39–5.10) [9▪▪,10▪▪,11], and this is a reflection of the complexity of SSc as well as the lack of a disease-modifying agent.

Renal crisis was a leading cause of death in the preangiotensin-converting enzyme (ACE) inhibitor era (before 1980s), which has since shifted to cardiopulmonary complications [pulmonary arterial hypertension (PAH), interstitial lung disease (ILD), pericardial and myocardial disease] [12–15]. More recently infections/septicemia and malignancies have been reported as leading non-SSc-related causes of deaths [9▪▪,16,17▪,35].

TREND IN MORTALITY AND SURVIVAL RATES

Trend of mortality can give us a better idea of how changes in preventive care, disease modification

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KEY POINTS

- Systemic sclerosis patients continue to have significantly higher morbidity and mortality, and are almost two to five times more likely to die compared with the general population.
- Systemic sclerosis, especially the diffuse cutaneous form of the disease is most aggressive during the initial years of its natural course and needs closer monitoring to prevent progression and early detection of organ complications.
- Although cardiopulmonary complications remain the most common, the burden of infections and malignancies seem to be increasing as more aggressive immunosuppressive treatment is being utilized.

and better defined guidelines for SSc may have impacted mortality rates. Elhai *et al.* analyzed death certificates of a large group of 2719 SSc patients in France (2000–2011). They found a gradual decline in mortality rate from 1.03 per 100 000 patients per year in 2000 to 0.6 in 2011 [9▪▪]. On a multiple-cause-of-death analysis, they found a rising trend of SSc as an ultimate cause of death with a decreasing trend of SSc as an associated cause of death. This, however, may reflect an overall improvement in survival among the general population and hint to a lack of progress in disease modification in SSc.

MORTALITY AND SURVIVAL IN INCIDENT DISEASE

Studies on prevalent cohorts (disease of any duration) neglect to capture very early deaths with resultant oversampling of individuals who averted early mortality, creating a survivor bias. Studying inception cohorts (captured within a very early period from disease onset) can mitigate this bias [10▪▪]. Hao *et al.* studied 4288 patients (1070 in the inception cohort within 4 years of onset and 3218 in the prevalent cohort) from Australia, Canada and Spain, and found a consistently lower survival in the inception cohort at 1, 3, 5 and 8 years (99, 94.8, 88.9 and 81.3%), versus the prevalent cohort (99.5, 98, 96.7, and 94.6%); $P < 0.0001$). In the same study, even lower survival (95.2, 85.2, 78, and 70.8% at 1, 3, 5, and 8 years, respectively) was noted among a subgroup of the inception cohort, which was recruited within 1 year of disease onset. The SMR of the prevalent and inception cohorts were 3.39 and 4.06 ($P = 0.001$), respectively [10▪▪]. These results support the argument that SSc-related morbidity and mortality are worse in early disease (specifically in diffuse systemic sclerosis (dcSSc) patients), mandating an aggressive approach to treatment and prevention of complications in this patient group.

ORGAN-SPECIFIC MORTALITY AND SURVIVAL

Organ involvement related to SSc accounts for around 50% of mortality, and has been reported as a very frequent cause of death (43–62.1%) even in more recent studies [8,9▪▪,10▪▪,17▪].

Pulmonary

Pulmonary involvement in the form of pulmonary hypertension and interstitial lung disease (ILD) is common in SSc [9▪▪,10▪▪,18,19]. Almost all of the studies looking at pulmonary involvement have described worse prognosis and higher mortality among this group of SSc patients – hazard ratios ranging from 1.68 to 9.49 [19], with one Brazilian study [17▪] showing an exceptionally high odds of mortality [odds ratio (OR) = 138.94, $P < 0.0001$] with pulmonary hypertension (as a primary cause). Patients with both pulmonary hypertension and ILD had worse survival at 1, 5 and 10 years as compared with SSc patients with pulmonary hypertension who did not have ILD (86, 54 and 54% vs. 96, 92, and 82%, respectively) [20]. In another study from Iran involving 446 hospitalizations by 181 SSc patients, SSc-related pulmonary events (pulmonary hypertension and/or ILD) accounted for almost a third of total deaths [21]. Another study looking at a large number of hospitalized SSc patients ($n = 9731$) in the US by Poudel *et al.* [35] reported pulmonary involvement as the primary reason for hospitalization in 20% of patients who died in hospital. The odds of dying in hospital was higher with the presence of pulmonary hypertension or ILD/pulmonary fibrosis were higher, at 1.82 and 2.23, respectively ($P < 0.05$ for both) [35]. Elhai *et al.* [9▪▪] showed that young men with SSc had significantly higher (up to 10-fold) rate of death and this was related to pulmonary involvement.

Cardiac

Cardiac complications also remain common cause of death in SSc [9▪▪,11,16,21,35]. Elhai *et al.* [9▪▪] studied 1072 deaths in a multinational cohort (EUSTAR) and 2719 death certificates from France where, in both groups, cardiac causes were the most common cause of death (26.7 and 31.1%, respectively).

Renal

Scleroderma renal crisis is a fatal renal complication of SSc but has been reported to occur less frequently

in the post-ACE-inhibitor era. Renal causes ranked seventh most common cause of death in the large sample study by Elhai *et al.* [9▪▪] with a frequency of 2.9% in both the EUSTAR cohort and the French death certificate study. In the inpatient database study by Poudel *et al.* [35], it was the ninth most common primary diagnosis among SSc patients who died in hospital (1.9%) but in itself was associated with a higher odds of dying (4.31, $P < 0.0001$). This rate was significantly higher in the cohort from New Zealand (10%) [22] whereas the mortality hazard ratios of renal crisis in an inception and prevalent cohort in Hao's study were 1.87 ($P = 0.048$) and 1.22 ($P = 0.2$), respectively [10▪▪].

Gastrointestinal

Gastrointestinal involvement is the most common visceral organ manifestation in SSc. Currently gastrointestinal-related mortality has surpassed renal-related mortality, with 3.9% deaths attributed to this in the most recent analysis of the EUSTAR cohort and in 6% among an Italian cohort [9▪▪,23].

NOVEL PREDICTORS AND DETERMINANTS OF MORTALITY AND SURVIVAL IN SYSTEMIC SCLEROSIS DISEASE

With time, newer disease characteristics have been established as predictors and determinants of severity, mortality, and survival. A recent study by Poudel *et al.* [19] has reviewed these factors and listed the magnitude of their relationships in detail. In that study, increasing age, male sex, lower BMI, lower hemoglobin, lower serum protein, higher white blood cell count and erythrocyte sedimentation rate (ESR) were associated with higher mortality.

Pestaña *et al.* [24] did a 30-year follow-up study of 1625 patients to distinguish if the mode of onset (Raynaud's phenomenon vs. non-Raynaud's phenomenon) had any difference in survival and found better survival and mortality if the mode of onset was Raynaud's phenomenon. Pavan *et al.* [25] found that low-avascular scores (≤ 1.5; based on capillary loss on nailfold capillary microscopy) at initial baseline evaluation had a significantly higher survival. Goh *et al.* [26] on the other hand, found that within the first year of disease diagnosis, a decline in forced vital capacity more accurately predicted survival, whereas serial gas transfer trends was more accurate at 2 years. Gheorghiu *et al.* [27] established digital ulcer history as a strong predictor of mortality with an OR of 13.1 ($P < 0.001$) in their EUSTAR cohort from Romania.

More recently, infections have been recognized as an important non-SSc-related cause of morbidity and mortality [12,13]. Although Cruz-Domínguez *et al.* [16,17▪] attributed infection as the overall cause in 25% of deaths, the Brazilian multiple-causes-of-death study reported infections (septicemia and pneumonia) as the leading secondary cause of death in 37.6% and the primary cause among 9.9%. Elhai *et al.* found infections to be less frequent (10.5%) but with a very high observed-to-expected number of death ratio (O/E ratio) of 5.61, surpassing cardiovascular (O/E 1.39) and respiratory (O/E 2.99) causes. A cohort study in Greece ($n = 72$) by Repa *et al.* [28] identified sepsis as being responsible for 30.8% deaths. Another study looking at a large number of hospitalized SSc patients ($n = 9731$) in the United States also reported infection/septicemia as the most common primary diagnosis (32.7%) among those who had died and reported a high adjusted mortality odds (3.36, $P < 0.0001$) with infections [35].

Development or presence of malignancy is another variable being explored more recently. Hao *et al.* listed malignancy as the most common cause of non-SSc-related deaths in both of their combined inception and combined prevalent cohorts (38.2 and 37.2%, respectively). It was, however, nonpredictive of mortality in their multivariate analysis [10▪▪]. Kang *et al.* [11] also reported similar findings (18.2% mortality because of malignancy) in Korea. Neoplasia has been reported as an overall cause of death in around 10% cases [16,17▪,23]. Among hospitalized patients, it was responsible for 3.3% deaths in an Iranian study [21] and 2.7% in the United States [35].

Malnutrition is an end-stage complication of gastrointestinal involvement in SSc and difficult to detect its contribution to mortality. However, it has been found responsible among 21% of a Mexican cohort, 5% of a New Zealand cohort, and has shown a higher hazard ratio for mortality (3.0, $P = 0.02$) in an Italian cohort [16,22,23].

In addition, some of the more well described mortality and survival predictors like dcSSc, male sex also has been confirmed in more recent work [16,22,29].

DATA ON MORTALITY AND SURVIVAL IN SYSTEMIC SCLEROSIS BASED ON DEATH CERTIFICATES

Studies based on death certificates can add important data about the causes leading up to mortality. Rezende *et al.* [17▪] and Elhai *et al.* [9▪▪] studied death certificates from Rio de Janeiro, Brazil ($n = 374$) and France ($n = 2719$), respectively. Although the Elhai study focused on looking at the trend of mortality and causes of death among SSc in excess to the

general population as already described above, the Brazilian study focused on a multiple-causes-of-death approach to discover nonprimary causes of death. Such approach allowed them to examine more than 1000 nonunderlying causes directly or indirectly related to the death process, which would otherwise have been overlooked. They concluded that, in the non-Caucasian ethnic group, cardiovascular (primary heart disease), respiratory (pulmonary hypertension and ILD) and infectious causes confer increased risk of death.

ROLE OF ETHNICITY ON MORTALITY AND SURVIVAL

Non-Caucasians account for a small percentage of the studied population in most of the publications. SSc in African-Americans has higher morbidity and mortality. Moore *et al.* [30] compared a group of African-American SSc patients ($n = 203$) with non-African-Americans ($n = 199$) and found a significantly higher rate of mortality (21.2 vs. 11.1%) among African-Americans with higher unadjusted (2.04, $P = 0.007$) and adjusted (1.26, $P = 0.633$) hazard ratios. Another study by Compton *et al.* [31] reported significantly younger age at diagnosis among African-Americans (41.8 ± 13.3 vs. 48.7 ± 13.2 years, $P < 0.01$) with almost double the rate of mortality than non-African-Americans (7.2 vs. 3.9%). The GRASP (Genome Research in African American Scleroderma Patients) clinical database, a multicenter SSc cohort database containing more than 1000 African-American patients from all over the Unitede States may give us more discriminative data on this population. A recent study from this database looked at clinical and serological characteristics and confirmed that African-American SSc patients are younger, have more dcSSc and the prevalence of scleroderma renal crisis is higher compared with European/Caucasian cohorts [32].

Studies, which have focused on Asian SSc cohorts have also reported a higher proportion of dcSSc but lower mortality rates as compared with Caucasian patients, although the SMR remains high [11,29].

PROGNOSTIC SCORING SYSTEM

There are multiple prognostic scoring systems available though they are limited to an organ system or a particular sub-group of the disease. The utility of such a scoring system comes into play if it can detect the patients with poor-prognosis early enough to institute aggressive preventive and disease-modifying therapy. Elhai *et al.* studied a large cohort of patients (11 193 patients and 1072 deaths in the EUSTAR sample till May 2014) and developed a prognostic scoring system called SCOpE (SCleroderma mOrtality p Eustar) to predict survival based on demographics, disease characteristics, signs and symptoms, lab values and lung function studies (Table 1). The score varies from 0 to 32 and very discriminately predicts survival at 3 years based on four categories split as quartiles (0–4, 5–9, 10–14, ≥15). The respective survival rates for the four

Table 1. SCleroderma mOrtality p Eustar score to predict survival in systemic sclerosis

Variable	Simplified score
Age (year)	
50–65	3
>65	6
Male sex	1
Diffuse cutaneous disease	1
More than 5 years disease duration	–
Progressive digital vasculopathy[a]	–
Oesophageal or gastric disease manifestations	–
Intestinal involvement	–
Systemic hypertension	–
Scleroderma renal crisis	2
Palpitations	–
Prominent dyspnoea	3
Digital ulcers	1
Joint synovitis	–
Contracture	1
Tendon friction rub	–
Muscle weakness	1
Elevated C reactive protein	4
Elevated creatine kinase	–
Proteinuria	3
Left ventricular ejection fraction less than 50%	2
Pulmonary arterial hypertension[a,b]	–
Interstitial lung disease	1
Carbon monoxide diffusion capacity less than 60% predicted	4
Forced vital capacity less than 70% predicted	2
Disease activity score = 3	–
Antinuclear antibodies	–
Anti-Scl70 antibodies	–

Three-year survival rates are 98, 93, 80 and 53%, respectively, for score groups of 0–4, 5–9, 10–14 and ≥15.
[a]In the last month, dyspnoea was classified as prominent in presence of New York Heart Association functional class III or IV.
[b]Diagnosed at time of right heart catheterization; interstitial lung disease was considered present if visible on chest radiograph or on high-resolution computed tomography scan; disease was active if the disease activity score was at least 3.
Reproduced with permission from [9▪▪].

Table 2. SADL model and staging system to predict mortality with interstitial lung disease in systemic sclerosis

	Predictor	Points
S	Ever smoking history	
	No	0
	Yes	1
A	Age (years)	
	<55	0
	55–70	1
	>70	2
DL	D_{LCO} (% predicted)	
	>60%	0
	40–60%	3
	<40%	4
Total possible points		7

	Risk category		
	Low	Moderate	High
Points	0–3	4–5	6–7
3-year mortality	3.2%	22.2%	56.9%

SADL, ever smoking history, age, and diffusing capacity of the lung for carbon monoxide (D_{LCO}, percentage predicted).
Reproduced with permission from [34▪].

quartiles at 3 years are 98, 93, 80 and 53% [9▪▪]. This scoring system was more discriminative than the older Bryan score [33]. However, it involves predominantly Caucasian patients and has not been validated on an external cohort, limiting its generalizability. The mean disease duration at recruitment was 8 years, which again creates the potential of survival bias as explained before.

Another mortality risk prediction model by Morisset *et al.* looked at a simplified SADL Model [ever Smoking history, Age and Diffusing capacity of the Lung for carbon monoxide (% predicted)] to predict mortality in SSc-related ILD (Table 2). Derived from a University of California, San Francisco cohort ($n = 137$) and validated in an external cohort from the Mayo Clinic, Rochester, Minnesota ($n = 90$), this scoring system has good discrimination within both cohorts and performed better than an older SSc-ILD staging system by Goh *et al.* [26,34▪]. The score ranges from 0 to 7 and is classified into three categories (low 0–3; moderate 4–5, high 6–7). The 3-year mortality have been reported as 3.2, 22.2 and 56.9%, respectively, in low-risk, moderate-risk and high-risk categories.

This classification system again lacks generalizability as it also included mainly Caucasian patients and was derived from highly specialized centers using a small retrospective cohort.

CONCLUSION

Systemic sclerosis is a rare disease and continues to carry a high-case fatality rate compared with the general population, whereas organ system complications seem to vary widely based on geography and ethnicity. Although survival seems to be improving, the persistently elevated mortality rate is concerning with the lack of a universally acceptable disease-modifying agent. With slowly increasing options for organic-specific drug therapy and new immunotherapies, we have improved overall survival of SSc patients but this has led to newer challenges such as infections and malignancies. Cardiopulmonary complications still seem to be the most important aspect to focus on for future organ-specific therapies, though an Food and Drug Administration (FDA)-approved disease-modifying agent has and still is the ultimate goal of SSc research. Simpler but robust clinical prediction tools can play a central role on deciding among therapeutic options. With the striking ethnic differences in disease manifestations and natural course, a more targeted set of guidelines might be needed to manage different populations.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Nikpour M, Baron M. Mortality in systemic sclerosis: lessons learned from population-based and observational cohort studies. Curr Opin Rheumatol 2014; 26:131–137.
2. Denton CP, Khanna D. Systemic sclerosis. Lancetl 2017; 390:1685–1699.
3. Barsotti S, Bruni C, Orlandi M, *et al.* One year in review 2017: systemic sclerosis. Clin Exp Rheumatol 2017; 35(Suppl 106):3–20.
4. Nihtyanova SI, Schreiber BE, Ong VH, *et al.* Prediction of pulmonary complications and long-term survival in systemic sclerosis: pulmonary complications and survival in SSc. Arthritis Rheumatol 2014; 66: 1625–1635.
5. Jaeger VK, Wirz EG, Allanore Y, *et al.*, EUSTAR co-authors. Incidences and risk factors of organ manifestations in the early course of systemic sclerosis: a longitudinal EUSTAR Study. PloS One 2016; 11:e0163894.
6. Walsh SJ, Fenster JR. Geographical clustering of mortality from systemic sclerosis in the Southeastern United States. J Rheumatol 1997; 24: 2348–2352.
7. Ioannidis JPA, Vlachoyiannopoulos PG, Haidich A-B, *et al.* Mortality in systemic sclerosis: an international meta-analysis of individual patient data. Am J Med 2005; 118:2–10.
8. Rubio-Rivas M, Royo C, Simeón CP, *et al.* Mortality and survival in systemic sclerosis: systematic review and meta-analysis. Semin Arthritis Rheum 2014; 44:208–219.

9. Elhai M, Meune C, Boubaya M, *et al.*, EUSTAR group. Mapping and predicting ■■ mortality from systemic sclerosis. Ann Rheum Dis 2017; 76:1897–1905.
This study simultaneously studied a large multinational scleroderma cohort (EUSTAR) and large number of death certificates to elicit the trend of mortality in SSc over long period of time. This study allowed the authors to generate a discriminative prognosticating scoring system called SCOpE (SCleroderma mOrtality p Eustar).

10. Hao Y, Hudson M, Baron M, *et al.,* Canadian Scleroderma Research Group, ■■ Australian Scleroderma Interest Group. Early mortality in a multinational systemic sclerosis inception cohort. Arthritis Rheumatol Hoboken NJ 2017; 69:1067–1077.
This study utilized large multinational cohorts and compared mortality differences between incident and prevalent cohorts; the authors highlighted an excessive mortality among incident cases within first few years of diagnosis.

11. Kang GW, Jung KH, Lee YS, *et al.* Incidence, prevalence, mortality and causes of death in systemic sclerosis in Korea: a nationwide population-based study. Br J Dermatol 2018; 178:e37–e39.

12. Tyndall AJ, Bannert B, Vonk M, *et al.* Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. Ann Rheum Dis 2010; 69:1809–1815.

13. Strickland G, Pauling J, Cavill C, *et al.* Mortality in systemic sclerosis-a single centre study from the UK. Clin Rheumatol 2013; 32:1533–1539.

14. Woodworth TG, Suliman YA, Furst DE, Clements P. Scleroderma renal crisis and renal involvement in systemic sclerosis. Nat Rev Nephrol 2016; 12:678–691.

15. Rubio-Rivas M, Simeón-Aznar CP, Velasco C, *et al.,* RESCLE investigators, Autoimmune Diseases Study Group (GEAS). Changes in the pattern of death of 987 patients with systemic sclerosis from 1990 to 2009 from the nationwide Spanish Scleroderma Registry (RESCLE). Clin Exp Rheumatol 2017; 35(Suppl 106):40–47.

16. Cruz-Domínguez MP, García-Collinot G, Saavedra MA, *et al.* Malnutrition is an independent risk factor for mortality in Mexican patients with systemic sclerosis: a cohort study. Rheumatol Int 2017; 37:1101–1109.

17. de Rezende RPV, Gismondi RA, Maleh HC, *et al.* Distinct mortality profile in ■ systemic sclerosis: a death certificate study in Rio de Janeiro, Brazil (2006–2015) using a multiple causes of death analysis. Clin Rheumatol 2017. [Epub ahead of print]
This study utilized death certificates of large sample of SSc patients with a multiple-causes-of-death approach to highlight the non-SSc causes secondarily contributing to the death of SSc patients.

18. Ferri C, Valentini G, Cozzi F, *et al.,* Systemic Sclerosis Study Group of the Italian Society of Rheumatology (SIR-GSSSc). Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. Medicine (Baltimore) 2002; 81:139–153.

19. Poudel DR, Jayakumar D, Danve A, *et al.* Determinants of mortality in systemic sclerosis: a focused review. Rheumatol Int 2017. [Epub ahead of print]

20. Michelfelder M, Becker M, Riedlinger A, *et al.* Interstitial lung disease increases mortality in systemic sclerosis patients with pulmonary arterial hypertension without affecting hemodynamics and exercise capacity. Clin Rheumatol 2017; 36:381–390.

21. Shenavandeh S, Naseri R. Assessment of hospitalization and mortality of scleroderma in-patients: a thirteen-year study. Reumatologia 2017; 55:163–168.

22. Ooi C, Solanki K, Lao C, *et al.* Mortality in the Waikato Hospital Systemic Sclerosis Cohort. Int J Rheum Dis 2018; 21:253–260.

23. Codullo V, Cereda E, Klersy C, *et al.* Serum prealbumin is an independent predictor of mortality in systemic sclerosis outpatients. Rheumatol Oxf Engl 2016; 55:315–319.

24. Pestaña MF, Rivas MR, Vilella CT, *et al.* FRI0402 Onset clinical involvement as prognostic factor in systemic sclerosis (SSC). Ann Rheum Dis 2017; 76(Suppl 2):639–640.

25. Pavan TR, Bredemeier M, Hax V, *et al.* Capillary loss on nailfold capillary microscopy is associated with mortality in systemic sclerosis. Clin Rheumatol 2018; 37:475–481.

26. Goh NS, Hoyles RK, Denton CP, *et al.* Short-term pulmonary function trends are predictive of mortality in interstitial lung disease associated with systemic sclerosis. Arthritis Rheumatol 2017; 69:1670–1678.

27. Gheorghiu A, Radu A, Oneata R, *et al.* AB0608 Predictive factors for long-term survival and disease progression of systemic sclerosis – a longitudinal analysis. Ann Rheum Dis 2017; 76(Suppl 2):1264.

28. Repa A, Gergianaki I, Spyrou G, *et al.* SAT0330 lower prevalence but comparable clinical characteristics and prognosis of systemic sclerosis in crete-greece as compared to other european countries: a single center experience. Ann Rheum Dis 2017; 76(Suppl 2):896.

29. Wangkaew S, Prasertwitayakij N, Phrommintikul A, *et al.* Causes of death, survival and risk factors of mortality in Thai patients with early systemic sclerosis: inception cohort study. Rheumatol Int 2017; 37: 2087–2094.

30. Moore DF, Kramer E, Eltaraboulsi R, Steen VD. Morbidity and mortality of scleroderma in African Americans. ACR Meeting Abstracts. November 2017. Available at: http://acrabstracts.org/abstract/morbidity-and-mortality-of-scleroderma-in-african-americans/. (Accessed 6 March 2018)

31. Compton SM, Silver RM, Kamen DL. Unique characteristics of scleroderma among african americans: a population based study. ACR Meet Abstracts. November 2017. Available at: http://acrabstracts.org/abstract/unique-characteristics-of-scleroderma-among-african-americans-a-population-based-study/. (Accessed 6 March 2018)

32. Morgan ND, Shah AA, Mayes MD, *et al.* Clinical and serological features of systemic sclerosis in a multicenter African American cohort: analysis of the genome research in African American scleroderma patients clinical database. Medicine (Baltimore) 2017; 96:e8980.

33. Bryan C, Knight C, Black CM, Silman AJ. Prediction of five-year survival following presentation with scleroderma: development of a simple model using three disease factors at first visit. Arthritis Rheum 1999; 42: 2660–2665.

34. Morisset J, Vittinghoff E, Elicker BM, *et al.* Mortality risk prediction in scler- ■ oderma-related interstitial lung disease: the SADL model. Chest 2017; 152:999–1007.
This study developed a simplified scoring model (SADL) to predict mortality among SSc patients with interstitial lung disease.

35. Poudel DR, George M, Dhital R, *et al.* Mortality, length of stay and cost of hospitalization among patients with systemic sclerosis: results from the National Inpatient Sample. Rheumatology 2018. [Epub ahead of print]

T cells and cytokines in systemic sclerosis

Patrizia Fuschiotti

Purpose of review
Dysregulation of both the innate and the adaptive immune systems has been identified in systemic sclerosis (SSc). However, the mechanisms underlying aberrant immune cell function remain poorly understood. T cells represent a predominant cell type in the affected tissues of patients, particularly in the early inflammatory stage of the disease. Antigen specificity of infiltrating T cells has not been identified; however, recent studies implicate specific T-cell subsets and the cytokines they produce in SSc pathogenesis by modulating the development of autoimmunity, inflammation and fibrosis.

Recent findings
The phenotype and function of distinct T-cell subsets have been identified in the affected tissues of SSc patients as well as in SSc animal models, implying their contribution to disease process. The molecular mechanisms underlying cytokine dysregulation by specific T-cell subpopulations are also becoming clear.

Summary
A better understanding of SSc pathogenesis will allow the development of novel therapeutic strategies targeting specific cell types and the pathways that are abnormally activated as well as the cytokines produced that may be directly involved with disease process. A further goal is to tailor therapy to address dysregulation specific to individual patients, leading to better efficacy and reduced toxicity.

Keywords
autoimmunity, cytokines, fibrosis, systemic sclerosis, T cells

INTRODUCTION

Aberrant innate and adaptive immune responses have long been identified in systemic sclerosis (SSc) [1,2]. However, still unclear is whether activation of immune effector pathways drives the disease process or is instead a response to vasculopathy and fibrosis, which are the main characteristic features of SSc. T cells are known to play a critical role in SSc pathogenesis and infiltrate the affected tissue even before vasculopathy and endothelial cell damage are detected [3]. In-situ hybridization studies show that infiltrating T cells and macrophages are adjacent to myofibroblasts, providing a link between immune cells and fibrosis [4–6]. T lymphocytes isolated from the blood or fibrotic skin of SSc patients exhibit an oligoclonal repertoire suggesting an antigen-specific immune response [7,8]. Although T-cell specificity is still unknown, a breakdown in self-tolerance is believed to induce T-cell activation and secretion of proinflammatory and profibrotic cytokines, which contribute to vasculopathy and excessive collagen synthesis [1,2,9]. Indeed, the blood and affected skin of patients with active disease show increased numbers of $CD4^+$ and $CD8^+$ T cells producing profibrotic type-2 cytokines such as interleukin (IL)-4 and IL-13 as well as of cells producing IL-17-family cytokines, driving inflammatory responses involving fibroblasts, endothelial and epithelial cells [1,2,9]. A deficient or redirected function of T regulatory cells (Tregs) also appears to contribute to altered immune homeostasis and fibrosis in SSc. Thus, a better understanding of the immunological mechanisms underlying disease processes will lead to novel and targeted therapeutic approaches in SSc. The following paragraphs review recent literature on the effector functions and molecular mechanisms underlying cytokine production in selective T-cell subsets that were shown to play a critical role in the pathogenesis of SSc.

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KEY POINTS

- T cells and the cytokines they produce are key players in the pathogenesis of SSc.
- An imbalance in Treg subsets has been found in severe forms of SSc.
- Expression of checkpoint inhibitor receptors by T-cell subsets in SSc modulates cytokine production and their cross-talk with fibroblasts at sites of inflammation and/or fibrosis.
- Specific effector T-cell subsets have been identified in the affected tissues of patients and in animal models that likely contribute to disease inflammatory and fibrotic processes.
- Abnormal cytokine production in SSc is effected by cell signaling, the local microenvironment and epigenetic modifications.

REGULATION OF T-CELL RESPONSES IN SYSTEMIC SCLEROSIS

Abnormalities in Tregs are implicated in the pathogenesis of SSc, although no consensus has been reached on their frequency and function [10–12]. Recent work has examined the phenotype and function of SSc Treg subpopulations in relation to specific clinical manifestations and autoantibody expression as well as the modulation of SSc T-cell responses by checkpoint receptor inhibitors.

Ugor *et al.* [13] showed an increase in the proportion of total Tregs ($CD4^+CD25^+Foxp3^+CD127^-$) [11,14] in the peripheral blood of SSc patients, particularly in the most severe forms of the disease, characterized by Scl-70 and RNA-Pol-III autoantibody positivity and pulmonary fibrosis. Apart from total Tregs, an increase was also measured for $CD62L^+$ Tregs, which include natural Tregs and central memory Tregs [15▪▪], both recirculating into the lymphatic organs and suppressing the activation of naive T cells. In addition, the authors identified defects in suppressive cytokine production by the different Treg subsets, with reduced transforming growth factor beta (TGF-β) production by $CD62L^+$ Tregs and decreased IL-10 production by total Tregs, indicating that the effector memory CD62L– Treg subset (induced Tregs) [15▪▪,16] is defective in IL-10 production. Importantly, this subset was shown to inhibit inflammatory responses in the skin and mucosa, thereby accounting for the imbalanced immune homeostasis in SSc. Finally, analysis of epigenetically regulated methylation of the Foxp3 promoter and enhancer regions [17] in SSc Tregs revealed reduced methylation of Treg-specific Foxp3 enhancer regions, consistent with the elevated $CD62L^+$ Treg proportions observed in dcSSc and the unbalanced distribution of Treg subsets in patients.

The mechanisms underlying the functional changes in IL-10 and TGF-β production by SSc $FoxP3^+$ Treg cells are undefined, although one hypothesis is that functional exhaustion occurred [18,19]. In support, Fleury *et al.* [12] showed that peripheral blood Tregs significantly upregulate expression of coinhibitory receptors (co-IRs) that are involved with T-cell exhaustion processes [20–22]. In particular, they observed an increase in expression of PD-1 and TIGIT in Tregs from SSc patients. Although intriguing, additional studies in a larger cohort of well-defined SSc patients are needed to identify the specific role of co-IR expression in the function of Treg cells. Fleury *et al.* also observed that co-IRs are not only upregulated in patient Tregs but also in other T-cell subsets and natural killer cells. They subsequently assessed the role of co-IRs in regulating cytokine production by patient and control peripheral blood mononuclear cells (PBMCs) as well as their impact on fibroblast gene expression. PD-1, TIM-3 and TIGIT antibody blockade showed significant patient-specific variation of each co-IR in modulating activation-induced T-cell cytokine production. In most individuals, however, at least one co-IR was actively suppressing cytokine production, indicating the presence of exhausted cell populations. Finally, changes in the cytokines secreted by PBMCs after TIM-3 blockade resulted in altered fibroblast gene expression. Of note, Fukasawa *et al.* [23] showed that soluble forms of co-IRs also contribute with ligand availability to modulating co-IR activity in SSc, thus adding additional complexity to the regulation of cytokine production by co-IRs in SSc lymphocytes, and to their cross-talk with fibroblasts at sites of inflammation and/or fibrosis.

EFFECTOR T-CELL SUBSETS IN SYSTEMIC SCLEROSIS

Recent studies have identified specific effector T-cell subsets in the affected tissues of patients and in animal models that likely contribute to disease inflammatory and fibrotic processes.

T follicular helper-like cells

Taylor *et al.* [24] identified a novel subset of memory T helper (T_H) cells expressing the inducible T-cell costimulator (ICOS) receptor and characterized by a T follicular helper (T_{FH})-like phenotype. These cells infiltrate the skin of SSc patients and correlate with the extent of dermal fibrosis and disease activity.

The authors also report that $ICOS^+$ T_{FH}-like cells are numerous in the skin of the graft-versus-host-disease (GVDH)-based mouse model for SSc [25]. ICOS belongs to the B7/CD28 superfamily and is expressed by activated $CD4^+$ effector T cells and mainly by T_{FH} cells, playing a critical and nonredundant role in the regulation of adaptive immune responses, particularly humoral responses [26]. The T_{FH}-specific cytokine IL-21 and its cognate receptor (IL-21R) are known to be upregulated in patients with SSc [27,28] and Taylor *et al.* now show that IL-21 contributes to dermal fibrosis via activation of an IL-21 and matrix MMP-12-dependent mechanism in dermal fibroblasts of the GVDH mouse model. Treatment of mice with an anti-ICOS or an anti-IL-21 monoclonal antibody blocked the expansion of T_{FH}-like cells and inhibited production of interferon (IFN)-γ, interleukin-6, TGF-β, fibroblast growth factor 15, vascular cell adhesion 1 and matrix metalloproteinases, leading to reduced inflammation and dermal fibrosis. These data provide a link between the IL-21/IL-21R axis and dermal fibrosis, and further implicate IL-21-producing T_{FH}-like cells in disease, suggesting that therapeutic benefit might arise from inhibition of these cells.

T helper 9 cells

Th9 cells and IL-9 are involved in the pathogenesis of several autoimmune diseases [29–31]. Although increased serum levels of IL-9 have been found in SSc [32], their role in disease process has not been investigated. Recently, Guggino *et al.* [33] reported strong expression of IL-9, IL-9R and factors promoting Th9 differentiation, such as IL-4, TGF-β and TSLP, in skin lesions of patients with limited (lcSSc) and diffuse (dcSSc) cutaneous SSc. IL-9 was produced by skin-infiltrating mononuclear cells, mainly Th9, mast cells and neutrophils. Similarly, Th9 cells were also expanded in peripheral blood and their frequency correlated directly with the extent of cutaneous fibrosis. Overexpression of IL-9 was also detected in renal biopsies of SSc patients with renal crisis. Infiltrating mononuclear cells, mast cells and neutrophils also expressed IL-9R. The authors' in-vitro studies showed that stimulation of isolated neutrophils and B cells with recombinant IL-9 significantly induced neutrophil extracellular traps (NETs) release by dying cells (NETosis) in neutrophils, expansion of mast cells and increased production of SSc-related autoantibodies by B lymphocytes. These findings suggest that Th9 cells and IL-9 are implicated in the pathogenesis of SSc. Additional studies are necessary to exploit their potential as novel therapeutic targets as well as to determine whether NETosis in SSc activates autoimmune processes as found in other autoimmune diseases [34].

Skin-resident $CD8^+$ T cells

Fuschiotti *et al.* [35] reported increased proportions of $CD8^+$ T cells producing high levels of the profibrotic cytokine IL-13 in the peripheral blood of dcSSc patients. Recently, Li *et al.* [36] identified a subset of skin-resident effector memory $CD8^+$ ($CD8_{EM}$) T cells in the affected skin of patients with early active dcSSc that was not found in healthy skin. These cells expressed high levels of IL-13 and IFNγ as well as markers of cytotoxicity. Moreover, IL-13-producing $CD8_{EM}$ T cells from SSc patients induced a profibrotic phenotype in SSc and normal dermal fibroblasts that could be inhibited by an IL-13-neutralizing antibody. Strikingly, same patient analysis showed that skin-resident SSc $CD8_{EM}$ T cells coproduce extremely high levels of IL-13 and IFNγ compared to blood counterparts, indicating their critical involvement in SSc skin disease. Further support came from genome-wide gene expression profiling of SSc skin [37–40], showing an upregulated $CD8^+$ T-cell signature that correlated with skin thickness [41]. Skin-resident memory T cells provide rapid in-situ protection against most common pathogens [42,43], but their dysregulation can contribute to autoimmune and inflammatory skin diseases [44]. The results of Li *et al.* provide new insights into SSc pathogenesis by identifying a pathogenic $CD8^+$ T-cell subset that resides in the affected skin of SSc patients in the early stage of disease and which has direct cellular cytotoxicity and profibrotic function.

Angiogenic T cells (Tang)

The mechanisms underlying endothelium damage and defective repair in SSc [45,46▪,47,48] are unclear. A functional T-cell subpopulation, termed angiogenic T cells (Tang), plays a critical role in the repair of injured endothelium [49]. Recently, Manetti *et al.* [50] found that Tang cells ($CD3^+$ $CD31^+CXCR4^+$) are selectively expanded in the circulation of SSc patients displaying severe peripheral vascular complications such as late nailfold videocapillaroscopy (NVC) patterns and digital ulcers compared to SSc patients without digital ulcer or with early/active NVC patterns, and to healthy controls. In contrast, Tang frequencies did not differ between controls and SSc patients with moderate capillary damage, suggesting that the frequencies of Tang cells may reflect the severity of peripheral vascular disease in SSc patients. Manetti *et al.* also established that the proportions of Tang cells in

patients correlated inversely with the levels of SDF-1α and $CD34^+CD133^+VEGFR\text{-}2^+$ endothelial progenitor cells (EPCs), but positively with the levels of vascular endothelial growth factor and MMP-9. As $CXCR4^+$ Tang cells home to areas of ischemia where SDF-1α is highly expressed [49], these new observations suggest that the SDF-1/CXCR4 axis might exert a major role in homing Tang cells to the affected SSc skin. The authors also suggested that inverse correlation of Tang cell proportions with those of EPC in the circulation of SSc patients represents ineffective compensation for inefficient angiogenesis and EPC function. Thus, Tang cells may represent a novel biomarker reflecting the severity of SSc-related peripheral vasculopathy that could be exploited as a novel therapeutic target, although further studies are required to clarify their function in SSc.

T-CELL CYTOKINE DYSREGULATION IN SYSTEMIC SCLEROSIS

Gene expression of multiple cytokines can be altered by cell activation, the local microenvironment and epigenetic modifications. Although changes in cytokine production by T cells in SSc tissue may contribute to disease pathogenesis [1,2,9], the underlying mechanisms are only recently being uncovered.

Epigenetic regulation of type-I interferon production by systemic sclerosis T cells

Type-I IFNs contribute to the pathogenesis of several autoimmune diseases, including SSc [51,52]. A type-I IFN-signature is present in PBMCs [53–55] and in the affected skin [40,56] of SSc patients, and numerous type-I IFN-associated genes are linked to SSc pathogenesis [57–61]. Ding *et al.* [62] performed a genome-wide DNA methylation analysis of purified $CD4^+$ and $CD8^+$ T cells from the peripheral blood of SSc patients and found a global hypomethylation of genes involved in type-I IFN signaling in both cell types. In validation experiments, they found that these methylation patterns correlated with increased expression of type-I IFN-associated genes including *IFI44L* [63], *IFITM1* [64], *EIF2AK2* [65], *MX1* [66] and *PARP9* [67], as well as with higher levels of type-I IFNα/β in patient serum. Importantly, these correlations were more pronounced in $CD4^+$ than $CD8^+$ T cells, suggesting a stronger responsiveness of $CD4^+$ T cells to IFN stimulus. This study shows that the type-I IFN pathway is dysfunctional in SSc patients at the epigenetic level, indicating that hypomethylation effecting upregulation of type I IFN–associated genes might be critical in SSc pathogenesis.

Galectin-9 expression by systemic sclerosis fibroblasts

Th2/Th17 polarized responses are important for fibrogenesis in SSc [1,68,69]. Although multiple studies have focused on understanding the role of T cell-derived cytokines in SSc cutaneous fibrosis, the effect of SSc fibroblasts on T-cell function remains unclear. Intriguingly, SSc dermal fibroblasts were shown to induce skin-localized trans-differentiation of Tregs into Th2-like cells and likely contributing to fibrosis [12]. A recent study from Saigusa *et al.* [70] supports a role for galectin-9 in fibroblast-dependent modulation of cytokine production in SSc lesional skin. Galectin-9 is a potent inducer of Th2 cytokines and a negative regulator of Th1/Th17 responses [71,72]. The authors found increased expression of galectin-9 in SSc dermal fibroblasts as well as in SSc patient serum that correlated positively with the skin score. This upregulation depended on autocrine endothelium stimulation [73] and Fli1 deficiency. Fli1 is broadly suppressed in the bulk skin and dermal fibroblasts from SSc patients [74,75]. Coculture of $Fli1^{+/-}$ dermal fibroblasts, which mimic SSc dermal fibroblasts [76,77], with $CD4^+$ T cells significantly increased the proportion of IL-4-producing $CD4^+$ T cells while suppressing IFNγ production, which was then restored by galectin-9 silencing. Furthermore, in-vivo silencing of galectin-9 suppressed dermal collagen deposition by increasing the production of skin-infiltrating $CD4^+IFN\gamma^+$ T cells in bleomycin-treated mice. Together, these results suggest that galectin-9 expression by SSc dermal fibroblasts promotes skin fibrosis by suppressing IFNγ production and inducing profibrotic Th2 cytokine expression by skin-infiltrating $CD4^+$ T cells.

Interleukin-13 production by systemic sclerosis $CD8^+$ T cells

IL-13 overproduction by peripheral blood SSc $CD8^+$ T cells is caused by upregulation of the Th2-specific transcription factor GATA-3 [78]. Cascio *et al.* [79] recently investigated the underlying molecular mechanism by focusing on the Th1-specific transcription factor T-bet, which induces IFNγ production and inhibits type-2 cytokines by antagonizing GATA-3 expression and/or function [80–82]. The authors showed that circulating and skin-resident $CD8_{EM}$ T cells from patients with active dcSSc express high levels of IL-13 and GATA-3 but similar levels of IFNγ and T-bet compared to controls. However, they observed that the levels of the active phosphorylated form of T-bet as well as the direct interaction between T-bet and GATA-3 are reduced in the nucleus of SSc $CD8^+$ T cells, allowing more

GATA-3 to bind to the IL-13 promoter and inducing IL-13. This defect results from the interaction of T-bet with the adaptor protein 14–3–3 [83] in the cytosol of SSc $CD8^+$ T lymphocytes, reducing T-bet translocation into the nucleus and thus its modulation of GATA-3 activity. Interestingly, they show that this mechanism is also found during Tc2 polarization of healthy donor $CD8^+$ T cells. Thus, by detailing a novel mechanism underlying type-2 cytokine production by $CD8^+$ T cells, Cascio *et al.* revealed a more complete picture of the complex pathway leading to SSc disease pathogenesis.

CONCLUSION

Immune cell dysfunction is a major component of SSc pathogenesis. Patients have few therapeutic options, and a better understanding of the molecular and cellular mechanisms underlying loss of self-tolerance, activation of effector immune pathways and of the interactions between the immune and stromal cells will lead to innovative therapies that selectively target the aberrant immune response, resulting in better efficacy and less toxicity.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Fuschiotti P. Current perspectives on the immunopathogenesis of systemic sclerosis. Immunotargets Ther 2016; 5:21–35.
2. Chizzolini C, Boin F. The role of the acquired immune response in systemic sclerosis. Semin Immunopathol 2015; 37:519–528.
3. Prescott RJ, Freemont AJ, Jones CJ, *et al.* Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol 1992; 166:255–263.
4. Fleischmajer R, Perlish JS, Reeves JR. Cellular infiltrates in scleroderma skin. Arthritis Rheum 1977; 20:975–984.
5. Kahari VM, Sandberg M, Kalimo H, *et al.* Identification of fibroblasts responsible for increased collagen production in localized scleroderma by in situ hybridization. J Invest Dermatol 1988; 90:664–670.
6. Scharffetter K, Lankat-Buttgereit B, Krieg T. Localization of collagen mRNA in normal and scleroderma skin by in-situ hybridization. Eur J Clin Invest 1988; 18:9–17.
7. Kreuter A, Hoxtermann S, Gambichler T, *et al.* Detection of clonal T cells in the circulation of patients with nephrogenic systemic fibrosis. Arch Dermatol 2009; 145:1164–1169.
8. Sakkas LI, Xu B, Artlett CM, *et al.* Oligoclonal T cell expansion in the skin of patients with systemic sclerosis. J Immunol 2002; 168:3649–3659.
9. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007; 117:557–567.
10. Slobodin G, Rimar D. Regulatory T cells in systemic sclerosis: a comprehensive review. Clin Rev Allergy Immunol 2017; 52:194–201.
11. Radstake TR, van Bon L, Broen J, *et al.* Increased frequency and compromised function of T regulatory cells in systemic sclerosis (SSc) is related to a diminished CD69 and TGFbeta expression. PLoS One 2009; 4:e5981.
12. Fleury M, Belkina AC, Proctor EA, *et al.* Increased expression and modulated regulatory activity of coinhibitory receptors PD-1, TIGIT, and TIM-3 in lymphocytes from patients with systemic sclerosis. Arthritis Rheumatol 2018; 70:566–577.
13. Ugor E, Simon D, Almanzar G, *et al.* Increased proportions of functionally impaired regulatory T cell subsets in systemic sclerosis. Clin Immunol 2017; 184:54–62.
14. Hartigan-O'Connor DJ, Poon C, Sinclair E, McCune JM. Human CD4+ regulatory T cells express lower levels of the IL-7 receptor alpha chain (CD127), allowing consistent identification and sorting of live cells. J Immunol Methods 2007; 319:41–52.
15. ■■ Dominguez-Villar M, Hafler DA. Regulatory T cells in autoimmune disease. Nat Immunol 2018; 19:665–673.
This review summarizes the newer concepts of T_{reg} cell instability, T_{reg} cell plasticity and tissue-specific T_{reg} cells, and their relationship to autoimmunity.
16. Kassan M, Wecker A, Kadowitz P, *et al.* CD4+CD25+Foxp3 regulatory T cells and vascular dysfunction in hypertension. J Hypertens 2013; 31: 1939–1943.
17. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. Immunity 2013; 38:414–423.
18. Jin HT, Jeong YH, Park HJ, Ha SJ. Mechanism of T cell exhaustion in a chronic environment. BMB Rep 2011; 44:217–231.
19. Wherry EJ. T cell exhaustion. Nat Immunol 2011; 12:492–499.
20. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity 2016; 44:989–1004.
21. McKinney EF, Lee JC, Jayne DR, *et al.* T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. Nature 2015; 523:612–616.
22. Day CL, Kaufmann DE, Kiepiela P, *et al.* PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 2006; 443:350–354.
23. Fukasawa T, Yoshizaki A, Ebata S, *et al.* Contribution of soluble forms of programmed death 1 and programmed death ligand 2 to disease severity and progression in systemic sclerosis. Arthritis Rheumatol 2017; 69: 1879–1890.
24. Taylor DK, Mittereder N, Kuta E, *et al.* T follicular helper-like cells contribute to skin fibrosis. Sci Transl Med 2018; 10:431–445.
25. Ruzek MC, Jha S, Ledbetter S, *et al.* A modified model of graft-versus-host-induced systemic sclerosis (scleroderma) exhibits all major aspects of the human disease. Arthritis Rheum 2004; 50:1319–1331.
26. Hutloff A, Dittrich AM, Beier KC, *et al.* ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. Nature 1999; 397:263–266.
27. Distler JH, Jungel A, Kowal-Bielecka O, *et al.* Expression of interleukin-21 receptor in epidermis from patients with systemic sclerosis. Arthritis Rheum 2005; 52:856–864.
28. Pesce J, Kaviratne M, Ramalingam TR, *et al.* The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest 2006; 116:2044–2055.
29. Rojas-Zuleta WG, Vasquez G. Th9 lymphocytes: a recent history from IL-9 to its potential role in rheumatic diseases. Autoimmun Rev 2016; 15: 649–655.
30. Deng Y, Wang Z, Chang C, *et al.* Th9 cells and IL-9 in autoimmune disorders: pathogenesis and therapeutic potentials. Hum Immunol 2017; 78:120–128.
31. Ciccia F, Guggino G, Ferrante A, *et al.* Interleukin-9 and T helper type 9 cells in rheumatic diseases. Clin Exp Immunol 2016; 185:125–132.
32. Yanaba K, Yoshizaki A, Asano Y, *et al.* Serum interleukin 9 levels are increased in patients with systemic sclerosis: association with lower frequency and severity of pulmonary fibrosis. J Rheumatol 2011; 38:2193–2197.
33. Guggino G, Lo Pizzo M, Di Liberto D, *et al.* Interleukin-9 over-expression and T helper 9 polarization in systemic sclerosis patients. Clin Exp Immunol 2017; 190:208–216.
34. Lee KH, Kronbichler A, Park DD, *et al.* Neutrophil extracellular traps (NETs) in autoimmune diseases: a comprehensive review. Autoimmun Rev 2017; 16:1160–1173.
35. Fuschiotti P, Medsger TA Jr, Morel PA. Effector CD8+ T cells in systemic sclerosis patients produce abnormally high levels of interleukin-13 associated with increased skin fibrosis. Arthritis Rheum 2009; 60:1119–1128.
36. Li G, Larregina AT, Domsic RT, *et al.* Skin-resident effector memory CD8+CD28- T cells exhibit a profibrotic phenotype in patients with systemic sclerosis. J Invest Dermatol 2017; 137:1042–1050.
37. Greenblatt MB, Sargent JL, Farina G, *et al.* Interspecies comparison of human and murine scleroderma reveals IL-13 and CCL2 as disease subset-specific targets. Am J Pathol 2012; 180:1080–1094.
38. Milano A, Pendergrass SA, Sargent JL, *et al.* Molecular subsets in the gene expression signatures of scleroderma skin. PLoS One 2008; 3:e2696.

39. Rice LM, Padilla CM, McLaughlin SR, *et al.* Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. J Clin Invest 2015; 125:2795–2807.
40. Rice LM, Stifano G, Ziemek J, Lafyatis R. Local skin gene expression reflects both local and systemic skin disease in patients with systemic sclerosis. Rheumatology (Oxford) 2016; 55:377–379.
41. Fuschiotti P. Current perspectives on the role of CD8+ T cells in systemic sclerosis. Immunol Lett 2018; 195:55–60.
42. Watanabe R, Gehad A, Yang C, *et al.* Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. Sci Transl Med 2015; 7:279ra239.
43. Thome JJ, Farber DL. Emerging concepts in tissue-resident T cells: lessons from humans. Trends Immunol 2015; 36:428–435.
44. Clark RA. Resident memory T cells in human health and disease. Sci Transl Med 2015; 7:269rv261.
45. Altorok N, Wang Y, Kahaleh B. Endothelial dysfunction in systemic sclerosis. Curr Opin Rheumatol 2014; 26:615–620.
46. ■ Mostmans Y, Cutolo M, Giddelo C, *et al.* The role of endothelial cells in the vasculopathy of systemic sclerosis: a systematic review. Autoimmun Rev 2017; 16:774–786.

This review describes the evidence that endothelial cell dysfunction in cell survival, angiogenesis and vasculogenesis as well as the interactions between endothelial cells and various other cells contribute to SSc vasculopathy.

47. Borghini A, Manetti M, Nacci F, *et al.* Systemic sclerosis sera impair angiogenic performance of dermal microvascular endothelial cells: therapeutic implications of cyclophosphamide. PLoS One 2015; 10:e0130166.
48. Kuwana M, Okazaki Y, Yasuoka H, *et al.* Defective vasculogenesis in systemic sclerosis. Lancet 2004; 364:603–610.
49. Hur J, Yang HM, Yoon CH, *et al.* Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. Circulation 2007; 116:1671–1682.
50. Manetti M, Pratesi S, Romano E, *et al.* Angiogenic T cell expansion correlates with severity of peripheral vascular damage in systemic sclerosis. PLoS One 2017; 12:e0183102.
51. Ronnblom L. The importance of the type I interferon system in autoimmunity. Clin Exp Rheumatol 2016; 34:21–24.
52. Ronnblom L, Eloranta ML. The interferon signature in autoimmune diseases. Curr Opin Rheumatol 2013; 25:248–253.
53. Eloranta ML, Franck-Larsson K, Lovgren T, *et al.* Type I interferon system activation and association with disease manifestations in systemic sclerosis. Ann Rheum Dis 2010; 69:1396–1402.
54. Tan FK, Zhou X, Mayes MD, *et al.* Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. Rheumatology (Oxford) 2006; 45:694–702.
55. York MR, Nagai T, Mangini AJ, *et al.* A macrophage marker, Siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and toll-like receptor agonists. Arthritis Rheum 2007; 56:1010–1020.
56. Brkic Z, van Bon L, Cossu M, *et al.* The interferon type I signature is present in systemic sclerosis before overt fibrosis and might contribute to its pathogenesis through high BAFF gene expression and high collagen synthesis. Ann Rheum Dis 2016; 75:1567–1573.
57. Arismendi M, Giraud M, Ruzehaji N, *et al.* Identification of NF-kappaB and PLCL2 as new susceptibility genes and highlights on a potential role of IRF8 through interferon signature modulation in systemic sclerosis. Arthritis Res Ther 2015; 17:71.
58. Rezaei R, Mahmoudi M, Gharibdoost F, *et al.* IRF7 gene expression profile and methylation of its promoter region in patients with systemic sclerosis. Int J Rheum Dis 2017; 20:1551–1561.
59. Wang J, Yi L, Guo X, *et al.* Association of the IRF5 SNP rs2004640 with systemic sclerosis in Han Chinese. Int J Immunopathol Pharmacol 2014; 27:635–638.
60. Xu Y, Wang W, Tian Y, *et al.* Polymorphisms in STAT4 and IRF5 increase the risk of systemic sclerosis: a meta-analysis. Int J Dermatol 2016; 55:408–416.
61. Yi L, Wang JC, Guo XJ, *et al.* STAT4 is a genetic risk factor for systemic sclerosis in a Chinese population. Int J Immunopathol Pharmacol 2013; 26:473–478.
62. Ding W, Pu W, Wang L, *et al.* Genome-wide DNA methylation analysis in systemic sclerosis reveals hypomethylation of IFN-associated genes in CD4(+) and CD8(+) T cells. J Invest Dermatol 2018; 138:1069–1077.
63. Schoggins JW, Wilson SJ, Panis M, *et al.* A diverse range of gene products are effectors of the type I interferon antiviral response. Nature 2011; 472:481–485.
64. Bergmann C, Distler JH. Canonical Wnt signaling in systemic sclerosis. Lab Invest 2016; 96:151–155.
65. Gil J, Alcami J, Esteban M. Activation of NF-kappa B by the dsRNA-dependent protein kinase, PKR involves the I kappa B kinase complex. Oncogene 2000; 19:1369–1378.
66. Haller O, Kochs G. Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res 2011; 31:79–87.
67. Zhang Y, Mao D, Roswit WT, *et al.* PARP9-DTX3L ubiquitin ligase targets host histone H2BJ and viral 3C protease to enhance interferon signaling and control viral infection. Nat Immunol 2015; 16:1215–1227.
68. Arthur CM, Baruffi MD, Cummings RD, Stowell SR. Evolving mechanistic insights into galectin functions. Methods Mol Biol 2015; 1207:1–35.
69. Raja J, Denton CP. Cytokines in the immunopathology of systemic sclerosis. Semin Immunopathol 2015; 37:543–557.
70. Saigusa R, Asano Y, Nakamura K, *et al.* Systemic sclerosis dermal fibroblasts suppress Th1 cytokine production via galectin-9 overproduction due to Fli1 deficiency. J Invest Dermatol 2017; 137:1850–1859.
71. Zhang Q, Luan H, Wang L, *et al.* Galectin-9 ameliorates anti-GBM glomerulonephritis by inhibiting Th1 and Th17 immune responses in mice. Am J Physiol Renal Physiol 2014; 306:F822–F832.
72. Niwa H, Satoh T, Matsushima Y, *et al.* Stable form of galectin-9, a Tim-3 ligand, inhibits contact hypersensitivity and psoriatic reactions: a potent therapeutic tool for Th1- and/or Th17-mediated skin inflammation. Clin Immunol 2009; 132:184–194.
73. Akamata K, Asano Y, Aozasa N, *et al.* Bosentan reverses the pro-fibrotic phenotype of systemic sclerosis dermal fibroblasts via increasing DNA binding ability of transcription factor Fli1. Arthritis Res Ther 2014; 16:R86.
74. Kubo M, Czuwara-Ladykowska J, Moussa O, *et al.* Persistent down-regulation of Fli1, a suppressor of collagen transcription, in fibrotic scleroderma skin. Am J Pathol 2003; 163:571–581.
75. Wang Y, Fan PS, Kahaleh B. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. Arthritis Rheum 2006; 54:2271–2279.
76. Ichimura Y, Asano Y, Akamata K, *et al.* Progranulin overproduction due to Fli-1 deficiency contributes to the resistance of dermal fibroblasts to tumor necrosis factor in systemic sclerosis. Arthritis Rheumatol 2015; 67: 3245–3255.
77. Taniguchi T, Asano Y, Akamata K, *et al.* Fibrosis, vascular activation, and immune abnormalities resembling systemic sclerosis in bleomycin-treated Fli-1-haploinsufficient mice. Arthritis Rheumatol 2015; 67:517–526.
78. Medsger TA Jr, Ivanco DE, Kardava L, *et al.* GATA-3 upregulation in CD8+ T cells is a biomarker of immune dysfunction in systemic sclerosis, resulting in excess IL-13 production. Arthritis Rheum 2011; 63:1738–1747.
79. Cascio S, Medsger TA Jr, Hawse WF, *et al.* 14-3-3z sequesters cytosolic T-bet, upregulating IL-13 levels in TC2 and CD8(+) lymphocytes from patients with scleroderma. J Allergy Clin Immunol 2018; 142:109–119.
80. Szabo SJ, Kim ST, Costa GL, *et al.* A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 2000; 100:655–669.
81. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol 2002; 2:933–944.
82. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol 2010; 28:445–489.
83. Muslin AJ, Xing H. 14-3-3 proteins: regulation of subcellular localization by molecular interference. Cell Signal 2000; 12:703–709.

Innate immunity and Toll-like receptor signaling in the pathogenesis of scleroderma: advances and opportunities for therapy

Max Brown and Steven O'Reilly

Purpose of review
Systemic sclerosis (SSc) is an autoimmune connective tissue disease in which inflammation and cytokine dysregulation leads to skin fibrosis. Toll-like receptors (TLRs) are conserved pattern recognition receptors, recognizing pathogens danger-associated molecular patterns (DAMPs) that elicit a cascade of proinflammatory signaling. Recently, TLRs have been found to be critically important in SSc pathogenesis, with increased levels of the TLRs and their ligands present in the disease. Animal models have also been pivotal in delineating the role of these innate immune receptors in SSc. This current review examines the role of TLRs and the most recent evidence of the role of DAMPs and how these may be exploited therapeutically.

Recent findings
Increasingly, studies have demonstrated the key roles of TLR4 and other intracellular TLRs in mediating fibrosis in SSc patients and animal models. TLR4 activation appears a key point and novel DAMPs, expressed upon tissue damage, appear critical in mediating the profibrotic effect through a downstream enhancement of transforming growth factor β. Deletion of Tenascin-C or a splice variant of fibronectin ameliorates animal models of skin fibrosis. Intracellular, nucleic acid sensing, TLR8 is critical in activating macrophages to secrete profibrotic molecules. The mechanism involves histone modification through epigenetic modifying enzymes.

Summary
TLRs are key therapeutic targets in SSc.

Keywords
danger-associated molecular patterns, macrophages, myeloid differentiation primary response gene 88, systemic sclerosis, Toll-like receptor

INTRODUCTION

A key mechanism of innate immunity is the activation of a group of pattern recognition receptors (PRRs) that recognize certain motifs on pathogens or internal signals to elicit inflammation via nuclear factor kappa-light-chain-enhancer of activated B cells. Recognition of the invading pathogen is critical to elicit protective responses. Due to evolutionarily pressure we have evolved PRRs. Innate immune recognition engages germline-encoded PRRs that recognize conserved microbial patterns in pathogens known as pathogen-associated molecular patterns [1]. This leads to an inflammatory response in the form of increased inflammatory mediators being released and an increase in costimulatory molecules but also direct antigen-specific adaptive immune responses [2]. Chief among these PRRs are the Toll-like receptors (TLR) which are conserved from the nematode worm *Caenorhabditis elegans* to mammals [3] and these are by far the most extensively studied of the PRRs. Dysregulated TLRs are attributed to a wide variety of autoimmune diseases including the autoinflammatory disorders.

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease in which there are alterations of the immune system, a breakdown in tolerance to self and skin and lung fibrosis. In a subset of patients with generalized skin fibrosis (diffuse) the

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KEY POINTS

- TLRs are critical in host defense to pathogens and internal danger signals.
- TLRs are also critical in the fibrosis associated with SSc.
- Internal cytosolic TLR8 appears a critical TLR in mediating fibrosis through secreted factors such as tissue inhibitor of metalloproteinases-1.
- Therapeutic targeting in particular to TLR4, appears a realistic possibility in SSc.

prognosis is poor. The fundamental processes leading to the increased formation of fibrotic tissue is not clear but recent emerging data have highlighted the role of the innate immune system in SSc and in particular the TLRs [4,5] have emerged as pathogenic mediators. Evidence is clear that monocytes and macrophages are elevated in skin and blood in SSc [6] and gene signatures associated with innate immunity are present in SSc [7]. Due to the increasing evidence implicating the innate immune system and TLRs in the disease they are now becoming a real druggable target [8]. This review will summarize recent data on the role of innate immunity in SSc and how this may be used to target these systems for therapeutic gain.

TOLERANCE AND DANGER

For decades it was generally accepted that the immune system responded to non self and not self, with the implication that anything that belonged to the organism is non antigenic and anything that does not is antigenic. The danger theory that was proposed by Polly Matzinger, nearly 25 years ago [9], proposed that the immune system responds to danger and rather than just 'nonself'. This paradigm suggests that proteins that are normally 'hidden' from the immune system as they are intracellular are recognized by the immune system upon released from the intracellular pool. This was all theoretical until the discovery of high-mobility group box 1 (HMGB-1) as the prototypical danger-associated molecular pattern (DAMP) [10]. HMGB-1 is a nuclear protein that is a DNA chaperone but can be released upon cell stress or death to be liberated into the extracellular space. Here, the HMGB-1 interacts with TLR4 to elicit inflammation. Since then a huge list of DAMPs has appeared and they do not seem to share structural characteristics yet can bind the same TLRs [11]. The DAMPs represent a diverse group of molecules that include, serum amyloid A, chaperone proteins and matrix fragments. Quite how such diversity can stimulate the same receptors is unknown. In SSc levels of various DAMPs are elevated including HMGB-1 [12], suggesting that these are contributing to disease. A key question arises from this 'What are the cellular sources of the DAMPs?' It is the authors' suggestion that these are derived from damaged endothelium as vascular damage is one of the earliest events in SSc. These liberated DAMPs from the endothelium can dually activate monocytes, leading to enhanced cytokine production, but also local stromal cells (Table 1).

SYSTEMIC SCLEROSIS AND TOLL-LIKE RECEPTOR 4

TLR4 is the most studied of all the TLRs and binds the bacterial component lipopolysaccharide (LPS) in Gram-negative bacteria. In SSc dermal fibroblasts stimulation with LPS leads to enhanced production of extracellular matrix (ECM) [13]. However, it is very unlikely that the endogenous trigger for stimulation of TLR4 is bacterial which suggests that other internal 'DAMPs' may be mediating the effect. Bhattacharyya *et al.* have demonstrated upregulation of TLR4 and its

Table 1. Toll-like receptors expressed in systemic sclerosis and their roles

TLR	Ligand	Location	Role in SSc
TLR4	Tenascin-C Fibronectin EDA	Cell surface	TLR4 KO protects mice from fibrosis Tenascin-C elevated in SSc and KO protects mice from fibrosis Tenascin-C FBG domain triggers myofibroblast phenotype in macrophages Fibronectin EDA domain triggers fibrosis. KO mice protected
TLR8	ssRNA	Intracellular	Enhances TIMP-1 expression RNA present in SSc serum mediates the increased expression of TIMP-1 TLR8 stimulation in plasmacytoid dendritic cells causes increase in interferon and CXCL4 Overexpression in animal models exacerbates SSc disease

TLR4 and 8 are expressed in SSc and their levels are perturbed as are internal DAMPs. Animal models where levels of TLRs are manipulated demonstrate their direct role in disease. CXCL, CXC chemokine ligand; DAMPs, danger-associated molecular pattern; EDA, Extra-Domain A; FBG, fibrinogen-like globe; KO, knockout; SSc, systemic sclerosis; ssRNA, single stranded RNA; TIMP-1, tissue inhibitor of metalloproteinases-1; TLR, Toll-like receptor.

activating ligands in lesional lung and skin in SSc donors. Significantly there appears to be a synergy between both LPS and the classic profibrotic cytokine transforming growth factor β 1 (TGF-β1). Although the prototypical DAMP HMGB-1 is elevated in SSc no functional activation of TLR4 in the disease has been demonstrated. Rather the glycoprotein Tenascin-C has been identified as being the chief DAMP activating TLR4 in SSc [14▪]. This remarkable work demonstrated that Tenascin-C is elevated in SSc and activated TLR4 to mediate profibrotic effects [14▪]. Mice deleted for Tenascin-C where protected from fibrosis as where TLR4 deleted mice [14▪]. Given the fact that Tenascin-C is expressed in embryonic development and highly restricted postnatally it fulfills the role of a DAMP. It is likely that tissue damage leads to liberation of Tenascin-C leading to a wound healing response necessary to instruct and direct tissue repair. A failure of termination of such a wound repair program would lead to fibrosis. Importantly TLR4 knockout mice are protected from fibrosis not only in the bleomycin model of fibrosis but also in the tight skin mouse [15].

Tenascin-C protein comprises four domains: Tenascin Assembly domain, epidermal growth factor-like repeats, up to 17 fibronectin-like repeats and a fibrinogen-like globe (FBG) domain. Significantly the FBG-domain of Tenascin-C was used compared with the standard TLR4 ligand LPS in monocyte-derived macrophages led to divergent signaling [16]. Stimulation of the macrophages with FBG led to increased synthesis and phosphorylation of collagen [16]. Macrophages do not normally express a great deal of collagen, if any, and this suggest that this is pushing the macrophage to a more myofibroblast phenotype that would be useful in wound repair. This is in agreement with a differential pro-repair phenotype as there was a down regulation also of matrix metallopeptidase 1 (MMP-1) [16]. Thus the prorepair (myofibroblasts) transition pathway may be activated by the FBG domain of Tenascin but LPS mediated through a pathogen leads to a protease-rich environment. In support of this Tenascin-C knock out mice are protected from experimental cardiac fibrosis [17]. Significantly Tenascin-C is higher both in lung fibroblasts from SSc patients but also in the serum with SSc patients whom have pulmonary hypertension [18].

Another possible DAMP is the Extra-Domain A (EDA) domain of fibronectin which encodes the type II extra repeat domain. This fibronectin spliced form is only expressed in damaged tissue and not in homeostasis. The fibronectin EDA was found to be markedly elevated in SSc skin and blood of patients and in the bleomycin model of skin fibrosis [19]. This appears to be regulated by TGF-β1 and stimulation of fibroblasts with this variant leads to myofibroblasts generation and matrix stiffening [19]. Deletion of either this variant of fibronectin or TLR4 markedly reduced skin fibrosis [19]. Because this variant is only produced upon damage it is suggested that this is a reparative response to ensure wound healing occurs. A recent study has also suggested that the fibronectin EDA domain interacts and binds latent TGF-β-binding protein [20] which could help activate TGF-β1 from its latent form.

In recent years, mitochondrial DNA (mtDNA) has been suggested as a DAMP that causes sterile inflammation [21] and blood levels of mtDNA are associated with clinical outcome in sepsis [22]. Although no data exist in relation to SSc it has been demonstrated that in idiopathic pulmonary fibrosis that there are elevated extracellular mtDNA and that this mtDNA is also elevated in the broncheoalveolar lavage [23]. Furthermore, stimulation of fibroblasts with mtDNA augmented alpha-Smooth muscle actin expression [23]. Significantly as a DAMP HMGB-1 can also be hyperacetylated which facilitates its expulsion from the cell to bind TLRs [24] it could be possible that other DAMPs relevant in SSc are also hyperacetylated by acetylase enzymes.

INTRACELLULAR TOLL-LIKE RECEPTORS

Intracellular TLRs include TLR3, 7 and 8. These intracellular TLRs are nucleic acid sensors that sense nucleic acid often of viral origin. TLR7/8 is the receptor for single stranded RNA (ssRNA) [25] and endogenous nucleic acids have been found to trigger a systemic lupus erythematosus disease [26]. We have previously demonstrated a major role for TLR8 in SSc-mediated fibrosis through enhanced tissue inhibitor of metalloproteinases-1 (TIMP-1) secretion [27]. TIMP-1 is the natural inhibitor of MMPs which means a balance that favors ECM deposition as opposed to breakdown. We could further show that a RNA species was present in SSc serum that can mediate the increased TIMP-1 in a myeloid differentiation primary response gene 88 (MyD88)-dependant fashion [27]. We subsequently demonstrated that this is mediated through a TLR-dependent and fos-related antigen 2 (Fra2)-dependent mechanism [28]. Fra2 is a subunit of the activator protein 1 transcription family that has been shown to be important in fibrosis mediated by fibroblasts [29]. We further show that this is mediated via a histone methylation modification [28].

TLR8 was recently shown to play a key role in SSc in plasmacytoid dendritic cells. Transgenic mice for TLR8 had exacerbated fibrotic disease [30▪▪]. Mechanistically the dendritic cells, through TLR8 stimulation, led to increased levels of Interferon and CXC

chemokine ligand 4, both hallmarks of the disease [30▪▪]. High TLR7 levels have also been demonstrated in SSc peripheral blood mononuclear cells [31]. Further evidence for a role for interferons comes from the fact that interferons are elevated in SSc monocytes and upregulate siglec-1 [32]. Significantly it appears that the DAMP HMGB-1, which is elevated in SSc, and TLR responses are interlinked with the response to nucleic acid contingent on HMGB-1 [33]. This suggests that higher levels of this DAMP may enhance TLR-mediated responses. It also appears that plasmacytoid dendritic cells have an increased response to TLR ligands in SSc leading to enhanced IL-6 production [34], which itself is profibrotic. Increased numbers of monocytes and myeloid dendritic cells are also found in SSc blood which may potentiate any signaling [35].

Although it is now clear that TLR8 is critical in SSc and in animal models of the disease (where TLR8 overexpression leads to an exacerbated diseases phenotype) what is the ligand and where is it derived? In our studies we found elevated levels of RNA in sera from SSc patients [27], however its source could not be ascertained. We speculate this is derived from internal endothelial cells that have been damaged (Fig. 1). Alternatively this could be derived from a virus. A recent study demonstrated that Epstein–Barr virus (EBV)-activated TLR8 to mediate profibrotic effects [36]. Significantly EBV itself can activate the fibroblast to myofibroblast conversion [37]. Also at least in an animal model gammaherpesvirus infection-induced fibrosis [38] whilst latent herpes virus exacerbates experimental lung fibrosis [39].

In support of the role of viruses in the pathogenesis of the disease a recent study demonstrated that an interferon gene signature is present in SSc in the earliest phase of the disease, even before overt skin fibrosis is present [40]. The presence of this interferon gene signature is correlated with B-cell activating factor suggesting some interaction between the two [40]. This suggests that possibly a viral infection is activating the interferon response early in the disease that drives activation of the immune and stromal cell compartment [40].

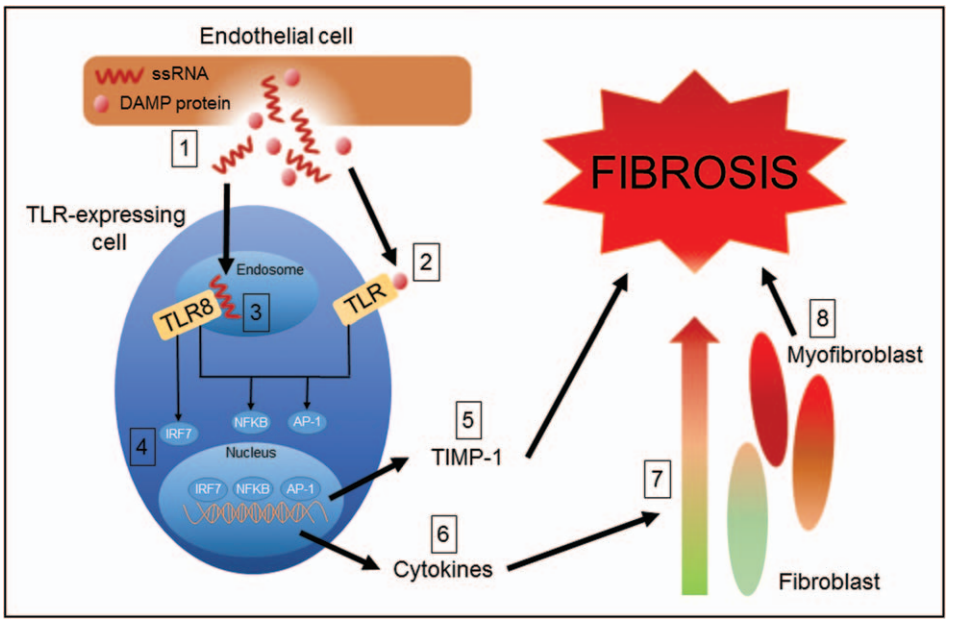

FIGURE 1. Proposed mechanisms through which damage to endothelium leads to fibrosis in systemic sclerosis. Damaged endothelial cells release danger-associated molecular pattern proteins (e.g. high-mobility group box 1) and ssRNA to be detected by Toll-like receptor-expressing cells such as monocytes and dendritic cells. Danger-associated molecular pattern proteins bind to and activate Toll-like receptors on the surface of cells. ssRNA enters cells through the formation of endosomes from which they activate Toll-like receptor 8. Once activated a Toll-like receptor can instigate a cascade of signaling events leading to the activation of proinflammatory transcription factors interferon regulatory factor 7, nuclear factor kappa-light-chain-enhancer of activated B cells and activator protein 1. Activator protein 1 activation induces the expression of tissue inhibitor of metalloproteinases-1. Tissue inhibitor of metalloproteinases-1 inhibits matrix metallopeptidases and as such promotes fibrosis. The proinflammatory transcription factors upregulate the expression of various cytokines (e.g. CXC chemokine ligand 4, CXC chemokine ligand 10, IL-6 and interferons). Cytokines cause proliferation and differentiation of fibroblasts into myofibroblasts. Myofibroblasts express high levels of collagen, Alpha-Sma and other extracellular matrix proteins leading to fibrosis. CXCL, CXC chemokine ligand; IFN, interferon; IRF7, interferon regulatory factor 7; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ssRNA, single stranded RNA; TIMP-1, tissue inhibitor of matrix metalloproteinase-1.

TLR9 which is activated by nucleosomes, has been shown to be elevated in SSc and a weak correlation with skin score [41]. Indeed TLR9 has been shown to be elevated and colocalized with myofibroblasts and that stimulation of TLR9 in healthy dermal fibroblasts with TLR9 agonists leads to increased generation of myofibroblasts that is dependent on MyD88 and through enhanced activation of TGF-β1 [42]. Significantly, this can be blocked by a proteasome inhibitor [42]. Also TLR has been shown to be upregulated on adaptive immune cells in SSc, namely B and T cells [43]. Nucleosome stimulation via TLR in these cells leads to increased IL-4 and IL-17, important profibrotic mediators [43,44].

THERAPEUTIC TARGETING OF INNATE IMMUNITY IN SYSTEMIC SCLEROSIS

Recent enthusiasm in innate immune research and in particular TLRs has led to a number of drugs that could target this system therapeutically. Multiple ways of targeting this could occur including blockade of the ligand that is the DAMP, blockade of the TLR receptor and/or blockade of downstream signaling adapters. Small molecule inhibitors are now attractive therapies for autoimmune diseases because they are specific and generally well tolerated.

Blocking TLR2 may be mediated through an anti-TLR2 antibody (Opsona, Dublin, Ireland) and this is being evaluated in delayed graft function in kidney transplant [45]. In support of this TLR2 knock out mice have reduced cardiac fibrosis due to pressure overload [46].

Targeting TLR4 may be a promising therapeutic due to the prominent role this plays. Eritoran is a specific TLR4 inhibitor that has shown promise in animal models. No studies have used this in SSc and it is currently under investigation in sepsis [47]. The small molecule TAK-242, binds to the intracellular domain of TLR4 and inhibit signaling by blocking the interaction with downstream signaling molecules [48]. This may be useful in blocking TLR4-mediated skin fibrosis. Bajo *et al.* developed T5342126 to target to MD2-TLR4 interaction and this was used *in vivo* to modify ethanol dependent behavior. This may prove useful in targeting TLR4-mediated fibrosis. Intracellular inhibition of adaptor molecules such as MyD88 may be useful and has been effective in inhibiting inflammatory responses in synovial membrane cultures [49].

Because of the importance of the intracellular TLR8 in SSc it may be beneficial to target this TLR. Idera pharmaceuticals have a TLR7 antagonist in clinical trials for plaque psoriasis. AZD1419 is a TLR agonist developed by Dynavax. Epigenetic therapy could also be a viable way to alter TLR signaling to reduce fibrosis as microRNA155 is rapidly upregulated with TLR4 activation ligands [50] as a negative regulator.

CONCLUSION

Innate immunity and in particular TLRs appear key in SSc pathogenesis. Evidence is now compiling of the role of DAMPs including Tenascin-C [14▪] activating a wound healing response that does not show natural resolution. Therapeutic targeting of TLRs may represent a new therapeutic avenue and should be tested in preclinical models. However, targeting of such pathways would be beneficial only if responses to natural pathogens are not diminished also.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001; 2:675–680.
2. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124:783–801.
3. Charles A, Janeway J, Medzhitov R. Innate immune recognition. Ann Rev Immunol 2002; 20:197–216.
4. O'Reilly S. Toll like receptors in systemic sclerosis: an emerging target. Immunol Lett 2018; 195:2–8.
5. Dowson C, Simpson N, Duffy L, O'Reilly S. Innate immunity in systemic sclerosis. Curr Rheumatol Rep 2017; 19:2.
6. Higashi-Kuwata N, Jinnin M, Makino T, *et al.* Characterization of monocyte/macrophage subsets in the skin and peripheral blood derived from patients with systemic sclerosis. Arthritis Res Ther 2010; 12:R128.
7. Johnson ME, Mahoney JM, Taroni J, *et al.* Experimentally-derived fibroblast gene signatures identify molecular pathways associated with distinct subsets of systemic sclerosis patients in three independent cohorts. PLoS One 2015; 10:e0114017.
8. O'Reilly S. Innate immunity in systemic sclerosis pathogenesis. Clin Sci 2014; 126:329–337.
9. Matzinger P. Tolerance, danger, and the extended family. Ann Rev Immunol 1994; 12:991–1045.
10. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002; 418:191–195.
11. O'Reilly S. Pound the alarm: danger signals in rheumatic diseases. Clin Sci 2015; 128:297–305.
12. Yoshizaki A, Komura K, Iwata Y, *et al.* Clinical significance of serum HMGB-1 and sRAGE levels in systemic sclerosis: association with disease severity. J Clin Immunol 2009; 29:180–189.
13. Bhattacharyya S, Kelley K, Melichian DS, *et al.* Toll-like receptor 4 signaling augments transforming growth factor-β responses: a novel mechanism for maintaining and amplifying fibrosis in scleroderma. Am J Pathol 2013; 182:192–205.
14. Bhattacharyya S, Wang W, Morales-Nebreda L, *et al.* Tenascin-C drives ▪ persistence of organ fibrosis. Nat Commun 2016; 7:11703.
The article is the first to demonstrate a role for Tenascin-C in systemic sclerosis mediated through Toll-like receptor 4 (TLR4) and both knock out mice are protected.

15. Takehiro T, Yoshihide A, Yohei I, *et al.* Amelioration of tissue fibrosis by Toll-like receptor 4 knockout in murine models of systemic sclerosis. Arthritis Rheumatol 2015; 67:254–265.

16. Piccinini AM, Zuliani-Alvarez L, Lim JMP, Midwood KS. Distinct microenvironmental cues stimulate divergent TLR4-mediated signaling pathways in macrophages. Sci Signal 2016; 9:ra86–ra186.

17. Podesser BK, Kreibich M, Dzilic E, *et al.* Tenascin-C promotes chronic pressure overload-induced cardiac dysfunction, hypertrophy and myocardial fibrosis. J Hypertens 2018; 36:847–856.

18. Brissett M, Veraldi KL, Pilewski JM, *et al.* FBC: localized expression of tenascin in systemic sclerosis-associated pulmonary fibrosis and its regulation by insulin-like growth factor binding protein 3. Arthritis Rheum 2012; 64:272–280.

19. Bhattacharyya S, Tamaki Z, Wang W, *et al.* FibronectinEDA promotes chronic cutaneous fibrosis through toll-like receptor signaling. Sci Transl Med 2014; 6:232ra50.

20. Klingberg F, Chau G, Walraven M, *et al.* The fibronectin ED-A domain enhances recruitment of latent TGF-β-binding protein-1 to the fibroblast matrix. J Cell Sci 2018; 131. pii: jcs201293. doi: 10.1242/jcs.201293.

21. Zhang Q, Raoof M, Chen Y, *et al.* Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010; 464:104–107.

22. Qiongyuan H, Jianan R, Jie W, *et al.* Elevated levels of plasma mitochondrial DNA are associated with clinical outcome in intra-abdominal infections caused by severe trauma. Surg Infect 2017; 18:610–618.

23. Ryu C, Sun H, Gulati M, *et al.* Extracellular mitochondrial DNA is generated by fibroblasts and predicts death in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2017; 196:1571–1581.

24. Bonaldi T, Talamo F, Scaffidi P, *et al.* Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J 2003; 22:5551–5560.

25. Heil F, Hemmi H, Hochrein H, *et al.* Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 2004; 303:1526–1529.

26. Barrat FJ, Meeker T, Gregorio J, *et al.* Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. J Exp Med 2005; 202:1131–1139.

27. Ciechomska M, Huigens CA, Hügle T, *et al.* Toll-like receptor-mediated, enhanced production of profibrotic TIMP-1 in monocytes from patients with systemic sclerosis: role of serum factors. Ann Rheum Dis 2013; 72:1382–1389.

28. Ciechomska M, O'Reilly S, Przyborski S, *et al.* Histone demethylation and toll-like receptor 8-dependent cross-talk in monocytes promotes transdifferentiation of fibroblasts in systemic sclerosis via Fra-2. Arthritis Rheumatol 2016; 68:1493–1504.

29. Reich N, Maurer B, Akhmetshina A, *et al.* The transcription factor Fra-2 regulates the production of extracellular matrix in systemic sclerosis. Arthritis Rheum 2010; 62:280–290.

30. ■■ Ah Kioon MD, Tripodo C, Fernandez D, *et al.* Plasmacytoid dendritic cells promote systemic sclerosis with a key role for TLR8. Sci Transl Med 2018; 10. pii: eaam8458. doi: 10.1126/scitranslmed.aam8458.

The article demonstrates a key role in plasmacytoid dendritic cells for TLR8 in mediating skin fibrosis through increased CXC chemokine ligand 4 secretion. Overexpression of TLR8 intransgenic mice exacerbates skin fibrosis.

31. Vreća M, Zeković A, Damjanov N, *et al.* Expression of TLR7, TLR9, JAK2, and STAT3 genes in peripheral blood mononuclear cells from patients with systemic sclerosis. J Appl Genet 2018; 59:59–66.

32. York MR, Nagai T, Mangini AJ, *et al.* A macrophage marker, siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and toll-like receptor agonists. Arthritis Rheum 2007; 56:1010–1020.

33. Yanai H, Ban T, Wang Z, *et al.* HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature 2009; 462: 99–103.

34. van Bon L, Popa C, Huijbens R, *et al.* Distinct evolution of TLR-mediated dendritic cell cytokine secretion in patients with limited and diffuse cutaneous systemic sclerosis. Ann Rheum Dis 2010; 69:1539–1547.

35. Carvalheiro T, Horta S, van Roon JAG, *et al.* Increased frequencies of circulating CXCL10-, CXCL8- and CCL4-producing monocytes and Siglec-3-expressing myeloid dendritic cells in systemic sclerosis patients. Inflamm Res 2018; 67:169–177.

36. Farina A, Peruzzi G, Lacconi V, *et al.* Epstein–Barr virus lytic infection promotes activation of Toll-like receptor 8 innate immune response in systemic sclerosis monocytes. Arthritis Res Ther 2017; 19:39.

37. Farina A, Cirone M, York M, *et al.* Epstein–Barr virus infection induces aberrant TLR activation pathway and fibroblast–myofibroblast conversion in scleroderma. J Invest Dermatol 2014; 134:954–964.

38. Stoolman JS, Vannella KM, Coomes SM, *et al.* Latent infection by γherpesvirus stimulates profibrotic mediator release from multiple cell types. Am J Physiol Lung Cell Mol Physiol 2011; 300:L274–L285.

39. Vannella KM, Luckhardt TR, Wilke CA, *et al.* Latent herpesvirus infection augments experimental pulmonary fibrosis. Am J Respir Crit Care Med 2010; 181:465–477.

40. Brkic Z, van Bon L, Cossu M, *et al.* The interferon type I signature is present in systemic sclerosis before overt fibrosis and might contribute to its pathogenesis through high BAFF gene expression and high collagen synthesis. Ann Rheum Dis 2016; 75:1567–1573.

41. Gheita TA, Sayed S, Azkalany GS, *et al.* Toll-like receptor 9 in systemic sclerosis patients: relation to modified Rodnan skin score, disease severity, and functional status. Clin Rheumatol 2018; 37:757–763.

42. Feng F, Goncalves MR, Xingchun Z, *et al.* Toll-like receptor 9 signaling is augmented in systemic sclerosis and elicits transforming growth factor β-dependent fibroblast activation. Arthritis Rheumatol 2016; 68: 1989–2002.

43. Yoshizaki A, Taniguchi T, Saigusa R, *et al.* Nucleosome in patients with systemic sclerosis: possible association with immunological abnormalities via abnormal activation of T and B cells. Ann Rheum Dis 2016; 75:1858–1865.

44. O'Reilly S, van Laar JM. Nucleosomes and systemic sclerosis. Nat Rev Rheumatol 2016; 12:138–139.

45. Arslan F, Houtgraaf JH, Keogh B, *et al.* Treatment with OPN-305, a humanized anti-toll-like receptor-2 antibody, reduces myocardial ischemia/reperfusion injury in pigs. Circulation 2012; 5:279–287.

46. Wang J-W, Fontes MSC, Wang X, *et al.* Leukocytic toll-like receptor 2 deficiency preserves cardiac function and reduces fibrosis in sustained pressure overload. Sci Rep 2017; 7:9193.

47. Kalil AC, LaRosa SP, Gogate J, *et al.* Influence of severity of illness on the effects of eritoran tetrasodium (E5564) and on other therapies for severe sepsis. Shock 2011; 36:327–331.

48. Matsunaga N, Tsuchimori N, Matsumoto T, Ii M. TAK-242 (Resatorvid), a small-molecule inhibitor of toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Mol Pharmacol 2011; 79:34–41.

49. Sacre SM, Andreakos E, Kiriakidis S, *et al.* The Toll-like receptor adaptor proteins MyD88 and Mal/TIRAP contribute to the inflammatory and destructive processes in a human model of rheumatoid arthritis. Am J Pathol 2007; 170:518–525.

50. O'Connell RM, Taganov KD, Boldin MP, *et al.* MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci U S A 2007; 104:1604–1609.

Recent developments in classification criteria and diagnosis guidelines for idiopathic inflammatory myopathies

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Purpose of review
The aim of this review was to summarize key developments in classification and diagnosis of the idiopathic inflammatory myopathies (IIMs).

Recent findings
The recently published European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for the IIMs provide a comprehensive, accurate and data-driven approach to identification of IIM cases appropriate for inclusion in research studies. Further, recent studies have advanced understanding of clinical manifestations of the IIMs and delineated the role of imaging, particularly magnetic resonance.

Summary
The recent publication of the EULAR/ACR classification criteria will potentially greatly improve IIM research through more accurate case identification and standardization across studies.
Future inclusion of newly recognized clinical associations with the MSAs may further improve the criteria's accuracy and utility. Clear and comprehensive understanding of associations between clinical manifestations, prognosis and multisystem involvement can aid diagnostic assessment; recent advances include delineation of such associations and expansion of the role of imaging.

Keywords
classification, dermatomyositis, diagnosis, myositis, polymyositis

INTRODUCTION

The idiopathic inflammatory myopathies (IIMs) are a group of autoimmune diseases characterised by chronic muscle inflammation (myositis), internal organ inflammation and significant morbidity and mortality [1]. The wide spectrum of clinical manifestations, variable disease course and distinct subtypes makes accurate classification and diagnosis of paramount importance to ensure valid research and timely instigation of treatment.

This article aims to summarize recently published research pertinent to advances in IIM classification and diagnosis. A Medline search for research articles published between January 2017 and May 2018 was carried out using the MeSH term 'myositis'. Articles primarily focussing on myositis-specific autoantibodies were excluded, as they will be reviewed in detail in a separate article.

CLASSIFICATION

The IIMs have traditionally been classified and diagnosed according to the criteria by Bohan and Peter (Table 1) since publication in 1975 [2,3]. The Bohan and Peter criteria demonstrate a high degree of accuracy and usefulness in research and clinical settings. However, this usefulness is limited by a number of factors, including lack of specification of how to exclude other forms of myopathy and

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KEY POINTS

- The recently published EULAR/ACR classification criteria for the IIMs can potentially improve the validity and accuracy of future research studies.
- Recent advances in understanding of clinical manifestations of the IIMs can aid a clinician's patient assessment.
- Particular advances have highlighted the benefits of the use of MRI, which includes identification of multisystem involvement and associations with clinical features.
- Identification of cardiac involvement has been improved through identification of the utility of serum troponin levels.

nonexplicit definition of inclusion criteria; further, recent advances in myositis research, such as the identification of myositis-specific autoantibodies (MSAs), are not included. Another major drawback of the Bohan and Peter criteria is the noninclusion of more recently described and defined IIM subtypes, including immune-mediated necrotising myopathy (IMNM) and antisynthetase syndrome (ASS).

These drawbacks have limited accurate identification of well defined populations suitable for IIM research studies. Therefore, in 2004, the International Myositis Classification Criteria Project (IMCCP) was established with the aim of developing new IIM classification criteria. The IMCCP working committee was formed of experts from adult and paediatric rheumatology, neurology, dermatology, epidemiology and biostatistics. In 2017, the newly developed European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria were published [4▪▪]. Initial methodology identified 93 candidate variables for inclusion in the classification criteria. Variable domains included pattern of weakness, dermatological manifestations, disease course, systemic manifestations, response to treatment, pattern of muscle biopsy abnormalities, presence of MSAs, electromyogram (EMG) and MRI features.

Using data from 976 IIM cases and 624 comparators, the combination of candidate variables that could most accurately distinguish between IIM and non-IIM participants was identified. The variables included in the final classification criteria are displayed in Table 2. Each variable was assigned a weighted score, according to the coefficient from a logistic regression model. The sum of the scores that a potential study participant fulfils corresponds to their probability of having an IIM. Without muscle biopsy data, a score between 5.5 (55% probability) and 7.5 (90% probability) corresponds to 'probable IIM' and a score equal to or greater than 7.5 corresponds to 'definite IIM'. With biopsy data, a score between 6.7 (55% probability) and 8.7 (90% probability) corresponds to 'probable IIM' and a score equal to or greater than 8.7 is 'definite IIM'.

The likely IIM subtype of each case can also be ascertained, according to the published classification

Table 1. Bohan and Peter classification criteria for polymyositis and dermatomyositis

Criteria	Description
A	Proximal and symmetrical muscle weakness of the pelvic and scapular girdle, anterior flexors of the neck, progressing for weeks to months, with or without dysphagia or involvement of respiratory muscles
B	Elevation of the serum levels of skeletal muscle enzymes: creatine kinase, aspartate aminotransferase, lactate dehydrogenase and aldolase
C	Electromyography characteristic of myopathy (short and small motor units, fibrillation, positive pointy waves, insertional irritability and repetitive high-frequency firing)
D	Muscle biopsy showing necrosis, phagocytosis, regeneration, perifascicular atrophy, perivascular inflammatory exudate
E	Typical cutaneous changes: (1) Heliotrope rash with periorbital oedema and violaceous erythema (2) Gottron's sign: vasculitis in the elbow, metacarpophalangeal and proximal interphalangeal joints
Polymyositis	(1) Definite – all of A–D (2) Probable – any three of A–D (3) Possible – any two of A–D
Dermatomyositis	(1) Definite – E plus and three of A–D (2) Probable – E plus and two of A–D (3) Possible – E plus and one of A–D

Exclusion criteria: congenital muscular dystrophies, central or peripheral neurological disease, infectious myositis, metabolic/endocrine myopathies and myasthenia gravis.
Adapted with permission [2].

Table 2. The European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies

Domain	Feature	Definition	Score points	
			With muscle biopsy data	Without muscle biopsy data
Age of onset	Age of onset of first symptom assumed to be related to the disease ≥18 and <40 years		1.5	1.3
	Age of onset of first symptom assumed to be related to the disease ≥40 years		2.2	2.1
Weakness pattern	Objective symmetric weakness, usually progressive, of the proximal upper extremities	Weakness of proximal upper extremities as defined by manual muscle testing or other objective strength testing, which is present on both sides and is usually progressive over time	0.7	0.7
	Objective symmetric weakness, usually progressive, of the proximal lower extremities	Weakness of proximal lower extremities as defined by manual muscle testing or other objective strength testing, which is present on both sides and is usually progressive over time	0.5	0.8
	Neck flexors are relatively weaker than neck extensors	Muscle grades for neck flexors are relatively lower than neck extensors as defined by manual muscle testing or other objective strength testing	1.6	1.9
	In the legs, proximal muscles are relatively weaker than distal muscles	Muscle grades for proximal muscles in the legs are relatively lower than distal muscles in the legs as defined by manual muscle testing or other objective strength testing	1.2	0.9
Skin manifestations	Heliotrope rash	Purple, lilac-coloured or erythematous patches over the eyelids or in a periorbital distribution, often associated with periorbital oedema	3.2	3.1
	Gottron's papules	Erythematous to violaceous papules over the extensor surfaces of joints, which are sometimes scaly. May occur over the finger joints, elbows, knees, malleoli and toes	2.7	2.1
	Gottron's sign	Erythematous to violaceous macules over the extensor surfaces of joints, which are not palpable	3.7	3.3
Other clinical manifestations	Dysphagia or oesophageal dysmotility	Difficulty in swallowing or objective evidence of abnormal motility of the oesophagus	0.6	0.7
Laboratory results	Anti-Jo-1 positivity	Autoantibody testing in serum performed with standardized and validated test, showing positive result	3.8	3.9
	Elevated serum levels of CK or LDH or AST or ALT	The most abnormal test values during the disease course (highest absolute level of enzyme) above the relevant upper limit of normal	1.4	1.3
Muscle biopsy features	Endomysial infiltration of mononuclear cells surrounding, but not invading, myofibers	Muscle biopsy reveals endomysial mononuclear cells abutting the sarcolemma of otherwise healthy, nonnecrotic muscle fibres, but there is no clear invasion of the muscle fibres	1.7	
	Perimysial and/or perivascular infiltration of mononuclear cells	Mononuclear cells are located in the perimysium and/or located around blood vessels (in either perimysial or endomysial vessels)	1.2	
	Perifascicular atrophy	Muscle biopsy reveals several rows of muscle fibres, which are smaller in the perifascicular region than fibres more centrally located	1.9	
	Rimmed vacuoles	Rimmed vacuoles are bluish by haematoxylin and eosin staining and reddish by modified Gomori trichrome stain	3.1	

ALT, alanine transaminase; AST, aspartate transaminase; CK, creatine kinase; LDH, lactate dehydrogenase.
Adapted with permission [4▪▪].

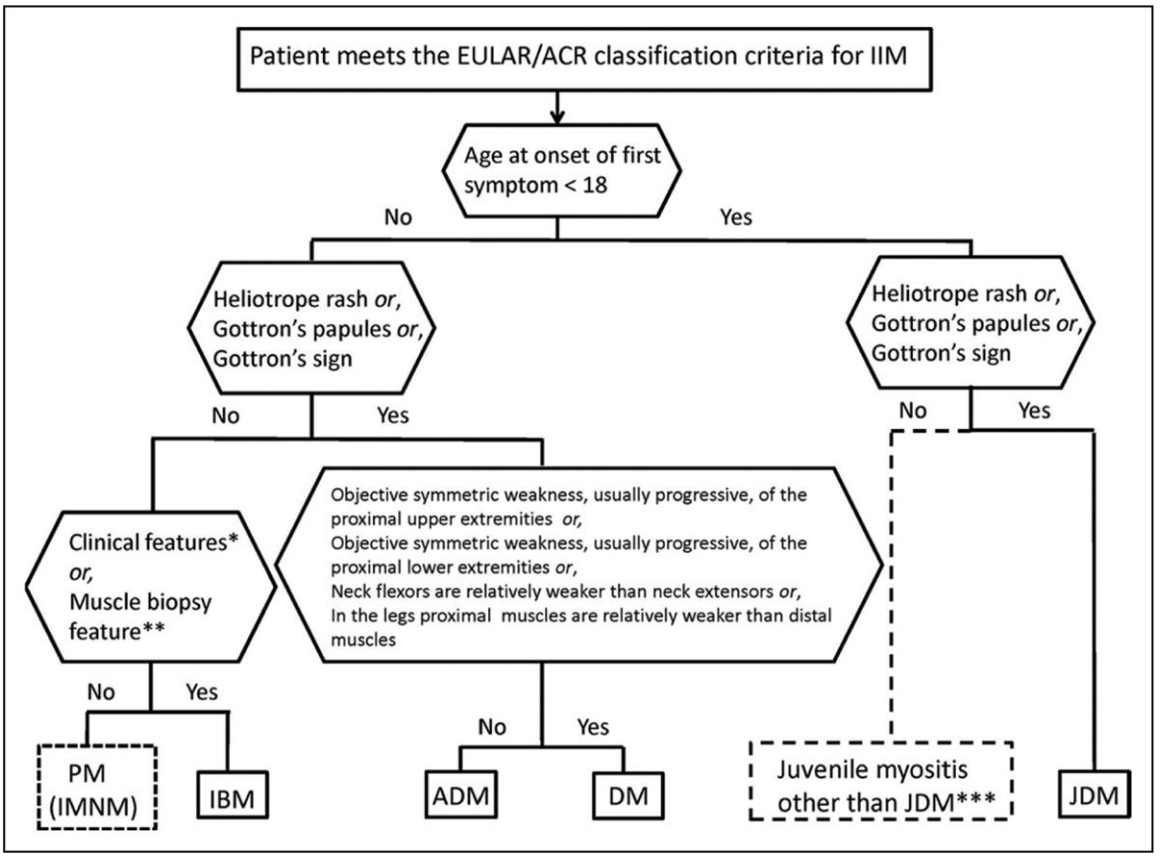

FIGURE 1. Classification tree for subtype of idiopathic inflammatory myopathies. ADM, amyopathic dermatomyositis; DM, dermatomyositis; EULAR/ACR, European League Against Rheumatism/American College of Rheumatology; IBM, inclusion body myositis; IMNM, immune-mediated necrotising myopathy; JDM, juvenile dermatomyositis; PM, polymyositis. For inclusion body myositis (IBM) classification, one of the following is required for classification: finger flexor weakness and response to treatment: not improved ([a]), or muscle biopsy: rimmed vacuoles ([b]). [c]Juvenile myositis other than juvenile dermatomyositis was developed based on expert opinion. Adapted with permission [4▪▪].

tree (Fig. 1). Subtype identification can only be carried out on cases with an IIM diagnostic probability of greater than 55%. Further, only those with polymyositis, inclusion body myositis (IBM), dermatomyositis, amyopathic dermatomyositis (ADM) and juvenile dermatomyositis (JDM) can be identified, in part due to the small number of cases of IMNM, hypomyopathic dermatomyositis, ASS and overlap myositis cases in the development population.

The EULAR/ACR criteria demonstrated high sensitivity (93%) and specificity (88%) when muscle biopsy data were included. Sensitivity and specificity also remained high when muscle biopsy data were not included: 87 and 82%, respectively. The accuracy of the newly created classification criteria was compared against previously developed criteria, including those by Bohan and Peter [2,3], Tanimoto *et al.* [5], Targoff *et al.* [6], Dalakas and Hohlfeld [7] and the European Neuromuscular Centre [8]. The Targoff criteria demonstrated slightly higher accuracy with 93% sensitivity and 89% specificity. No other criteria showed both higher sensitivity and specificity. Also, the new criteria demonstrated correct IIM subtype classification in the majority of cases. The inclusion of muscle biopsy data improved the accuracy of all subtypes, apart from ADM (94% without muscle biopsy data and 60% with) and JDM (97% without muscle biopsy data and 96% with). Agreement between the EULAR/ACR criteria and the Bohan and Peter criteria was found to be 89% without muscle biopsy data and 93% with biopsy data. External validation in the Euromyositis registry and the Juvenile Dermatomyositis Biomarker Study and Repository revealed sensitivity of 100% for both adult and juvenile populations. A website-based calculator has been developed to allow for further use in other study populations: www.imm.ki.se/biostatistics/calculators/iim.

The criteria have a number of major strengths. Their development methodology included a large IIM population with non-IIM comparators and

considered inclusion of a wide variety of candidate variables. The ease of use and provision of a website-based calculator are also major strengths. Drawbacks include the fact that a majority of the development population were white, thus potentially limiting the criteria's validity in Asian and African populations. Further, MRI and electromyography data were only available in 38 and 29% of cases, thus potentially excluding these variables from the criteria only due to missing data. A limitation of subtype identification is the inability to separately identify IMNM and ASS cases. The inclusion of only one myositis-specific autoantibody (anti-Jo-1) in the classification criteria is a major limitation, as recent studies have illustrated the important and distinctive clinical and subtype associations [9–11]. Therefore, the authors have recommended that a future update of the EULAR/ACR criteria should include more IMNM, ADM, hypomyopathic dermatomyositis, juvenile IIM other than JDM and MSA-positive cases, to allow accurate identification.

In summary, the newly developed EULAR/ACR classification criteria for the IIMs provide an accurate method through which clearly defined study populations can be formed, thus potentially improving validity of IIM research.

DIAGNOSIS

The newly developed classification criteria provide robust methods for identifying IIM cases for research purposes; however, their use is not designed nor recommended for use in clinical practice.

Accurate diagnosis of an IIM is key to appropriate treatment instigation, prognostication and prevention of complications. However, diagnosis and subtype identification in clinical settings can be challenging, in part due to potential multisystem involvement and wide variations between subtype manifestations. Currently, no clear diagnostic criteria for the IIMs exist. However, findings from clinically focused research studies can aid a clinician's diagnostic accuracy, identification of factors associated with prognosis and guide investigation of multisystem involvement.

CLINICAL FEATURES

Findings from epidemiological and observational studies can inform clinical practice and guide the diagnostic process. A number of observational studies have recently been published and these will be summarized, with a particular focus upon potential clinical applications. Cox *et al.* [12] recently described 'hiker's feet', hyperkeratosis of skin of the feet in cases of dermatomyositis. This newly described manifestation was detected in nine dermatomyositis cases out of a large IIM cohort ($N = 2145$). Interestingly, seven of the nine cases also fulfilled criteria for ASS and six cases were positive for anti-Jo-1 antibodies. This study highlights the importance of foot examination in IIM cases when trying to identify cutaneous manifestations. Mamyrova *et al.* [13▪] identified that only sun exposure and nonsteroidal anti-inflammatory use were significantly associated with an IIM flare, which highlights the importance of ascertaining a patient's symptom relationship with these factors. Svensson *et al.* [14] recently investigated if there is an association between IIM onset and preceding infections and respiratory tract disease. Preceding infections were significantly more common in IIM cases, than controls: 13 vs. 9%, respectively. They also identified that respiratory tract disease was associated with IIM onset. The authors therefore concluded that infections may increase the risk of IIM development through possible activation of the inflammatory response.

A recent study compared the utility of muscle testing via hand-held dynamometry against manual muscle testing in myositis cases [15]. They reported that hand-held dynamometry was particularly accurate at assessing the strength of single muscle groups, whereas manual muscle testing was only reliable in assessing generalised muscle weakness. Therefore, consideration should be given to the wider use of handheld dynamometry as a method of assessing and quantifying muscle strength and identifying weakness during a diagnostic assessment.

INTERSTITIAL LUNG DISEASE

Interstitial lung disease (ILD) is an important extramuscular manifestation to consider during the diagnostic process and subsequent assessments. A number of studies have recently investigated risk factors for ILD. A recent study by Schiffenbauer *et al.* [16] has highlighted important relationships between cigarette smoking, ILD risk and clinical and serological manifestations in the IIMs. White IIM cases who had ever smoked were more likely to have polymyositis and test positive for an antisynthetase or anti-Jo-1 autoantibodies; they were also less likely to test positive for antitranscriptional intermediary factor antibody. Whites who had ever smoked were more likely to develop ILD, whereas the risk of ILD was decreased for ever smokers in the African–American population. This complements the finding by Chinoy *et al.* [17] in 2012, who identified an association between smoking and development of anti-Jo-1 antibodies in cases

positive for HLA-DRB1*03, thus indicating a genetic interaction with smoking and IIM development. These studies therefore highlight the importance of ascertaining a patient's smoking history due to its potential diagnostic utility and estimation of ILD risk.

Investigation for predictors of poor survival in 497 IIM-associated ILD cases was carried out by Sato *et al.* [18]. They identified that older age of onset (>60 years), raised C-reactive protein, low peripheral capillary oxygen saturation (<95%) and positivity for the antibody against melanoma differentiation-associated gene 5 were all associated with increased mortality risk. Therefore, presence of these factors can aid prognostication. Ang *et al.* [19] reported cutaneous dermatomyositis features associated with ILD development in their cohort of 101 dermatomyositis cases. They reported that the presence of mechanics' hands was significantly associated with an increased risk of ILD, whereas the presence of Gottron's papules was associated with a significantly reduced risk, thus highlighting the potential prognostic value of pattern of cutaneous manifestations.

IMAGING

The role of imaging in IIM diagnosis has expanded in recent years and has a number of capabilities. Imaging techniques, such as MRI, can help identify the presence of myositis, delineate its extent and assess treatment response, through serial scans and also help focus appropriate areas for muscle biopsy, limiting the likelihood of a false negative sample.

Studies by both Yao *et al.* [20▪] and Andersson *et al.* [21] have recently identified associations between semi-quantitative MRI scores and a number of variables, including physician global assessment, the modified childhood myositis assessment scale and creatine kinase levels in IIM cases; Yao *et al.* [20▪] also compared changes in semi-quantitative scores prior to and following rituximab therapy and identified no consistent changes. It could be that the use of such semi-quantitative scoring systems may aid assessment during the diagnostic process; however, further evidence for its particular utility is required.

Pinal-Fernandez *et al.* [22] reported the particular MRI features of IMNM, compared with polymyositis, dermatomyositis, CADM and IBM. They reported that more widespread muscle oedema, atrophy and fat replacement were associated with IMNM and that positivity for the antibody against signal recognition particle was associated with more severe disease, than those that were positive for the antibody against HMG-CoA reductase. Further MRI features in IBM were characterized by Guimaraes *et al.* [23] in 12 biopsy-proven cases. MRI scans of upper and lower limbs were scored for muscle atrophy, fat infiltration and oedema pattern. They concluded that changes were most severe in the lower limbs and the most common abnormality was fat infiltration. Further, they identified that the number of muscles with fat infiltration was statistically associated with disease duration, muscle strength and functional status. Therefore, MRI may be a useful and sensitive method through which IIM subtype can be identified; however, correlation with clinical features, serological status and muscle biopsy examination will complement this.

Focused MRI scanning can identify focal areas on myositis; however, whole-body MRI offers the ability to completely delineate all muscle groups affected and potentially identify secondary organ involvement and the presence of associated malignancy. Whole-body MRI, as opposed to focused imaging, was advocated by Elessawy *et al.* [24▪] as the modality of choice due to the ability to detect all muscle groups affected, which may not be evident on clinical examination. Huang *et al.* [25] also reported the utility of whole-body MRI and advocated its use to help identify associated malignancy, extramuscular manifestations, such as ILD and cardiac involvement, and steroid-associated osteonecrosis.

Other imaging modalities also offer utility in IIM diagnosis. Burlina *et al.* [26▪] recently aimed to develop and evaluate the capability of ultrasound imaging to automatically identify myositis through 'machine learning' and 'deep learning' statistical techniques. Machine and deep learning allow the development of computational algorithms that can detect the presence of a disease, thus potentially aiding or superseding human diagnostic skills. The developed algorithm could therefore allow automatic detection of myositis through ultrasound imaging. The developed algorithms displayed accuracies ranging 69–87%. The developed algorithms also enabled distinction between IBM, polymyositis and dermatomyositis. This study therefore identified the capability of automated ultrasound imaging to detect myositis and highlighted potential future innovation.

Computed tomography (CT) scanning offers detailed assessment of the presence of ILD. A recent study by Ungprasert *et al.* [27] investigated the associations between pulmonary function tests and quantitative thoracic high-resolution CT analysis in an IIM population with ILD. After investigating for associations in 110 cases, they concluded that the quantitative measurements correlate well with pulmonary function test values, which included diffusing capacity for carbon monoxide, total lung capacity and oxygen saturations, thus extending patient assessment through CT imaging.

CARDIAC INVOLVEMENT

The ability to identify cardiac involvement (an uncommon but important extramuscular manifestation) and distinguish from other heart disease has undergone advances recently. The utility of MRI in identifying cardiac involvement has been confirmed in recent years by a small number of studies [28,29]. Recent advances include identification that raised serum levels of troponin I can be used as a reliable indicator of myocardial involvement in the IIMs and can distinguish between active myocardial and skeletal muscle disease [30]. Hughes *et al.* [31] have recently developed a pathway using cardiac troponins to screen for subclinical cardiac disease in IIM patients. Guerra *et al.* [32] aimed to identify the ability of 'global longitudinal strain' measurement via echocardiography to identify subclinical systolic impairment in IIM cases. Through investigation of 28 IIM cases and comparison with healthy controls, they concluded that subclinical systolic impairment is common and that the global longitudinal strain method may be useful in identification.

Huber *et al.* [33▪] confirmed the ability of MRI to identify inflammatory cardiac disease; however, they identified an inability to differentiate between inflammatory cardiac disease due to IIM or acute viral myocarditis. Differentiation was only possible with MRI examination of the thoracic skeletal muscles.

CONCLUSION

Accurate case identification is key to IIM research and the recent publication of the EULAR/ACR classification criteria will potentially greatly improve IIM research through accurate case identification and standardization across studies. Clear diagnosis of the IIMs is important to ensure appropriate diagnosis and treatment instigation. Recent advances in knowledge of clinical features will aid the clinician in prognostication, treatment stratification and investigation for multisystem involvement.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Oldroyd A, Lilleker J, Chinoy H. Idiopathic inflammatory myopathies: a guide to subtypes, diagnostic approach and treatment. Clin Med (Lond) 2017; 17:322–328.
2. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975; 292:344–347.
3. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975; 292:403–407.
4. ▪▪ Lundberg IE, Tjärnlund A, Bottai M, *et al.* 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis 2017; 76:1955–1964.

This study describes the recently published classification criteria. The data-driven approach and consideration of a wide range of candidate variables make these criteria the most accurate and valid to date.

5. Tanimoto K, Nakano K, Kano S, *et al.* Classification criteria for polymyositis and dermatomyositis. J Rheumatol 1995; 22:668–674.
6. Targoff IN, Miller FW, Medsger TA, Oddis CV. Classification criteria for the idiopathic inflammatory myopathies. Curr Opin Rheumatol 1997; 9: 527–535.
7. Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet 2003; 362:971–982.
8. Hoogendijk JE, Amato AA, Lecky BR, *et al.* 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10-12 October 2003, Naarden, The Netherlands. Neuromuscul Disord 2004; 14:337–345.
9. Dalakas MC. Inflammatory muscle diseases. N Engl J Med 2015; 372: 1734–1747.
10. Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 2016; 280:8–23.
11. Rider LG, Nistala K. The juvenile idiopathic inflammatory myopathies: pathogenesis, clinical and autoantibody phenotypes, and outcomes. J Intern Med 2016; 280:24–38.
12. Cox JT, Gullotti DM, Mecoli CA, *et al.* Hiker's feet': a novel cutaneous finding in the inflammatory myopathies. Clin Rheumatol 2017; 36:1683–1686.
13. ▪ Mamyrova G, Rider LG, Ehrlich A, *et al.* Environmental factors associated with disease flare in juvenile and adult dermatomyositis. Rheumatology 2017; 56:1342–1347.

This article identified that sun exposure and NSAID use were associated with an increased risk of flares in juvenile and adult dermatomyositis populations.

14. Svensson J, Holmqvist M, Lundberg IE, Arkema EV. Infections and respiratory tract disease as risk factors for idiopathic inflammatory myopathies: a population-based case-control study. Ann Rheum Dis 2017; 76:1803–1808.
15. Baschung Pfister P, de Bruin ED, Sterkele I, *et al.* Manual muscle testing and hand-held dynamometry in people with inflammatory myopathy: an intra- and interrater reliability and validity study. PLoS One 2018; 13:e0194531.
16. Schiffenbauer A, Faghihi-Kashani S, O'Hanlon TP, *et al.* The effect of cigarette smoking on the clinical and serological phenotypes of polymyositis and dermatomyositis. Semin Arthritis Rheum 2018. [Epub ahead of print]
17. Chinoy H, Adimulam S, Marriage F, *et al.* Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. Ann Rheum Dis 2012; 71:961–965.
18. Sato S, Masui K, Nishina N, *et al.* Initial predictors of poor survival in myositis-associated interstitial lung disease: a multicentre cohort of 497 patients. Rheumatology 2018; 57:1212–1221.
19. Ang CC, Anyanwu CO, Robinson E, *et al.* Clinical signs associated with an increased risk of interstitial lung disease: a retrospective study of 101 patients with dermatomyositis. Br J Dermatol 2017; 176:231–233.
20. ▪ Yao L, Yip AL, Shrader JA, *et al.* Magnetic resonance measurement of muscle T2, fat-corrected T2 and fat fraction in the assessment of idiopathic inflammatory myopathies. Rheumatology (Oxford) 2016; 55:441–449.

This study identified that semi-quantitative MRI changes do not change following rituximab therapy.

21. Andersson H, Kirkhus E, Garen T, *et al.* Comparative analyses of muscle MRI and muscular function in antisynthetase syndrome patients and matched controls: a cross-sectional study. Arthritis Res Ther 2017; 19:17.
22. Pinal-Fernandez I, Casal-Dominguez M, Carrino JA, *et al.* Thigh muscle MRI in immune-mediated necrotising myopathy: extensive oedema, early muscle damage and role of anti-SRP autoantibodies as a marker of severity. Ann Rheum Dis 2017; 76:681–687.
23. Guimaraes JB, Zanoteli E, Link TM, *et al.* Sporadic inclusion body myositis: MRI findings and correlation with clinical and functional parameters. Am J Roentgenol 2017; 209:1340–1347.

24. Elessawy SS, Abdelsalam EM, Abdel Razek E, Tharwat S. Whole-body MRI
■ for full assessment and characterization of diffuse inflammatory myopathy. Acta Radiol Open 2016; 5:205846011666821.
This study identified the utility of whole-body MRI scanning in the inflammatory myopathies.
25. Huang Z, Gao B, Chen H, *et al.* An efficacy analysis of whole-body magnetic resonance imaging in the diagnosis and follow-up of polymyositis and dermatomyositis. PLoS One 2017; 12:e0181069.
26. Burlina P, Billings S, Joshi N, Albayda J. Automated diagnosis of myositis from
■ muscle ultrasound: exploring the use of machine learning and deep learning methods. PLoS One 2017; 12:e0184059.
This study employed novel machine learning approaches to allow automated diagnosis of myositis via ultrasound scanning.
27. Ungprasert P, Wilton KM, Ernste FC, *et al.* Novel assessment of interstitial lung disease using the 'Computer-Aided Lung Informatics for Pathology Evaluation and Rating' (CALIPER) software system in idiopathic inflammatory myopathies. Lung 2017; 195:545–552.
28. Rosenbohm A, Buckert D, Gerischer N, *et al.* Early diagnosis of cardiac involvement in idiopathic inflammatory myopathy by cardiac magnetic resonance tomography. J Neurol 2015; 262:949–956.
29. Buckert D, Rosenbohm A, Kassubek J, *et al.* Early detection of myocardial involvement in idiopathic inflammatory myopathies detected by cardiac magnetic resonance imaging. J Cardiovasc Magn Reson 2015; 17:277.
30. Lilleker JB, Diederichsen ACP, Jacobsen S, *et al.* Using serum troponins to screen for cardiac involvement and assess disease activity in the idiopathic inflammatory myopathies. Rheumatology (Oxford) 2018; 57: 1041–1046.
31. Hughes M, Lilleker JB, Herrick AL, Chinoy H. Cardiac troponin testing in idiopathic inflammatory myopathies and systemic sclerosis-spectrum disorders: biomarkers to distinguish between primary cardiac involvement and low-grade skeletal muscle disease activity. Ann Rheum Dis 2015; 74: 795–798.
32. Guerra F, Gelardi C, Capucci A, *et al.* Subclinical cardiac dysfunction in polymyositis and dermatomyositis: a speckle-tracking case-control study. J Rheumatol 2017; 44:815–821.
33. Huber AT, Bravetti M, Lamy J, *et al.* Noninvasive differentiation of idiopathic
■ inflammatory myopathy with cardiac involvement from acute viral myocarditis using cardiovascular magnetic resonance imaging T1 and T2 mapping. J Cardiovasc Magn Reson 2018; 20:11.
This study highlights the challenges of diagnosing cardiac myositis via MRI.

New insights in myositis-specific autoantibodies

Anna Ghirardello and Andrea Doria

Purpose of review
The aim of this study was to provide the most recent evidence on clinical utility of myositis-specific autoantibodies (MSAs) in the management of patients with myositis.

Recent findings
In the last few years, several evidences have emerged on the clinical and pathogenetic role of established and novel MSA. Antisynthetase antibodies represent a reliable biomarker for pulmonary involvement also in patients with connective tissue diseases other than myositis. Antisignal recognition particle and antihydroxy-3-methylglutaryl coenzyme A reductase autoantibodies are able to induce complement-dependent muscle damage. Dermatomyositis-specific antibodies are useful indicators of clinical diversity. The pivotal role of antitranscription intermediary factor 1γ autoimmune response in adult-age paraneoplastic dermatomyositis has been further asserted. AnticN1A and antifour-and-a-half LIM protein 1 antibodies are newly conceived myositis-related antibody specificities, which can contribute to patients' stratification into more homogeneous groups.

Summary
Distinct autoantibody-associated clinical phenotypes can be predicted by extended MSA testing in serum. Standardization and validation of MSA laboratory detection methods is strongly recommended for better supporting myositis diagnosis, management and prognosis definition.

Keywords
autoantibodies, autoimmune myositis, myositis-specific antibodies

INTRODUCTION

Autoantibodies against intracellular self-antigens are serological biomarkers of connective tissue diseases (CTDs), including idiopathic inflammatory myopathies (IIMs) or myositis. The autoantibody detection represents a valuable tool in the diagnostic workup of adult and juvenile poly/dermatomyositis. They are categorized as myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs). MSA include mutually exclusive diagnostic markers of myositis [1], while MAAs, present in over 50% of the patients, are not disease-specific. MSA comprise various antibody reactivities: apart from the classic antiaminoacil tRNA synthetases (ARS), anti-Mi-2 and anti-SRP, novel established MSAs, such as anti-TIF1γ, anti-MDA5, anti-NXP2, anti-SAE and anti-HMGCR antibodies, are included. Moreover, two promising MSAs have recently been identified, that is anticytoplasmic nucleotidase 1A and antifour-and-a-half LIM protein 1 (anti-FHL1) antibodies.

Common MAAs include anti-Ro/SSA, anti-Ku antibodies, anti-PM/Scl and anti-U1 ribonucleoprotein (RNP). They can be associated with MSA, or mainly found in myositis-CTD overlap syndromes.

MSA target ubiquitous or muscle-specific enzymatic ribonucleoproteins involved in DNA transcription, chromatin remodelling, epigenetic modifications, protein synthesis or translocation, muscle cell metabolism and differentiation, innate immune response (Table 1).

Both MSA and MAA support the diagnosis of myositis, define distinctive phenotypes and predict disease progression and response to treatment (Table 1). Some MSAs are associated with an increased risk of cancer-associated myositis, others seem not confined to muscle inflammation, yet strictly associated with peculiar skin lesions or pulmonary involvement [2,3▪▪]. MSA laboratory detection is far from being standardized and validated, thus international collaborative efforts are mandatory on this concern.

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KEY POINTS

- Increasing evidence highlights the clinical relevance of MSA titration as useful tools for monitoring both muscle and extramuscular involvement and treatment response in myositis.
- An intriguing pathophysiological link between tumorigenesis and autoimmune response in myositis has been speculated.
- Autoantibody-associated necrotizing myopathies should be recognized as distinct immune-mediated myositis subsets.
- MSA are promising tools in identifying distinct disease entities within the clinicoserological spectrum of dermatomyositis.
- There is an urgent need to include internationally validated extended MSA testing in clinical laboratory practice.

This review provides recent evidences into the clinical and pathogenetic role of MSA and proposes guidelines for approaching MSA testing in clinical laboratory.

POLYMYOSITIS-SPECIFIC AUTOANTIBODIES AND SUBGROUP DEFINITION

The discovery of MSA has strongly contributed to the definition of immunopathological entities within heterogeneous clinical spectrum and prognosis of PM.

Antisynthetase antibodies

Anti-ARS antibodies target cytoplasmic aminoacyl-tRNA synthetases, which catalyze the binding of one amino acid to corresponding tRNA during protein synthesis. Anti-ARS antibodies interact with conformational epitopes in conserved regions of the enzyme to inhibit enzyme activity.

Eight mutually exclusive anti-ARS are identified so far: anti-Jo-1 (histidyl-tRNA synthetase), anti-PL7 (threonyl), anti-PL12, (alanyl), anti-EJ (glycyl), anti-OJ (isoleucyl), KS (asparaginyl), Zo (phenylalanyl) and Ha (tyrosyl). On average, anti-ARS antibodies are the most frequent in IIM, overall occurring in one-third of patients: anti-Jo-1 is found in 20–30% of patients, anti-PL7 or PL12, in about 5%, anti-KS, anti-OJ, anti-EJ, anti-Zo, anti-Ha, each in less than 2% of IIM patients. Specificity for the diagnosis of IIM is proximal to 100% for anti-Jo-1, not so high for the other anti-ARS [4▪], the latter being reported in other CTD, thus challenging the validity of the term 'MSA' when referred to non-Jo-1 antisynthetase [5▪]. In anti-Jo-1 positive patients, scattered necrotic muscle fibres are frequently observed, almost confined in perifascicular areas [6].

By definition, anti-ARS antibodies represent the serological marker of antisynthetase syndrome,

Table 1. Myositis-specific autoantibodies: target autoantigens and clinical associations

Autoantibody	Autoantigen	Autoantigen function	Associated phenotype	Frequency (%)
Antisynthetase	Aminoacyl-tRNA synthetases	Protein synthesis	PM/DM, antisynthetase syndrome	Overall: 30–40 Jo1: 15–24, Others: 5–10, JDM: <5
Anti-Mi-2	Nuclear DNA helicase	DNA transcription	'Classic' DM	Adult: 10–20, JDM: 3–5
Anti-TIF1γ	Transcriptional intermediary factor 1γ	DNA transcription and RNA metabolism	Adult: DM, CAM JDM: skin ulceration	Adult: 13–31, CAM: 50–75, JDM: 22–29
Anti-NXP2	Nuclear matrix protein 2	DNA transcription activation of p53	Adult: DM, CAM JDM: calcinosis	Adult: 1–17, CAM: 25–30, JDM: 23–25
Anti-MDA5	Melanoma differentiation associated gene 5	Innate immune response to viral infection	Adult: DM, ILD, CADM JDM: ILD	Adult: 7–26, CADM: 40–60, JDM: 7–38
Anti-SAE	SUMO-activating enzyme	DNA transcription Posttranslation modif.	Severe DM	Adult: 8, JDM: 1
Anti-SRP	Signal recognition particle	Protein transport	Necrotizing myositis	Adult: 5–8, JDM: 1–2
Anti-HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase *(muscle-spec.)*	Cholesterol synthesis	Necrotizing myositis	Adult PM: 5–7, Statin-associated: 40 JDM: 1
AnticN-1A	Cytosolic 5′-nucleotidase 1A *(muscle-specific)*	Muscle metabolism, RNA processing	Inclusion body myositis	IBM: 35–50, PM/DM: 5
Anti-FHL1	Four-and-a-half LIM protein 1 *(muscle-specific)*	Muscle differentiation, sarcomere assembly	Severe myopathy, dysphagia	Adult IIM: 25%

ARS, aminoacyl-tRNA synthetase; CADM, clinically amyopathic DM; CAM, cancer-associated myositis; IBM, inclusion body myositis; ILD, interstitial lung disease; JDM, juvenile DM; PM/DM, polymyositis/dermatomyositis.

characterized by the classic triad: arthritis, myositis and interstitial lung disease (ILD), usually associated with other CTD manifestations [7,8▪]. Disease prognosis is closely related to pulmonary involvement. Anti-ARS antibodies increase the likelihood of developing ILD, representing a feasible surrogate biomarker of CTD-ILD subgroup diagnosis [9▪].

Phenotypic differences are ascribed to specific anti-ARS antibody: anti-Jo-1 patients show more severe muscle disease, whereas anti-PL7 and anti-PL12 are characterized by delayed diagnosis, more severe ILD and CTD features [10▪]. Anti-ARS positivity does not seem to increase the risk of cancer [10▪]. Anti-Jo-1 antibody is associated with HLA-DRB1∗03 in polymyositis and dermatomyositis [11].

Antisignal recognition particle and anti3-hydroxy-3-methylglutaryl coenzyme A reductase antibodies

These two distinct MSAs are associated with a clinically similar disease subset. Antisignal recognition particle (anti-SRP) antibody targets a cytoplasmic ribonucleoprotein, consisting of a six-polypeptide-7SL RNA complex, which regulates the transport of nascent proteins into the endoplasmic reticulum. Major epitopes reside on SRP54 and SRP72 protein moieties. Anti-hydroxy-3-methylglutaryl coenzyme A reductase (anti-HMGCR) antibody recognizes the catalytic domain of HMGCR, the key enzyme in cholesterol biosynthesis, which is pharmacologically inhibited by statin and overexpressed in endoplasmic reticulum of muscle fibre following statin exposure. Anti-SRP or anti-HMGCR antibodies are reported in 5–10% of adult polymyositis/dermatomyositis and about 1% of juvenile forms. They are distinct markers for immune-mediated necrotizing myopathy, a chronic treatment-resistant autoimmune myositis with very high creatine phosphokinase (CK) levels, histologically characterized by a broad spectrum of prominent myofiber necrosis/regeneration, numerous macrophages and aspecific lymphocytic infiltration and sarcolemmal C5b-9 deposits, both correlated with the amount of necrosis [12▪▪]. Extramuscular involvement, that is ILD, may occasionally be observed. Both SRP and HMGCR are expressed in the sarcolemma of regenerating muscle fibres, and respective antibodies can ectopically bind their cognate autoantigen and induce complement-mediated muscle damage [12▪▪]. Anti-SRP myositis seems persistently active and more severe than anti-HMGCR one, especially in younger patients at disease onset [13▪▪]. Anti-SRP or anti-HMGCR necrotizing myositis do not seem to be triggered by cancer [13▪▪,14▪]. Genotyping revealed the association of anti-SRP antibodies with HLA DRB1∗08 : 03 [11].

Anti-HMGCR myopathy is a rare condition mostly induced by statin therapy, yet also observed in adult-onset and juvenile-onset IIM patients never exposed to statins [15▪], suggesting that various triggers might occur in disease pathogenesis. Intriguingly, anti-HMGCR myopathy is associated with HLA-DRB1∗11 : 01 in adults, while association with DRB1∗07 : 01 has been reported in children [15▪].

DERMATOMYOSITIS-SPECIFIC AUTOANTIBODIES AND SUBGROUP DEFINITION

Dermatomyositis-specific autoantigens consist of nuclear transcription factors, involved in dynamic posttranscriptional/epigenetic regulation of nuclear processes, that is gene expression, genome stability, cell cycle control and differentiation. They comprise small protein-modified factors, such as small ubiquitin-like modifier (SUMO) activating enzymes, called SAE, or SUMOylated (SUMO-activated) proteins, that is Mi-2, NXP2, TIF1γ, which participate in SUMO-mediated transcriptional repression and gene silencing. Deregulation of SUMO pathways have implications in cancer development, possibly playing a role in cancer-associated dermatomyositis [16].

Dermatomyositis-specific antibodies are valuable tools to improve diagnosis and subclassification, especially when muscle biopsy could otherwise be inconclusive (Fig. 1) [17–20].

Anti-Mi-2 antibody

Anti-Mi-2 antibody recognizes the nucleosome remodelling histone-deacetylase (NuRD) nuclear protein complex, which promotes SUMO-mediated transcriptional repression via chromatine structure remodelling. DRB1∗0302 and DRB1∗0701 are the primary HLA allelic risk factors [11,20].

Anti-Mi-2 positive dermatomyositis is usually characterized by photodistributed skin lesions, lung sparing, low frequency of cancer or other internal organ involvement and good response to treatment [21,22].

Antitranscription intermediary factor 1γ antibody

This autoantibody primarily binds the nuclear transcription factor transcription intermediary factor 1γ (TIF1γ), which acts as an E3-ubiquitin ligase, as a histone-binding protein or SUMO-interacting

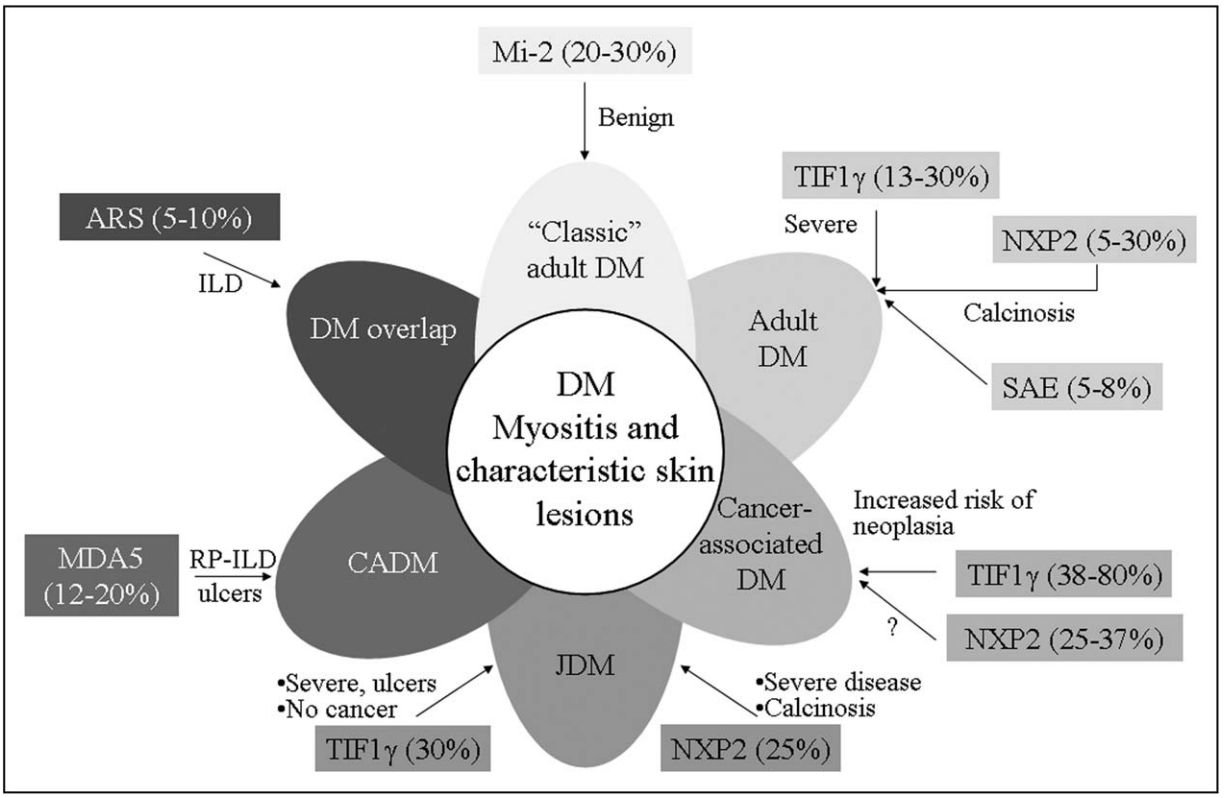

FIGURE 1. The broad clinicoserological spectrum of dermatomyositis syndromes. CADM, clinically amyopathic dermatomyositis; JDM, juvenile dermatomyositis.

protein in many cellular processes, including cell differentiation and carcinogenesis. In particular, TIF1γ inhibits transforming growth factor (TGF)-β induced transcription through ubiquitination of SMAD4. Intriguingly, TIF1 family proteins are known as tumour suppressors involved in the regulation of p53 oncogene and overexpressed in solid tumours [23▪▪].

Anti-TIF1γ antibody is almost exclusively found in 20–30% of adult and juvenile dermatomyositis worldwide, mainly associated with severe characteristic skin involvement, less likely calcinosis or systemic features [20]. Antibody positivity is strongly associated with an increased risk of cancer in middle-old age dermatomyositis patients [standardized incidence ratio (SIR) = 17.24, confidence interval (CI) 11.94–24.14] [24▪], while such an association was not confirmed in children or young adult patients. Anti-TIF1γ correlates with the onset and progression of malignancy and with shorter time interval between cancer onset and dermatomyositis diagnosis [25]. Anti-TIF1γ is undetectable in adults with primary solid tumours or other paraneoplastic rheumatic syndromes [26▪]. Intriguingly, somatic mutations in tumour *TIF1* family genes were found in anti-TIF1 antibody-positive patients with cancer-associated myositis [23▪▪], suggesting a role for TIF1 autoimmune response in the pathogenesis of paraneoplastic DM.

Antinuclear matrix protein 2 antibody

This autoantibody targets the nuclear matrix protein 2 (NXP2 or MORC3), a SUMO-interacting nuclear protein involved in SUMO-mediated transcriptional repression, chromatin remodelling, DNA repair and p53-induced tumour suppression. Anti-NXP2 antibody is detected more frequently in juvenile or young-adult white dermatomyositis patients (20–25%), than in adult ones (10–25%), although it is rarely described in Japanese populations (2–5%) [20]. Anti-NXP2 associated phenotype usually consists of young-onset severe dermatomyositis with systemic features such as distal-proximal muscle weakness, myalgia, dysphagia and mild typical skin involvement [20,27▪,28▪]; in children, gastrointestinal vasculitis, calcinosis, muscle atrophy and cramps, and muscle ischemic features can worsen disease prognosis [20,29▪]. Intriguingly, anti-NXP2 antibody predicts calcinosis in dermatomyositis (odds ratio 9.8) [3▪▪,30]. Likewise, anti-TIF1γ, the presence of anti-NXP2 antibody may represent a risk factor for cancer in adults [24▪,27▪]; however, such finding still remains a matter of debate [3▪▪,28▪].

Antismall ubiquitin-like modifier 1 activating enzyme antibody

Anti-small ubiquitin-like modifier 1 activating enzyme (anti-SAE) antibody recognizes the nuclear heterodimer called SUMO-1 activating enzyme (SAE1/2) as autoantigen, which epigenetically controls gene expression by transcription factors' sumoylation. Antibody positivity, strictly related to distinct HLA haplotypes [11], has been described in a small proportion (5–10%) of patients presenting 'classic' severe adult-onset dermatomyositis, without peculiar disease features or evolution [3▪▪,20].

Antimelanoma differentiation-associated gene 5 antibody

Melanoma differentiation-associated gene 5 (*MDA5*) target antigen belongs to retinoic acid-inducible gene (RIG)-I receptor family, cytoplasmic interferon (IFN)-induced innate sensors for viral dsRNA, which initiate signalling cascade leading to IFN-mediated antiviral host response. In addition, activation of MDA5 induces cell growth inhibition and apoptosis in cancer cells [31].

Anti-MDA5 antibody has been detected in Asian more than white populations, as a specific marker of adult or juvenile dermatomyositis often presenting with peculiar hypo/amyopathic picture, severe mucocutaneous ulcerative lesions, arthralgia/arthritis and/or refractory rapidly progressive ILD (RPILD) [32,33]. A systematic meta-analysis confirmed the diagnostic value of anti-MDA5 antibody for dermatomyositis-RPILD [34▪▪]. Anti-MDA5 antibody-associated RPILD may be present in the absence of muscle or skin manifestations [35]. High serum anti-MDA5 antibody and ferritin levels, suggestive of macrophage activation, represent reliable prognostic factors in patients with dermatomyositis-RPILD [36▪,37]. No association with cancer has clearly emerged so far [3▪▪,38].

NOVEL PUTATIVE MYOSITIS-SPECIFIC AUTOANTIBODY AND IDIOPATHIC INFLAMMATORY MYOPATHY PATIENTS' STRATIFICATION

Novel autoantibody specificities have demonstrated clinical utility for patients' stratification in defined myositis subsets.

Anticytosolic 5'-nucleotidase 1A antibody

Recently, anticytosolic 5'-nucleotidase 1A (anti-cN-1A) antibody has been proposed as serological tool for early diagnosis of inclusion body myositis (IBM) and patients' stratification [39▪]. Sporadic IBM, a typically over 50 years-old disabilitating myopathy, might be initially misdiagnosed as inflammatory myositis, particularly polymyositis. The cN-1A enzyme, highly expressed in skeletal muscle, regulates cell metabolism, replication and energy balance. In IBM muscle biopsies, the autoantigen is aberrantly aggregated in rimmed vacuoles and myofiber perinuclear regions, and anticN-1A antibodies from IBM patients may affect myofiber protein degradation, thus playing a pathogenic role in the disease [40▪▪].

Given the variability in patient population, detection methods and epitopes' identification, findings on anticN-1A antibody specificity are discordant [40▪▪,41–43]. The antibody is not only present in approximately one-third of IBM patients but also in small proportion of adult or juvenile myositis, or other CTDs. Antibody positivity may aid in stratifying IBM patients at a higher risk for severe phenotype, respiratory involvement and mortality [39▪,44▪].

Antifour-and-a-half LIM protein 1 antibody

By screening a muscle-specific cDNA library, FHL1 protein has been identified as a novel specific target of IIM autoimmune response [2]. Anti-FHL1 antibodies were found in 25% of IIM patients, but not in healthy/disease controls [45]. Of note, *FHL1* mutations are known to be associated with rare FHL1-related hereditary myopathies, characterized by generalized progressive muscle dysfunction and damage. FHL1 is essential for sarcomere assembly and normal skeletal muscle function. Anti-FHL1 antibody may be predictive of treatment-resistant IIM, characterized by dysphagia, severe muscle weakness and atrophy, and peculiar immunohistopathological features such as muscle fibre necrosis, fat accumulation and FHL1 abnormal patchy aggregation [45].

MYOSITIS-SPECIFIC AUTOANTIBODY AS CLUES IN THE PATHOGENESIS OF MYOSITIS

Increasing evidence suggests that myositis autoantigens drive pathogenic antigen-specific autoimmune response towards muscle and extramuscular tissues [40▪▪,46▪▪]. Both immunogenetic background and different environmental triggers, for example cigarette smoking, statins, ultraviolet (UV) light, or tumorigenesis, contribute to the generation of MSA [11,47▪▪,48]. Myositis-specific autoantigens are overexpressed and modified in apoptotic and regenerating muscle fibres, as well as in extramuscular tissues and tumour cells of

myositis patients [23▪▪,49]. Intriguingly, during tumorigenesis, somatically mutated antigens, that is TIF-1γ, become neoepitopes for initial MHC-restricted antitumor immune response [23▪▪]. Thus, regenerating muscle fibres and tumour cells share similar immunostimulating phenotypes, suggesting a pathogenetic link between cancer and autoimmunity in myositis. In genetically predisposed individuals, the generation of neoepitopes in normal or tumour tissues can induce autoreactive B cell immune response to produce MSA. The aberrant autoantigens' expression in several tumoral tissues may drive a misdirected immunologic antitumoral response, which might or might not efficiently eradicate the tumour.

Immune complexes containing myositis antigens and cognate autoantibodies likely stimulate the type I IFN signalling, which is markedly upregulated in myositis tissues [50,51▪▪], thus in turn increasing antigen availability and propagation of the immune response. The autoantigens show proinflammatory and chemoattractant properties, contributing *per se* to innate immune activation. Intrinsic nonimmune changes in the muscle, that is transcriptional deregulation of specific long noncoding RNAs involved in muscle proliferation and differentiation, might also contribute to muscle inflammation [52▪▪].

RECENT ADVANCES AND PERSPECTIVES IN LABORATORY DETECTION OF MYOSITIS-SPECIFIC AUTOANTIBODY

In the context of high pretest probability of myositis, the utility of extended MSA testing has being increasingly emphasized. However, anti-Jo-1 is the sole routinely detected MSA considered in the new IIM classification criteria [53▪], thus opening a heated debate [54,55]. The antinuclear antibody test is unreliable, due to scarce and preferential cytoplasmic expression of MSA target antigens [56]. Immunoprecipitation, together with high-sensitive immunoprecipitation-western blot techniques, represent the gold-standard tools for MSA determination, yet too complex and time-consuming for adopting in clinical setting [57▪▪]. Alternatively, commercial antigen-specific assays, based on single-analyte ELISA tests or similar, or multiplex line/dot blot and bead-based immunoassays, have recently been assessed, resulting in reliable diagnostic performance and clinical adherence of autoantibody titration for many of them [4▪,26▪,58,59] (Fig. 2). Strong positivity is highly predictive of IIM, yet the antibody cut-off definition remains a challenge. Unusual MSA coexistence could increasingly become a matter of concern and should be interpreted with caution. Crucial for testing accuracy is the quality of recombinant human proteins

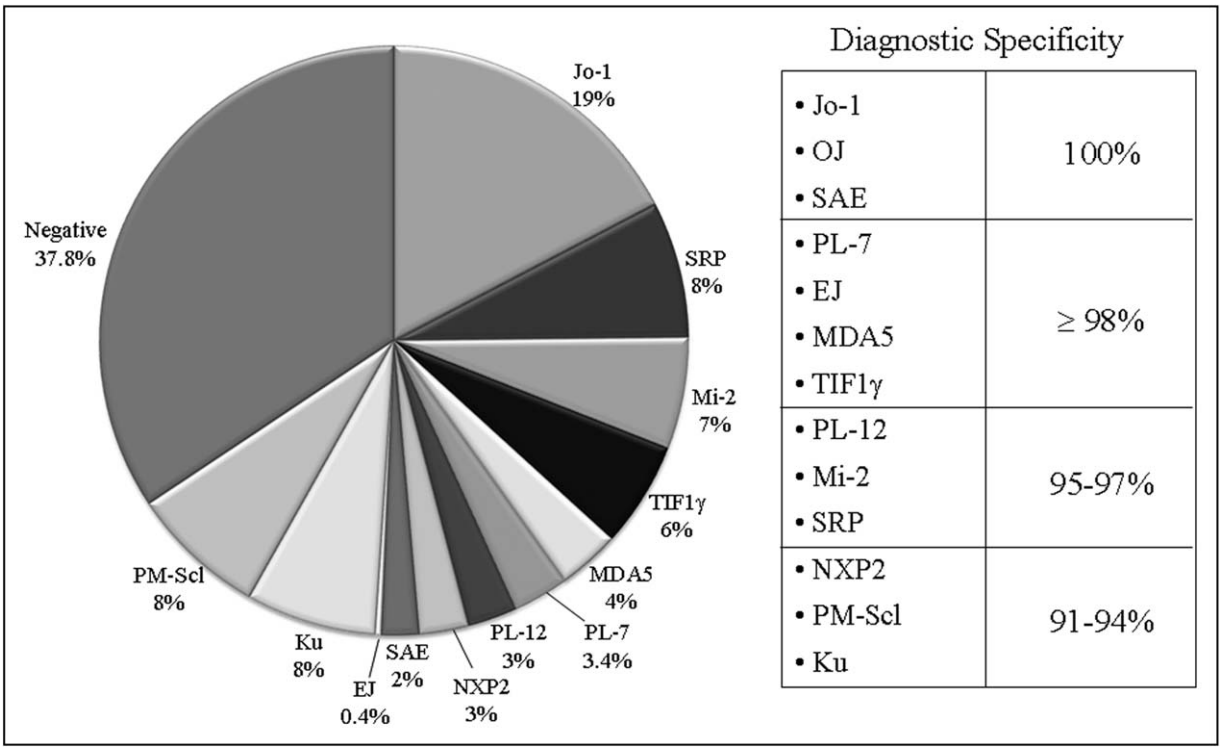

FIGURE 2. Determination of major MSA/MAA by commercial line blot immunoassay. Frequency distribution (percentual values of positivity) and diagnostic specificity in a multicentre cohort of 267 patients with IIM and 258 controls.

used as antigens, and the use of proper equipment and data processing. Major limitations to application of MSA testing in clinical laboratories is the lack of reference materials and procedures [60]. There needs to be a concerted effort to establish consensus guidelines for MSA detection, desumed from international standardization studies on core patients and controls populations involving both clinical laboratories and industry [60]. Appropriate MSA laboratory flowchart should include extended multianalyte immunoassay as first-choice screening test, followed by antibody confirmation and titration by antigen-specific ELISA-based assays [60].

CONCLUSION

MSA are diagnostic markers for autoimmune myositis, helpful in identifying distinct immunopathological entities and addressing to a more appropriate treatment. Robust detection of MSA in early phase of the disease predicts clinical course and disease prognosis. The strong associations between certain immunologic triggers and MSA provide clues into the etiopathogenetic mechanisms leading to loss of tolerance in IIM.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Mammen AL, Casciola-Rosen L, Christopher-Stine L, *et al.* Myositis-specific autoantibodies are specific for myositis compared to genetic muscle disease. Neurol Neuroimmunol Neuroinflamm 2015; 2:e172.
2. Lundberg IE. New tools for diagnosis and therapy. Nat Rev Rheumatol 2017; 13:74–76.
3. ■■ Best M, Jachiet M, Molinari N, *et al.* Distinctive cutaneous and systemic features associated with specific antimyositis antibodies in adults with dermatomyositis: a prospective multicentric study of 117 patients. J Eur Acad Dermatol Venereol 2018; 32:1164–1172.

This seminal study prospectively assessed the predictive role of MSA for distinctive antibody-associated clinical phenotypes in adult dermatomyositis. One-hundred and seventeen patients were included in a 3.5-year multicentre prospective study. Recognizing the MSA-associated phenotype in adult dermatomyositis is helpful for a more accurate appraisal of the risk of cancer, interstitial lung disease and calcinosis.

4. ■ Ghirardello A, Bettio S, Bassi N, *et al.* Autoantibody testing in patients with myositis: clinical accuracy of a multiparametric line immunoassay. Clin Exp Rheumatol 2017; 35:176–177.

This interesting letter reports data concerning the multicentre validation of a new generation line blot assay for extended MSA testing. The good diagnostic accuracy of multiparameter line immunoassay for serum MSA determination is demonstrated.

5. ■ Pauling JD, Salazar G, Lu H, *et al.* Presence of antieukaryotic initiation factor-2B, anti-RuvBL1/2 and antisynthetase antibodies in patients with antinuclear antibody negative systemic sclerosis. Rheumatology (Oxford) 2018; 57: 712–717.

This study underlines the lower than expected specificity of non-Jo-1 antisynthetase antibodies for myositis, by reporting antisynthetase antibodies in antinuclear antibody-negative systemic sclerosis without features of overt myositis.

6. Mescam-Mancini L, Allenbach Y, Hervier B, *et al.* Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis. Brain 2015; 138:2485–2492.
7. Monti S, Montecucco C, Cavagna L. Clinical spectrum of anti-Jo-1-associated disease. Curr Opin Rheumatol 2017; 29:612–617.
8. ■ González-Gay MA, Montecucco C, Selva-O'Callaghan A, *et al.* Timing of onset affects arthritis presentation pattern in antisynthetase syndrome. Clin Exp Rheumatol 2018; 36:44–49.

The study describes in detail the timing of onset of major clinical features of antisynthetase syndrome in a large multicentre cohort of longitudinally investigated patients.

9. ■ Cotton CV, Spencer LG, New RP, Cooper RG. The utility of comprehensive autoantibody testing to differentiate connective tissue disease associated and idiopathic interstitial lung disease subgroup cases. Rheumatology (Oxford) 2017; 56:1264–1271.

This comprehensive review article exhaustively emphasizes the utility of extended CTD-specific serology, including MSA profiling, as a reliable tool for improving accurate ILD subgroup diagnosis and clinical care.

10. ■ Pinal-Fernandez I, Casal-Dominguez M, Huapaya JA, *et al.* A longitudinal cohort study of the antisynthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology (Oxford) 2017; 56:999–1007.

This study longitudinally assessed the prognostic value of antisynthetase antibody specificity as an independent risk factor for ILD severity in antisynthetase syndrome.

11. McHugh NJ, Tansley SL. Autoantibodies in myositis. Nat Rev Rheumatol 2018; 14:290–302.
12. ■■ Allenbach Y, Arouche-Delaperche L, Preusse C, *et al.* Necrosis in anti-SRP+ and anti-HMGCR+myopathies: role of autoantibodies and complement. Neurology 2018; 90:e507–e517.

A well detailed histopathological, immunohistochemical and gene expression analysis on muscle biopsies from SRP+ or HMGCR+ IMNM patients is reported, together with in-vitro studies on autoantibody-induced myotube damage. The study conclusively demonstrated that both anti-SRP and anti-HMGCR antibodies are able to induce complement-mediated myofiber damage.

13. ■■ Pinal-Fernandez I, Parks C, Werner JL, *et al.* Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. Arthritis Care Res (Hoboken) 2017; 69: 263–270.

This longitudinal study elegantly assessed the disease course and independent prognostic factors influencing recovery in anti-SRP+ compared with anti-HMGCR+ myositis patients. Younger age at onset and persistent disease activity despite treatment were the main adverse prognostic factors in anti-SRP+ patients.

14. ■ Tiniakou E, Pinal-Fernandez I, Lloyd TE, *et al.* More severe disease and slower recovery in younger patients with anti3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. Rheumatology (Oxford) 2017; 56:787–794.

Similar to the study above, the authors longitudinally investigated disease severity and response to therapy in a large cohort of patients with anti-HMGCR antibody-associated myositis. Younger age at onset and persistently high CK levels have been found as the main factors related to adverse outcome and poor treatment response.

15. ■ Kishi T, Rider LG, Pak K, *et al.* Association of anti3-hydroxy-3-methylglutaryl-Coenzyme A reductase autoantibodies with DRB1*07:01 and severe myositis in juvenile myositis patients. Arthritis Care Res (Hoboken) 2017; 69:1088–1094.

This is a seminal research that first characterized the clinical phenotype and genetic background of juvenile anti-HMGCR antibody-positive myositis patients.

16. Eifler K, Vertegaal ACO. SUMOylation-mediated regulation of cell cycle progression and cancer. Trends Biochem Sci 2015; 40:779–790.
17. Iaccarino L, Ghirardello A, Bettio S, *et al.* The clinical features, diagnosis and classification of dermatomyositis. J Autoimmun 2014; 48-49:122–127.
18. Deakin CT, Yasin SA, Simou S, *et al.* Muscle biopsy findings in combination with myositis-specific autoantibodies aid prediction of outcomes in juvenile dermatomyositis. Arthritis Rheumatol 2016; 68:2806–2816.
19. Uruha A, Suzuki S, Nishino I. Diagnosis of dermatomyositis: autoantibody profile and muscle pathology. Clin Exp Neuroimmunol 2017; 8:302–312.
20. Wolstencroft RW, Fiorentino DF. Dermatomyositis clinical and pathological phenotypes associated with myositis-specific autoantibodies. Curr Rheumatol Rep 2018; 20:28.

21. Fujimoto M, Murakami A, Kurei S, *et al.* Enzyme-linked immunosorbent assays for detection of antitranscriptional intermediary factor-1 gamma and anti-Mi-2 autoantibodies in dermatomyositis. J Dermatol Sci 2016; 84:272–281.

22. Matsuda T, Ueda-Hayakawa I, Kambe N, *et al.* Four cases of anti-Mi-2 antibody-positive dermatomyositis: relationship between anti-Mi-2 antibody titre and disease severity and activity. J Eur Acad Dermatol Venereol 2018; 32:e233–e234.

23. ■■ Pinal-Fernandez I, Ferrer-Fabregas B, Trallero-Araguas E, *et al.* Tumour TIF1 mutations and loss of heterozygosity related to cancer-associated myositis. Rheumatology (Oxford) 2018; 57:388–396.

This is a seminal research first reporting that *TIF1* genes show increased number of somatic mutations and loss of heterozygosity in tumours from paraneoplastic anti-TIF1γ-positive patients, thus likely suggesting a causative pathophysiological link between carcinogenesis and cancer-associated myositis. Genetic alterations in *TIF1* genes together with the known increased expression of misfunctional TIF1 proteins in tissues from paraneoplastic anti-TIF1γ-positive patients intriguingly suggest that autoantibody response would be a misdirected antitumour immune response.

24. ■ Yang H, Peng Q, Yin L, *et al.* Identification of multiple cancer-associated myositis-specific autoantibodies in idiopathic inflammatory myopathies: a large longitudinal cohort study. Arthritis Res Ther 2017; 19:259.

This large longitudinal cohort study in Chinese population estimated the cancer risk associated with different MSA in patients with IIM by SIR. The study demonstrated that some MSA, that is anti-TIF1γ, anti-NXP2 and anti-SAE1, are associated with an increased risk of paraneoplastic myositis, and that other MSA might also be driven by malignancy in some cases.

25. Ogawa-Momohara M, Muro Y, Mitsuma T, *et al.* Strong correlation between cancer progression and antitranscription intermediary factor 1γ antibodies in dermatomyositis patients. Clin Exp Rheumatol 2018. [Epub ahead of print]

26. ■ Venalis P, Selickaja S, Lundberg K, *et al.* Association of antitranscription intermediary factor 1γ with paraneoplastic rheumatic syndromes other than dermatomyositis. Arthritis Care Res 2018; 70:648–651.

This research demonstrated that IgG anti-TIFγ autoimmune response is specifically observed almost exclusively in cancer-associated dermatomyositis, although not in primary solid tumours or other paraneoplastic rheumatic syndromes.

27. ■ Albayda Y, Pinal-Fernandez I, Huang W, *et al.* Antinuclear matrix protein 2 autoantibodies and edema, muscle disease, and malignancy risk in dermatomyositis patients. Arthritis Care Res 2017; 69:1771–1776.

In this brief report, the Authors exhaustively described the anti-NXP2 antibody-associated clinical phenotype in a longitudinal cohort of adult dermatomyositis patients, mainly focusing on the characteristics of skeletal muscle involvement and peculiar extramuscular features related to skin and pulmonary involvement.

28. ■ Rogers A, Chung L, Li S, *et al.* Cutaneous and systemic findings associated with nuclear matrix protein 2 antibodies in adult dermatomyositis patients. Arthritis Care Res 2017; 69:1909–1914.

Similar to the study above, the adult anti-NXP2 antibody-associated dermatomyositis subset has been defined as mainly characterized by severe myositis often accompanied by mild typical skin presentation and calcinosis.

29. ■ Aouizerate J, De Antonio M, Bader-Meunier B, *et al.* Muscle ischaemia associated with NXP2 autoantibodies: a severe subtype of juvenile dermatomyositis. Rheumatology (Oxford) 2018; 57:873–879.

Peculiar clinical and histopathological findings strictly related to anti-NXP2 antibody positivity in juvenile dermatomyositis patients were described in this well performed and detailed study.

30. Fredi M, Bartoli F, Cavazzana I, *et al.* Calcinosis in poly-dermatomyositis: clinical and laboratory predictors and treatment options. Clin Exp Rheumatol 2017; 35:303–308.

31. Wu Y, Wu X, Wu L, *et al.* The anticancer functions of RIG-I-like receptors, RIG-I and MDA5, and their applications in cancer therapy. Transl Res 2017; 190:51–60.

32. Sontheimer RD. MDA5 autoantibody-another indicator of clinical diversity in dermatomyositis. Ann Transl Med 2017; 5:160.

33. Moghadam-Kia S, Oddis CV, Sato S, *et al.* Antimelanoma differentiation-associated gene 5 antibody: expanding the clinical spectrum in North American patients with dermatomyositis. J Rheumatol 2017; 44:319–325.

34. ■■ Li L, Wang Q, Wen X, *et al.* Assessment of anti-MDA5 antibody as a diagnostic biomarker in patients with dermatomyositis-associated interstitial lung disease or rapidly progressive interstitial lung disease. Oncotarget 2017; 8:76129–76140.

By means of a systematic meta-analysis, this study assessed the high diagnostic value of anti-MDA5 antibody as a noninvasive biomarker for dermatomyositis-associated RPILD, a refractory and potentially fatal disease complication.

35. González-Moreno J, Raya-Cruz M, Losada-Lopez I, *et al.* Rapidly progressive interstitial lung disease due to anti-MDA5 antibodies without skin involvement: a case report and literature review. Rheumatol Int 2018; 38:1293–1296.

36. ■ Matsushita T, Mizumaki K, Kano M, *et al.* Antimelanoma differentiation-associated protein 5 antibody level is a novel tool for monitoring disease activity in rapidly progressive interstitial lung disease with dermatomyositis. Br J Dermatol 2017; 176:395–402.

This longitudinal study demonstrated the utility of serum anti-MDA5 antibody level determination for monitoring disease activity and response to therapy in patients with dermatomyositis-associated rapidly progressive ILD. The importance of serial measurements of anti-MDA5 antibody levels for predicting pulmonary involvement relapse is emphasized.

37. Fujiki K, Kotani T, Isoda K, *et al.* Evaluation of clinical prognostic factors for interstitial pneumonia in anti-MDA5 antibody-positive dermatomyositis patients. Mod Rheumatol 2018; 28:133–140.

38. Sato S, Murakami A, Kuwajima A, *et al.* Clinical utility of an enzyme-linked immunosorbent assay for detecting antimelanoma differentiation-associated gene 5 autoantibodies. PLoS One 2016; 11:e0154285.

39. ■ Lilleker JB, Rietveld A, Pye SR, *et al.* Cytosolic 5'-nucleotidase 1A autoantibody profile and clinical characteristics in inclusion body myositis. Ann Rheum Dis 2017; 76:862–868.

A detailed retrospective analysis of pooled data from various European IBM registries demonstrated that anticN1A antibody is useful to identify patients with more severe clinical and histopathological features.

40. ■■ Tawara N, Yamashita S, Zhang X, *et al.* Pathomechanisms of anticytosolic 5'-nucleotidase 1A autoantibodies in sporadic inclusion body myositis. Ann Neurol 2017; 81:512–525.

This study described the peculiar clinicopathological features of muscle biopsies from anticN1A antibody-positive IBM patients, and showed for the first time by in-vitro and in-vivo passive immunization model that anticN1A autoantibodies might be pathogenic, by inducing protein degradation and accumulation in myofibers and macrophage infiltration in muscle tissue.

41. Herbert MK, Stammen-Vogelzangs J, Verbeek MM, *et al.* Disease specificity of autoantibodies to cytosolic 5'-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. Ann Rheum Dis 2016; 75:696–701.

42. Lloyd TE, Christopher-Stine L, Pinal-Fernandez I, *et al.* Cytosolic 5'-nucleotidase 1A as a target of circulating autoantibodies in autoimmune diseases. Arthritis Care Res 2016; 68:66–71.

43. Muro Y, Nakanishi H, Katsuno M, *et al.* Prevalence of anti-NT5C1A antibodies in Japanese patients with autoimmune rheumatic diseases in comparison with other patient cohorts. Clin Chim Acta 2017; 472:1–4.

44. ■ Yeker RM, Pinal-Fernandez I, Kishi T, *et al.* Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis. Ann Rheum Dis 2018; 77:714–719.

This large study extensively describes for the first time the clinical features of anticN1A antibody-positive juvenile myositis patients, finding that anticN1A autoantibodies seem to be associated with more severe disease also in juvenile patients.

45. Albrecht I, Wick C, Hallgren Å, *et al.* Development of autoantibodies against muscle-specific FHL1 in severe inflammatory myopathies. J Clin Invest 2015; 125:4612–4624.

46. ■■ Arouche-Delaperche L, Allenbach Y, Amelin D, *et al.* Pathogenic role of antisignal recognition protein and anti3-hydroxy-3-methilglutaryl-CoA reductase antibodies in necrotizing myopathies: myofiber atrophy and impairment of muscle regeneration in necrotizing autoimmune myopathies. Ann Neurol 2017; 81:538–548.

The Authors demonstrated that SRP and anti-HMGCR antibodies induce muscle fibre atrophy and regeneration impairment with high levels of inflammatory cytokines, supporting the pathogenic role of these autoantibodies in immune-mediated necrotizing myopathy.

47. ■■ Schiffenbauer A, Faghihi-Kashani S, O'Hanlon TP, *et al.* The effect of cigarette smoking on the clinical and serological phenotypes of polymyositis and dermatomyositis. Semin Arthritis Rheum 2018; pii:S0049-0172(17)30630-3. doi:10.1016/j.semarthrit.2018.02.003. [Epub ahead of print]

The study explored for the first time the possible gene–environment interactive effects in susceptibility to IIM. By a large population study using extensive autoantibody profiling and high-resolution HLA data, the authors elegantly demonstrated that both HLA allele risk factors and cigarette smoking have an impact on the frequencies of defined clinical and serological phenotypes in autoimmune myositis. The study was conducted on 465 racially diverse patients.

48. Adler BL, Christopher-Stine L. Triggers of inflammatory myopathy: insights into pathogenesis. Discov Med 2018; 25:75–83.

49. Selva-O'Callaghan A, Alvarado-Cardenas M, Pinal-Fernández I, *et al.* Statin-induced myalgia and myositis: an update on pathogenesis and clinical recommendations. Expert Rev Clin Immunol 2018; 14:215–224.

50. De Paepe B. Interferons as components of the complex web of reactions sustaining inflammation in idiopathic inflammatory myopathies. Cytokine 2015; 74:81–87.

51. ■■ Huard C, Gullà SV, Bennett DV, *et al.* Correlation of cutaneous disease activity with type 1 interferon gene signature and interferon β in dermatomyositis. Br J Dermatol 2017; 176:1224–1230.

This interesting study demonstrates that type I IFN-inducible gene signature and IFN-β in blood are highly differentially deregulated in dermatomyositis. High type I IFN pathway gene signature in blood and high levels of IFN-β, but not IFN-α, correlate with cutaneous disease activity indices, such as CDASI, thus offering as promising surrogate biomarkers of cutaneous disease activity in clinical trials.

52. ■■ Hamann PD, Roux BT, Heward JA, *et al.* Transcriptional profiling identifies differential expression of long noncoding RNAs in Jo-1 associated and inclusion body myositis. Sci Rep 2017; 7:8024.

This elegant study presented original data on the possible contribution of intrinsic nonimmune changes in the muscle to the pathogenesis of anti-Jo-1 associated myositis and IBM. By next-generation sequencing analysis of the transcriptome in the muscle biopsies, the authors demonstrated that transcriptional profiles of certain long noncoding RNAs involved in muscle proliferation and differentiation are differentially upregulated in autoimmune myositis and IBM. The involvement of nonimmune pathogenetic mechanisms in myositis could explain the partial response to anti-inflammatory and immunosuppressive treatments.

53. Lunberg IE, Tjärnlund A, Bottai M, *et al.* 2017 European League Against ■ Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis 2017; 76:1955–1964.
In this study, the new 2017 EULAR/ACR classification criteria for adult and juvenile IIM and their major subgroups have been proposed by international multidisciplinary expert groups and partially validated.
54. Malaviya AN. EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: little emphasis on autoantibodies, why? Ann Rheum Dis 2017; pii:annrheumdis-2017-212701. doi:10.1136/annrheumdis-2017-212701. [Epub ahead of print]
55. Vulsteke JB, De Langhe E, Claeys KG, *et al.* Detection of myositis-specific antibodies. Ann Rheum Dis 2018; pii:annrheumdis-2017-212915. doi:10.1136/annrheumdis-2017-212915. [Epub ahead of print]
56. Fritzler MJ, Choi MY, Mahler M. The antinuclear antibody test in the diagnosis of antisynthetase syndrome and other autoimmune myopathies. J Rheumatol 2018; 45:444–445.
57. Ceribelli A, Isailovic N, De Santis M, *et al.* Myositis-specific autoantibodies and ■■ their association with malignancy in Italian patients with polymyositis and dermatomyositis. Clin Rheumatol 2017; 36:469–475.
This study described in detail the analytical and clinical accuracy of protein and RNA immunoprecipitation for MSA laboratory detection, and the high reliability of immunoprecipitation-western blot methodology for identifying novel myositis-related autoantibody specificities.
58. Musset L, Allenbach Y, Benveniste O, *et al.* Anti-HMGCR antibodies as a biomarker for immune-mediated necrotizing myopathies: a history of statins and experience from a large international multicenter study. Autoimmun Rev 2016; 15:983–993.
59. Gono T, Okazaki Y, Murakami A, Kuwana M. Improved quantification of a commercial enzyme-linked immunosorbent assay kit for measuring anti-MDA5 antibody. Mod Rheumatol 2018; 1–6; doi:10.1080/14397595.2018.1452179. [Epub ahead of print]
60. Mahler M, Fritzler MJ. Detection of myositis-specific antibodies: additional notes. Ann Rheum Dis 2018; pii:annrheumdis-2018-213153. doi:10.1136/annrheumdis-2018-213153. [Epub ahead of print]

Dermatomyositis etiopathogenesis: a rebel soldier in the muscle

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Purpose of review
The purpose of this article is to review the etiopathogenesis of dermatomyositis, including the predisposing factors, triggers, inflammatory cells, pathways and target antigens associated with dermatomyositis.

Recent findings
During the last few years, we have made considerable progress in unveiling the etiopathogenesis of dermatomyositis. In the first place, we have defined genes within the major histocompatibility complex locus as the greatest genetic risk factor for the disease. Second, we have demonstrated that certain environmental factors, as well as tumors, may trigger certain dermatomyositis subtypes. Moreover, we have established the importance of the interferon pathway in dermatomyositis pathogenesis compared with other myositis subtypes. But probably, the most remarkable advance has been the discovery of multiple autoantibodies that define groups of patients with characteristic clinical features, prognosis and response to treatment.

Summary
Dermatomyositis cause and pathogenesis have proven to be a complex and fascinating task for the scientific community and the last decade has been full of new findings on how the disease starts and how it causes damage to different organ systems. However, we have still more questions than answers in this topic, answers that will be critical to understanding autoimmunity and finding effective therapies to dermatomyositis.

Keywords
autoantibodies, cause, dermatomyositis, immunology, pathogenesis

INTRODUCTION

Dermatomyositis is a rare group of autoimmune diseases directed against the muscle and the skin with occasional involvement of other organs systems like the lungs or the joints [1].

However, our understanding of dermatomyositis has changed dramatically over the years. From the original conception that dermatomyositis was a single but highly heterogeneous entity characterized by various degrees of skin and muscle involvement, we have transitioned to a model in which dermatomyositis is recognized by most experts to be a composite of individual diseases defined by the presence of autoantibodies recognizing specific autoantigens (i.e. Mi-2 [2], NPX2 [3], TIF1γ [4▪▪], SAE [5] or MDA5 [6]). Supporting this model, data not only suggests that these different dermatomyositis subsets have very different clinical features, but also that they have different etiopathogenesis, prognosis and responses to treatment [1].

Dermatomyositis etiopathogenesis comprises a broad field of research, including the causes of the disease, the predisposing factors, the inflammatory cells implicated and the proteins that are used to modulate inflammation and cause damage. Being that dermatomyositis is a disorder that affects multiple organs and systems, all these categories have been studied not just in the muscle, but also in the rest of the tissues affected by the disease, such as the skin or the lungs [1]. Moreover, to increase the complexity of the topic, much of the early research focused in general mechanisms of dermatomyositis, but in the recent years we have started also to understand the specific differences between autoantibody subsets [1].

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KEY POINTS

- Autoantibodies define specific dermatomyositis subgroups.
- Genes within the major histocompatibility complex are the most important genetic risk factor in dermatomyositis.
- Tumors and infections are the most likely dermatomyositis triggers.
- The interferon pathway has been implicated in dermatomyositis pathogenesis.

In this review, we will discuss the etiopathogenesis of dermatomyositis. To structure it in a systematic and clear way first we will go over the dermatomyositis risk factors. Later we will analyze the proposed triggers of the disease and discuss the autoantigens, inflammatory cells and proteins implicated both in modulating inflammation and causing harm. Finally, we will discuss the different theories that have been proposed to explain dermatomyositis pathogenesis (Table 1).

THE SUBSTRATE: PREDISPOSING FACTORS

Several epidemiological factors have been associated with a higher risk of developing dermatomyositis. For example, females are more likely to develop dermatomyositis [7] with children and middle-aged adults being the most common ages of onset [7]. Moreover, black patients may be comparatively more susceptible to develop the disease [8] and some dermatomyositis subsets (anti-MDA5) are more prevalent in Asian cohorts [9,10].

Regarding the environmental risk factors of dermatomyositis, it has been reported that juvenile forms of the disease occur more commonly in spring and summer [11], and anti-MDA5 dermatomyositis has been reported to occur more often from August to March [12]. In addition, areas with higher ultraviolet radiation intensity (closer to the equator) showed higher relative frequency of dermatomyositis compared with other types of myositis [13▪]. Moreover, smoking has been inversely associated with the TIF1γ myositis [14] while the presence of pollutants and tobacco during pregnancy has been implicated with a higher risk of developing juvenile dermatomyositis [15].

There is also convincing evidence supporting a genetic predisposition in patients with dermatomyositis [16▪▪]. Even if very few cases of purely familial dermatomyositis have been described (none with a definitive genetic diagnosis) [17–19], a family history of autoimmune diseases is known to increase the chances of developing dermatomyositis [20]. The predisposing genetic factors of dermatomyositis have been extensively studied and it is known that

Table 1. Proposed factors implicated in dermatomyositis etiopathogenesis

Predisposing factors	
Epidemiological	Female predominance
	Children and middle-aged adults
	Race
Environmental	Seasonality
	Ultraviolet radiation
	Smoking, air pollutants
Genetic	Major histocompatibility complex
	Cytokine polymorphisms
Triggers	Cancer
	Virus
	Drugs
Antigens	Anti-Mi-2
	Anti-NXP2
	Anti-TIF1γ
	Anti-MDA5
	Anti-SAE
Tissue involvement	Muscle
	Skin
	Lungs
	Joints
	Gastrointestinal
Inflammatory cells	CD4+ T cells
	B cells
	Macrophages
	Plasma cells
Proinflammatory proteins	IFN type I
	MHC-I
	RIG-1
	MxA
	Other IFN-inducible genes
	Interleukins and other cytokines
	Cytotoxic proteins (perforin and granzyme B)
	Soluble intercellular adhesion proteins
Theories	
Antibody-centered	Trigger → antibody → endothelial cell → ischemia → target tissue damage
IFNpathy	Trigger → IFN type I production → target tissue damage and maintenance of immune process

IFN, interferon; MHC, major histocompatibility complex.

certain variants of the major histocompatibility complex (MHC) located on chromosome 6 are the strongest genetic markers in these patients [16▪▪]. Characteristically, a combination of MHC alleles denominated the 8.1 ancestral haplotype defines the increased risk of developing myositis [16▪▪]. This haplotype is the most common in northern Europe and has been associated with predisposition to multiple other autoimmune diseases [16▪▪]. Particularly, the DRB1∗03:01 allele showed slightly stronger associations both in adult and juvenile dermatomyositis [16▪▪]. In addition, genetic alterations in cytokine genes may increase the risk of developing juvenile dermatomyositis (Table 1) [21].

THE SPARK THAT INITIATES THE FIRE: THE TRIGGERS

Over the years many factors have been proposed to be the trigger of dermatomyositis, including cancer, infectious diseases, drugs and pregnancy.

The association between cancer and dermatomyositis has been known for decades and repeatedly confirmed around the world [22]. This risk of cancer is not homogeneous in all dermatomyositis subtypes, being particularly high in anti-TIF1γ patients [4▪▪] and lower, but also significant, in anti-NXP2 patients [3]. Regarding anti-TIF1γ, it has been recently shown that tumors from patients with dermatomyositis and this autoantibody show mutations and loss of heterozygosity (LOH) in *TIF1* genes more frequently than tumors from anti-TIFγ negative dermatomyositis patients [23▪]. This would suggest that tumor mutations may trigger dermatomyositis and also that the tumors of these patients may avoid immune surveillance by deleting the mutated genetic region through LOH [23▪].

In addition to cancer, infections have been also proposed to be possible dermatomyositis triggers by means of molecular mimicry. Although no specific organisms have been proven to cause dermatomyositis so far, the abovementioned seasonality of certain forms of the disease [11], the implication of certain dermatomyositis autoantigens (MDA5) [6] in the intracellular recognition of viruses [24], the presence of an interferon signature [25▪▪], usually implicated in antiviral responses, in the muscle [25▪▪], skin [26] and blood [27] of these patients, and the homology of certain of these autoantigens with viral proteins (data not published) suggest that infections could be an important dermatomyositis trigger.

Complementarily, several drugs have proposed to induce dermatomyositis, including D-penicillamine, anti-TNF-α drugs [28], IFN-α [29] and checkpoint inhibitors (ipilimumab) [30]. However, the evidence supporting these associations are low.

Finally, pregnancy was also proposed to be a possible dermatomyositis trigger [31]. However, the only study specifically designed to address this point found that the chances of developing dermatomyositis during pregnancy were the same as in any other period during childbearing age, ruling out pregnancy as a possible trigger for dermatomyositis [32]. Alternatively, this same study confirmed previous observations suggesting that pregnancy has a benign immunomodulatory effect on the activity of the disease that stops immediately after delivery, which is accompanied by frequent flares of the disease during puerperium [32]. Significantly, during pregnancy, cells are interchanged bidirectionally between the fetus and the mother in a phenomenon called microchimerism. These cells can persist in the host recipient and were studied as candidate triggers for juvenile [33,34] and adult dermatomyositis [35]. However, their pathogenic role could not be confirmed (Table 1) [35,36].

THE TARGETS: AUTOANTIGENS

As mentioned earlier, one of the greatest advances on myositis etiopathogenesis was finding that these patients harbor autoantibodies directed against nuclear or cytoplasmic autoantigens [1]. Anti-Mi-2 [2], anti-TIF1γ [4▪▪], anti-NXP2 [3], anti-SAE [5] or anti-MDA5 [6] are the most common dermatomyositis autoantibodies and one of these is present in around 70% of dermatomyositis patients [37]. These autoantibodies have repeatedly been demonstrated to be associated with very specific clinical phenotypes [1]. In brief, anti-Mi-2 [2], anti-TIF1γ [38], anti-NXP2 [3], anti-SAE [5] myositis are characterized by muscle and skin involvement with no lung involvement. In contrast, anti-MDA5-positive patients often have amyopathic dermatomyositis with rapidly progressive forms of interstitial lung disease [6]. As mentioned earlier, anti-TIF1γ and, to a lesser extent, anti-NXP2 are associated with cancer [3,4▪▪,39]. Moreover, anti-NXP2 patients are most likely to develop calcinosis [3].

Although the primary source of dermatomyositis autoantigens is unknown, several myositis autoantigens are overexpressed during muscle regeneration [40,41] Whether overexpression of these autoantigens is restricted to the muscle lineage or whether they are also expressed in the stem cells of other target tissues, like the skin, the lung or the blood vessels, remains to be shown.

To date, it is not well understood how the immune synapses between the antigen and the effector cells are established. That is, we do not know

if the autoantigen is presented exclusively by the MHC-I of the tissues affected by the disease, if it is just the autoantigen released by the apoptotic and necrotic cells that is digested by macrophages and dendritic cells and presented by them to the T and B cells or even, if the apoptotic blebs or exosomes may be mediating antigen presentation directly (Table 1).

THE VICTIMS: IMPLICATED TISSUES

Regardless of what triggers the different types of dermatomyositis, a variety of organ systems are damaged in most patients. Here we will focus on the muscle and the skin as the two best-studied tissues in these patients. However, it's important to recognize that dermatomyositis may also affect the lungs (particularly anti-MDA5) [6], the joints or the gastrointestinal system [1].

Although most patients with dermatomyositis have muscle involvement, the severity of weakness is generally less than in other types of myositis like immune-mediated necrotizing myositis or inclusion body myositis [42]. The most characteristic pathologic finding in the muscle of dermatomyositis patients is the presence of perifascicular atrophy, which is highly specific but only modestly sensitive, being found in just 25–50% of dermatomyositis patients [43,44]. Moreover, as we will mention later, it is typical to find inflammatory infiltrates surrounding the vessels and invading the perimysium [1]. Importantly up to 16% of dermatomyositis biopsies have prominent necrosis without focal collection of endomysial infiltrates similar to the biopsies of patients with immune-mediated necrotizing myopathy [43].

The presence of heliotrope and Gottron's sign and papules are the most characteristic skin lesions in dermatomyositis patients. In addition, certain types of dermatomyositis are also associated with skin ulcers (anti-MDA5) [45] or calcinosis (anti-NXP2) [3]. Dermatomyositis skin biopsy findings are characterized by the presence of an interface dermatitis with hyperkeratosis, epidermal hyperplasia with focal areas of atrophy, vacuolar degeneration and necrotic keratinocytes along the basal membrane [46]. Moreover, there are inflammatory infiltrates at the dermal–epidermal junction and surrounding the vessels [46]. However, these skin biopsy features are unspecific and indistinguishable from other autoimmune diseases like lupus erythematosus [46].

Importantly, as mentioned earlier, there is an important immune response against the blood vessels in both the skin and in the muscle of dermatomyositis patients. As a reflection of this in the muscle, there is a reduced capillary density. Furthermore, in dermatomyositis patients, tubuloreticular inclusions are found within the endothelial cells and immunoglobulins and complement are found on the surface of endothelial cells [47,48]. Likewise, the capillary involvement in the skin can be detected in a skin biopsy, but also by less invasive methods such as nailfold capillaroscopy [49], the latter of which will show capillary enlargement and neovascularization with frequent capillary hemorraghes (Table 1) [49].

THE ATTACKERS: INFLAMMATORY CELLS

The inflammatory infiltrates in the muscle dermatomyositis patients are predominantly composed of CD4+ T cells, dendritic cells and B lymphocytes with a lesser presence of macrophages [25▪▪,50]. The distribution of this infiltrate is primarily in the septa, inside of the fascicles or surrounding the blood vessels [50,51].

The abovementioned CD4+ T cells have been reported to be mostly CD28−, which is a subtype of CD4+ cells with proinflammatory and direct cytotoxic potential [51]. Moreover, most reports suggest that in dermatomyositis there is no restriction of the repertoire of T-cell receptors, suggesting a broad immune response as opposed to polymyositis or inclusion body myositis [52,53].

Likewise, as mentioned before, the skin of these patients also shows a predominance of CD4+ T cells and macrophages in the dermal–epidermal junction and surrounding the dermal vasculature (Table 1) [46].

THE WEAPONS: MANY WAYS TO CAUSE HARM

What are the actual mechanisms by which invading immune cells cause harm to target tissues? First, autoantibodies themselves may have a direct pathogenic role on the disease, either by activating complement or effector cells. The reported presence of membrane attack complex, the active form of complement, on the surface of the muscle fibers and vessels of these patients [47,48] supports this possibility but requires further verification.

Second, cytokines are proteins that mediate intercellular immunologic communication. Multiple cytokines have been implicated in dermatomyositis pathogenesis, but the best documented are the interferons. Interferons are so-called because of their capacity to interfere with viral replication. There are several types, but in dermatomyositis the most relevant ones are type I interferons (IFN-α and IFN-β). Type I interferons have a potent antiviral activity by acting at different levels. Thus, they upregulate MHC-I proteins to expose viral proteins on the surface of the cell and target host cells for recognition by dendritic cells, lymphocytes and natural killer cells,

which are also activated by these molecules. Both the muscle [25▪▪], skin [26] and the blood [27] of adult and juvenile [54] forms of dermatomyositis patients show an overexpression of interferon-induced proteins, which is often called the interferon signature.

Moreover, the abovementioned MHC-I overexpression that can be induced by interferon has been proposed to cause muscle damage in and of itself. This was reported based on in-vitro [55] and animal models [56] showing severe myopathy after overexpressing MHC-I genes in muscle. Complementarily, MHC-I and other proteins related with the interferon pathway, like RIG-1 [44] or MxA [57], have been found to be overexpressed in the perifascicular areas of patients with myositis, and it was suggested that they be more sensitive markers of perifascicular involvement that the atrophy itself.

Numerous other cytokines have been implicated in dermatomyositis pathogenesis, including IL-1 [58], 2, 4, 6, 8, 10 [59], 15 [60], 17 [61], 18 [62] and 23 [61], TNF-α [63], TGF-β [64], and the B-cell activating factor [65]. However, most of them are not specific for dermatomyositis and are found in many other inflammatory processes.

Finally, cytotoxic proteins [66] and soluble intercellular adhesion molecules (ICAM) [51,67] have also been implicated in dermatomyositis pathogenesis. Thus, both granzyme B [68] and perforin [66], which are characteristically expressed in the CD4+CD28− cells of the muscle biopsies of these patients [51], have been proposed to be associated with dermatomyositis. Alternatively, ICAM, vascular cell adhesion molecules [67,69] and the selectins [69], are overexpressed in the tissues of these patients and may be responsible of attracting the inflammatory cells to the target tissues in these patients (Table 1).

THE BATTLE: PATHOGENIC THEORIES

In the preceding paragraphs, we have reviewed many of the different components implicated in the immunologic phenomenon that is dermatomyositis. But how are they orchestrated to work together? In other words, what are the models of dermatomyositis pathogenesis, from the trigger to the symptoms and signs of the disease?

Two main theories have been proposed. A longstanding one proposes that the trigger of the disease breaks tolerance of the immune system to certain autoantigen, producing autoantibodies that bind to endothelial cells, causing vascular damage and ischemia of muscle and skin, which would cause the characteristic perifascicular atrophy observed in muscle biopsies from these patients [47,70]. However, perifascicular muscle fibers are not more vulnerable to ischemia than other areas of the muscle [71,72] and ischemic lesions in the perifascicular area, which are often found in the antisynthetase syndrome [73], are rarely found in patients with pure dermatomyositis. Moreover, this theory does not explain why there is a preferential expression of interferon-related genes in the perifascicular area. Alternatively, a more recent model hypothesizes that the abovementioned triggers sensitize the immune-system, stimulating interferon production, similar to a viral infection. As a consequence of the interferon production, the affected tissues are damaged. This results in increased autoantigen expression, establishing a feedforward loop of B, T-cell activation, and autoantibody production. In this model, endothelial lesions and perifascicular atrophy would not be cause and consequence, but two manifestations of the same process. However, recent data suggest that hypoxia may induce IFN-I production in the muscle [74], and thus, the real mechanism of dermatomyositis pathogenesis may be more complex than accounted for by the two theories outlined here (Table 1).

CONCLUSION

Dermatomyositis is an extremely complicated immune phenomenon. In the last few decades, we have identified some of the predisposing factors, triggers and pathogenic mechanisms most likely implicated in this phenomenon. However, the puzzle is still far from being solved, and we still have many questions to answer before we can fully understand the disease and win the war against those rebel cells in the muscle.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Mammen AL. Dermatomyositis and polymyositis: clinical presentation, autoantibodies, and pathogenesis. Ann N Y Acad Sci 2010; 1184:134–153.
2. Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. Arthritis Rheum 1985; 28:796–803.
3. Albayda J, Pinal-Fernandez I, Huang W, *et al.* Antinuclear matrix protein 2 autoantibodies and edema, muscle disease, and malignancy risk in dermatomyositis patients. Arthritis Care Res 2017; 69:1771–1776.

4. Trallero-Araguas E, Rodrigo-Pendas JA, Selva-O'Callaghan A, *et al.* Usefulness of antip155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. Arthritis Rheum 2012; 64:523–532.

Highest level of evidence supporting the association between anti-TIFg and cancer.

5. Tarricone E, Ghirardello A, Rampudda M, *et al.* Anti-SAE antibodies in autoimmune myositis: identification by unlabelled protein immunoprecipitation in an Italian patient cohort. J Immunol Methods 2012; 384:128–134.
6. Sato S, Hirakata M, Kuwana M, *et al.* Autoantibodies to a 140-kD polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. Arthritis Rheum 2005; 52:1571–1576.
7. Oddis CV, Conte CG, Steen VD, Medsger TA Jr. Incidence of polymyositis–dermatomyositis: a 20-year study of hospital diagnosed cases in Allegheny County, PA 1963–1982. J Rheumatol 1990; 17:1329–1334.
8. Smoyer-Tomic KE, Amato AA, Fernandes AW. Incidence and prevalence of idiopathic inflammatory myopathies among commercially insured, Medicare supplemental insured, and Medicaid enrolled populations: an administrative claims analysis. BMC Musculoskelet Disord 2012; 13:103.
9. Hoshino K, Muro Y, Sugiura K, *et al.* Anti-MDA5 and anti-TIF1-gamma antibodies have clinical significance for patients with dermatomyositis. Rheumatology (Oxford) 2010; 49:1726–1733.
10. Ceribelli A, Fredi M, Taraborelli M, *et al.* Prevalence and clinical significance of anti-MDA5 antibodies in European patients with polymyositis/dermatomyositis. Clin Exp Rheumatol 2014; 32:891–897.
11. Kim E, Cook-Mills J, Morgan G, *et al.* Increased expression of vascular cell adhesion molecule 1 in muscle biopsy samples from juvenile dermatomyositis patients with short duration of untreated disease is regulated by miR-126. Arthritis Rheum 2012; 64:3809–3817.
12. Hosono Y, Nakashima R, Serada S, *et al.* Splicing factor proline/glutamine-rich is a novel autoantigen of dermatomyositis and associated with antimelanoma differentiation-associated gene 5 antibody. J Autoimmun 2017; 77:116–122.
13. Love LA, Weinberg CR, McConnaughey DR, *et al.* Ultraviolet radiation intensity predicts the relative distribution of dermatomyositis and anti-Mi-2 autoantibodies in women. Arthritis Rheum 2009; 60:2499–2504.

Comprehensive study demonstrating the association between the relative prevalence of dermatomyositis and the level of ultraviolet radiation.

14. Schiffenbauer A, Faghihi-Kashani S, O'Hanlon TP, *et al.* The effect of cigarette smoking on the clinical and serological phenotypes of polymyositis and dermatomyositis. Semin Arthritis Rheum 2018. [Epub ahead of print]
15. Orione MA, Silva CA, Sallum AM, *et al.* Risk factors for juvenile dermatomyositis: exposure to tobacco and air pollutants during pregnancy. Arthritis Care Res 2014; 66:1571–1575.
16. Miller FW, Chen W, O'Hanlon TP, *et al.* Genome-wide association study identifies HLA 8.1 ancestral haplotype alleles as major genetic risk factors for myositis phenotypes. Genes Immun 2015; 16:470–480.

Study proving that the HLA is the most significant genetic predisposing factor in myositis.

17. Plamondon S, Dent PB, Reed AM. Familial dermatomyositis. J Rheumatol 1999; 26:2691–2692.
18. Davies MG, Hickling P. Familial adult dermatomyositis. Br J Dermatol 2001; 144:415–416.
19. Rider LG, Gurley RC, Pandey JP, *et al.* Clinical, serologic, and immunogenetic features of familial idiopathic inflammatory myopathy. Arthritis Rheum 1998; 41:710–719.
20. Ginn LR, Lin JP, Plotz PH, *et al.* Familial autoimmunity in pedigrees of idiopathic inflammatory myopathy patients suggests common genetic risk factors for many autoimmune diseases. Arthritis Rheum 1998; 41:400–405.
21. Mamyrova G, O'Hanlon TP, Sillers L, *et al.* Cytokine gene polymorphisms as risk and severity factors for juvenile dermatomyositis. Arthritis Rheum 2008; 58:3941–3950.
22. Hill CL, Zhang Y, Sigurgeirsson B, *et al.* Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet 2001; 357:96–100.
23. Pinal-Fernandez I, Ferrer-Fabregas B, Trallero-Araguas E, *et al.* Tumour TIF1 mutations and loss of heterozygosity related to cancer-associated myositis. Rheumatology (Oxford) 2018; 57:388–396.

First evidence of an increased rate of mutations and loss of heterozygosity in tumors from anti-TIF1γ+ patients, suggesting that these mutations may trigger dermatomyositis in these patients and the tumor may escape immune surveilance by deleting regions of its genome.

24. Kato H, Takeuchi O, Sato S, *et al.* Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 2006; 441:101–105.
25. Greenberg SA, Pinkus JL, Pinkus GS, *et al.* Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. Ann Neurol 2005; 57:664–678.

Comprehensive study demonstrating the importance of the interferon pathway in dermatomyositis.

26. Wong D, Kea B, Pesich R, *et al.* Interferon and biologic signatures in dermatomyositis skin: specificity and heterogeneity across diseases. PLoS One 2012; 7:e29161.
27. Walsh RJ, Kong SW, Yao Y, *et al.* Type I interferon-inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. Arthritis Rheum 2007; 56:3784–3792.
28. Brunasso AM, Aberer W, Massone C. New onset of dermatomyositis/polymyositis during anti-TNF-alpha therapies: a systematic literature review. ScientificWorldJournal 2014; 2014:179180.
29. Dietrich LL, Bridges AJ, Albertini MR. Dermatomyositis after interferon alpha treatment. Med Oncol 2000; 17:64–69.
30. Sheik Ali S, Goddard AL, Luke JJ, *et al.* Drug-associated dermatomyositis following ipilimumab therapy: a novel immune-mediated adverse event associated with cytotoxic T-lymphocyte antigen 4 blockade. JAMA Dermatol 2015; 151:195–199.
31. Kanoh H, Izumi T, Seishima M, *et al.* A case of dermatomyositis that developed after delivery: the involvement of pregnancy in the induction of dermatomyositis. Br J Dermatol 1999; 141:897–900.
32. Pinal-Fernandez I, Selva-O'Callaghan A, Fernandez-Codina A, *et al.* Pregnancy in adult-onset idiopathic inflammatory myopathy': report from a cohort of myositis patients from a single center. Semin Arthritis Rheum 2014; 44:234–240.
33. Ye Y, van Zyl B, Varsani H, *et al.* Maternal microchimerism in muscle biopsies from children with juvenile dermatomyositis. Rheumatology (Oxford) 2012; 51:987–991.
34. Artlett CM, Ramos R, Jiminez SA, *et al.* Chimeric cells of maternal origin in juvenile idiopathic inflammatory myopathies. Childhood Myositis Heterogeneity Collaborative Group. Lancet 2000; 356:2155–2156.
35. Selva-O'Callaghan A, Boeckh-Behrens TM, Balada-Prades E, *et al.* Fetal microchimerism and inflammatory myopathies. Lancet 2001; 357:887.
36. Artlett CM, Sassi-Gaha S, Ramos RC, *et al.* Chimeric cells of maternal origin do not appear to be pathogenic in the juvenile idiopathic inflammatory myopathies or muscular dystrophy. Arthritis Res Ther 2015; 17:238.
37. Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 2016; 280:8–23.
38. Fujimoto M, Hamaguchi Y, Kaji K, *et al.* Myositis-specific anti155/140 autoantibodies target transcription intermediary factor 1 family proteins. Arthritis Rheum 2012; 64:513–522.
39. Fiorentino DF, Chung LS, Christopher-Stine L, *et al.* Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1gamma. Arthritis Rheum 2013; 65:2954–2962.
40. Casciola-Rosen L, Nagaraju K, Plotz P, *et al.* Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. J Exp Med 2005; 201:591–601.
41. Mammen AL, Casciola-Rosen LA, Hall JC, *et al.* Expression of the dermatomyositis autoantigen Mi-2 in regenerating muscle. Arthritis Rheum 2009; 60:3784–3793.
42. Pinal-Fernandez I, Casal-Dominguez M, Carrino JA, *et al.* Thigh muscle MRI in immune-mediated necrotising myopathy: extensive oedema, early muscle damage and role of anti-SRP autoantibodies as a marker of severity. Ann Rheum Dis 2017; 76:681–687.
43. Pinal-Fernandez I, Casciola-Rosen LA, Christopher-Stine L, *et al.* The prevalence of individual histopathologic features varies according to autoantibody status in muscle biopsies from patients with dermatomyositis. J Rheumatol 2015; 42:1448–1454.
44. Suarez-Calvet X, Gallardo E, Pinal-Fernandez I, *et al.* RIG-I expression in perifascicular myofibers is a reliable biomarker of dermatomyositis. Arthritis Res Ther 2017; 19:174.
45. Narang NS, Casciola-Rosen L, Li S, *et al.* Cutaneous ulceration in dermatomyositis: association with antimelanoma differentiation-associated gene 5 antibodies and interstitial lung disease. Arthritis Care Res 2015; 67:667–672.
46. Dourmishev LA, Wollina U. Dermatomyositis: immunopathologic study of skin lesions. Acta Dermatovenerol Alp Pannonica Adriat 2006; 15:45–51.
47. Kissel JT, Mendell JR, Rammohan KW. Microvascular deposition of complement membrane attack complex in dermatomyositis. N Engl J Med 1986; 314:329–334.
48. Kissel JT, Halterman RK, Rammohan KW, Mendell JR. The relationship of complement-mediated microvasculopathy to the histologic features and clinical duration of disease in dermatomyositis. Arch Neurol 1991; 48:26–30.
49. Bertolazzi C, Cutolo M, Smith V, Gutierrez M. State of the art on nailfold capillaroscopy in dermatomyositis and polymyositis. Semin Arthritis Rheum 2017; 47:432–444.
50. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. III: Immunoelectron microscopy aspects of cell-mediated muscle fiber injury. Ann Neurol 1986; 19:112–125.
51. Fasth AE, Dastmalchi M, Rahbar A, *et al.* T cell infiltrates in the muscles of patients with dermatomyositis and polymyositis are dominated by CD28null T cells. J Immunol 2009; 183:4792–4799.
52. Benveniste O, Cherin P, Maisonobe T, *et al.* Severe perturbations of the blood T cell repertoire in polymyositis, but not dermatomyositis patients. J Immunol 2001; 167:3521–3529.
53. O'Hanlon TP, Dalakas MC, Plotz PH, Miller FW. The alpha beta T-cell receptor repertoire in inclusion body myositis: diverse patterns of gene expression by muscle-infiltrating lymphocytes. J Autoimmun 1994; 7:321–333.
54. Niewold TB, Kariuki SN, Morgan GA, *et al.* Elevated serum interferon-alpha activity in juvenile dermatomyositis: associations with disease activity at diagnosis and after thirty-six months of therapy. Arthritis Rheum 2009; 60:1815–1824.

55. Li CK, Knopp P, Moncrieffe H, *et al.* Overexpression of MHC class I heavy chain protein in young skeletal muscle leads to severe myositis: implications for juvenile myositis. Am J Pathol 2009; 175:1030–1040.

56. Nagaraju K, Raben N, Loeffler L, *et al.* Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. Proc Natl Acad Sci U S A 2000; 97:9209–9214.

57. Uruha A, Nishikawa A, Tsuburaya RS, *et al.* Sarcoplasmic MxA expression: a valuable marker of dermatomyositis. Neurology 2017; 88:493–500.

58. Gabay C, Gay-Croisier F, Roux-Lombard P, *et al.* Elevated serum levels of interleukin-1 receptor antagonist in polymyositis/dermatomyositis. A biologic marker of disease activity with a possible role in the lack of acute-phase protein response. Arthritis Rheum 1994; 37:1744–1751.

59. Gono T, Kaneko H, Kawaguchi Y, *et al.* Cytokine profiles in polymyositis and dermatomyositis complicated by rapidly progressive or chronic interstitial lung disease. Rheumatology (Oxford) 2014; 53:2196–2203.

60. Sugiura T, Harigai M, Kawaguchi Y, *et al.* Increased IL-15 production of muscle cells in polymyositis and dermatomyositis. Int Immunol 2002; 14:917–924.

61. Shen H, Xia L, Lu J, Xiao W. Interleukin-17 and interleukin-23 in patients with polymyositis and dermatomyositis. Scand J Rheumatol 2011; 40:217–220.

62. Gono T, Kawaguchi Y, Sugiura T, *et al.* Interleukin-18 is a key mediator in dermatomyositis: potential contribution to development of interstitial lung disease. Rheumatology (Oxford) 2010; 49:1878–1881.

63. Kim GT, Cho ML, Park YE, *et al.* Expression of TLR2, TLR4, and TLR9 in dermatomyositis and polymyositis. Clin Rheumatol 2010; 29:273–279.

64. Confalonieri P, Bernasconi P, Cornelio F, Mantegazza R. Transforming growth factor-beta 1 in polymyositis and dermatomyositis correlates with fibrosis but not with mononuclear cell infiltrate. J Neuropathol Exp Neurol 1997; 56:479–484.

65. Baek A, Park HJ, Na SJ, *et al.* The expression of BAFF in the muscles of patients with dermatomyositis. J Neuroimmunol 2012; 249:96–100.

66. Goebels N, Michaelis D, Engelhardt M, *et al.* Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. J Clin Invest 1996; 97:2905–2910.

67. Sallum AM, Marie SK, Wakamatsu A, *et al.* Immunohistochemical analysis of adhesion molecule expression on muscle biopsy specimens from patients with juvenile dermatomyositis. J Rheumatol 2004; 31:801–807.

68. Grassi M, Capello F, Bertolino L, *et al.* Identification of granzyme B-expressing CD-8-positive T cells in lymphocytic inflammatory infiltrate in cutaneous lupus erythematosus and in dermatomyositis. Clin Exp Dermatol 2009; 34:910–914.

69. Kubo M, Ihn H, Yamane K, *et al.* Increased serum levels of soluble vascular cell adhesion molecule-1 and soluble E-selectin in patients with polymyositis/dermatomyositis. Br J Dermatol 2000; 143:392–398.

70. De Visser M, Emslie-Smith AM, Engel AG. Early ultrastructural alterations in adult dermatomyositis. Capillary abnormalities precede other structural changes in muscle. J Neurol Sci 1989; 94:181–192.

71. Karpati G, Carpenter S, Melmed C, Eisen AA. Experimental ischemic myopathy. J Neurol Sci 1974; 23:129–161.

72. Hathaway PW, Engel WK, Zellweger H. Experimental myopathy after microarterial embolization; comparison with childhood x-linked pseudohypertrophic muscular dystrophy. Arch Neurol 1970; 22:365–378.

73. Mescam-Mancini L, Allenbach Y, Hervier B, *et al.* Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis. Brain 2015; 138:2485–2492.

74. De Luna N, Suarez-Calvet X, Lleixa C, *et al.* Hypoxia triggers IFN-I production in muscle: implications in dermatomyositis. Sci Rep 2017; 7:8595.

The diagnostic work-up of cancer-associated myositis

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Purpose of review
Despite the well-recognized association between malignancy and myositis, definite data indicating the best strategy for diagnosing cancer in myositis patients is lacking. In this article, we review the data on cancer screening in patients with myositis, and propose an algorithm for this purpose based on recently published data.

Recent findings
Evidence has recently emerged supporting blind screening in patients with certain myositis phenotypes. In addition to the clinical examination, imaging techniques such as PET/computed tomography scanning and whole-body MRI, and determination of the autoantibody profile beyond anti-TIF1γ antibody, the well known cancer biomarker in dermatomyositis, will help the clinician face this complex clinical situation. Molecules related to the checkpoint inhibitor pathway, specifically soluble programmed death 1, may also have a role in the diagnostic work-up of cancer in myositis. In the future, blood tests analysing circulating DNA will certainly help in detecting patients with cancer-associated myositis (CAM).

Summary
A step forward has been achieved in the pathway to establish optimal cancer screening for myositis patients. International consensus guidelines for an effective diagnostic work-up of CAM are in progress and will be of paramount importance to improving the outcome in these patients.

Keywords
autoantibodies, biomarkers, dermatomyositis, malignancy, screening

INTRODUCTION

The relationship between idiopathic inflammatory myopathies (IIM) and cancer has been recognized since the beginning of the past century, but consistent epidemiological data have only been available since the 1990s. A recent meta-analysis estimating the risk of malignancy in patients with IIM compared with the general population is in line with the previous data [1].

Diagnosing occult neoplastic disease in a patient with a recent diagnosis of myositis is a challenge for clinicians. IIMs are not homogeneous diseases and the risk of developing cancer varies between the various phenotypes. Although a systematic approach has not yet been designed, cancer risk stratification is advisable in the IIM population, and several factors should be taken into account. The clinical phenotype, autoantibody profile, and findings on muscle biopsy, as well as the ethnicity, age, and sex of the patient are some of the factors that will be addressed in this review. Available task-force recommendations for cancer screening from medical societies and local governments will be taken into account. A mainly experience-based and evidence-based (when possible) suggested strategy for the diagnostic work-up of cancer in patients with IIMs is presented.

RELEVANCE OF THE MYOSITIS PHENOTYPE

The specific IIM phenotype is an important element for establishing the risk of malignancy in these patients. Dermatomyositis, polymyositis, immune-mediated necrotizing myopathy (IMNM), sporadic inclusion body myositis (sIBM), and overlap myositis

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KEY POINTS

- Blind screening in patients with myositis, particularly dermatomyositis, is warranted.
- ''EUCLIDES'' (Epidemiological Useful Clinical-Laboratory-Imaging Development Screening) is a proposed strategy to help the physician succeed in the diagnostic work-up of malignancy in patients with myositis.
- In the near future, early detection of cancer by means of 'liquid biopsy' will likely change our management approach to CAM.

(including the antisynthetase syndrome) are now considered the five main IIM phenotypes [2].

Based on epidemiologic studies, the standardized incidence ratio (SIR) – that is, the number of cancer cases arising in patients with an IIM divided by the expected number of cancer cases according to national age-specific, sex-specific, and period-specific cancer rates – is highest in patients with dermatomyositis, and is much higher during the 1st year after the dermatomyositis diagnosis (SIR 17.29) [1]. Importantly, the risk of cancer is clearly associated with the presence of specific autoantibodies, as will be discussed later [3,4▪]. Polymyositis has also been commonly associated with cancer, but the lack of consistent criteria to homogeneously classify these patients has made it difficult to draw conclusion from these data. Recent evidence suggests that up to 60% of patients with sIBM may meet the criteria for T cell large granular lymphocytic leukaemia [5], a generally indolent form of cancer, but other cancer types are rarely described in these patients [6]. Regarding IMNM, positive status for anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (anti-HMGCR) has been inconsistently associated with an increased risk of cancer in patients with this condition [7–9], whereas those testing positive for anti-signal recognition particle autoantibodies do not seem to have an increased risk of neoplastic disease [10,11]. Autoantibody-negative IMNM was reported to have the highest risk of cancer, but this is still a poorly defined patient population [7]. Finally, overlap myositis is usually considered to have no association with cancer. This idea is best supported in patients with antisynthetase syndrome, who have shown no significant associations, regardless of the autoantibody type [12].

BIOMARKERS

Here, the broad-term *biomarkers* include tumour markers, autoantibodies, and other molecules. However, the use of tumour markers in this context is somewhat controversial, as they do not always help with the diagnosis and sometimes generate confusion [13].

As was briefly mentioned above, several autoantibodies have been related to cancer-associated myositis (CAM). The first and most widely known is anti-TIF1γ antibody, whose value resides in a high-negative predictive capability to rule out CAM. In dermatomyositis patients, the risk of developing cancer increases 27-fold when anti-TIF1γ antibody is present [3]. In contrast, this autoantibody has not been found in large cohorts of patients with other paraneoplastic rheumatic syndromes or solid cancers [14]. Significantly, the association with dermatomyositis may have a pathophysiological basis. A recent article published by Pinal-Fernandez *et al.* [15▪] reported a significantly larger number of TIF1 gene abnormalities in tumours of dermatomyositis patients with anti-TIF1γ-positive myositis than in those with anti-TIF1γ-negative disease. Six of seven patients with anti-TIF1γ-positive CAM presented mutations or loss of heterozygosity (LOH), a mechanism enabling the tumour to escape from the immune system response by deleting specific sections of its genome, whereas only one of six anti-TIF1γ-negative CAM patients showed these genetic features. These findings are consistent with the hypothesis that TIF1 gene mutations in the tumour of these patients could be the trigger leading to myositis, with LOH enabling the tumour to escape immune surveillance, as has been reported in cancer-associated scleroderma [16]. Data from a study by Scholtissek *et al.* [17], who reported upregulation of TIF1γ in skin and muscle of dermatomyositis patients, support a possible etiopathogenic role of these autoantibodies in the disease.

Other autoantibodies, such as those against nuclear matrix protein NXP2 (anti-NXP2) and against anti-HMGCR, have been also associated with cancer in myositis patients. In a large cohort of dermatomyositis patients, 56 of 235 were positive for anti-NXP2, and five of them had CAM, yielding an estimated 3.68-fold higher cancer risk than that of general population, but a much lower risk than that of anti-TIF1γ-positive dermatomyositis patients [4▪].

Finally, as was mentioned above, evidence of an association between anti-HMGCR antibodies and CAM is inconsistent; the risk of malignancy seems to be higher in autoantibody-negative IMNM patients than in those carrying these autoantibodies [7].

Although the association between the autoantibody profile and cancer is of great interest in IIM, the practical value of autoantibody determinations is to incorporate the results in risk stratification of

specific patients. Yang *et al.* [18▪] recently analysed the risk of cancer in myositis patients based on the autoantibody profile. In a cohort of 617 patients diagnosed with dermatomyositis and polymyositis, 72 of whom had a malignancy, the SIR for developing cancer associated with these autoantibodies was 17.28 in those anti-TIF1γ positive, 8.14 in those anti-NXP2 positive; and 12.92 in patients positive to anti-small ubiquitin-like modifier one activating enzyme (SAE1). Patients with antisynthetase antibodies or anti-HMGCR did not have an increased risk of cancer. In addition, patients testing negative to all the autoantibodies studied also had a significant risk of developing cancer in an interval of 3 years. Although patients with CAM had a poorer outcome than those without, the authors found no differences in survival rates or in the type of malignancy between the various autoantibodies.

In another recent study, Chen *et al.* [19▪▪] analysed the serum values of soluble programmed death ligand 1 (sPD-L1), a surrogate of the programmed death 1 checkpoint pathway, in several groups of patients with dermatomyositis and cancer, including patients with recent-onset therapy-naïve CAM, and patients treated and cured or in remission. A total of 40 CAM patients, 22 new-onset and 18 with stable cancer, were analysed. A control group of patients with solid tumours, patients diagnosed with other systemic autoimmune diseases including dermatomyositis without cancer, and a group of healthy controls, were also studied. The highest sPD-L1 values were observed in patients with solid tumours and the lowest in healthy controls. But the most interesting findings were that patients with dermatomyositis and cancer in remission showed values similar to those in the autoimmune diseases group, but recently diagnosed therapy-naïve patients were highly positive. The combination of sPD-L1 levels and anti-TIF1γ antibody increased the specificity for diagnosing CAM to 95% with a positive predictive value of 70%; hence, the combination of findings from these two tests may perform the best for cancer detection in dermatomyositis patients.

Studies analysing novel biomarkers related to energy metabolism proteins in muscle tissue of IIM patients using reverse phase protein microarrays have shown that the glycolytic pyruvate kinase isoform M2 and mitochondrial ATPase inhibitor factor (IF1) are significantly increased in dermatomyositis patients [20]. Given that this pyruvate kinase isoform plays a role in tumorigenesis and that IF1 is highly overexpressed in most carcinomas, the use of these molecules as biomarkers in cancer-associated dermatomyositis could be a valuable approach.

Finally, in a study in *Science*, Cohen *et al.* reported identification of eight different types of cancer, some of them usually very difficult to detect, using a multianalyte blood test, referred to as 'liquid biopsy'. Earlier detection of these and other cancers through analysis of proteins and mutations in cell-free DNA will undoubtedly change our approaches to the diagnostic work-up of CAM patients [21▪▪].

USEFULNESS OF IMAGING TECHNIQUES

Conventional imaging techniques may be of help in diagnosing occult malignancies in CAM patients. Most of the screening methods in use today include a mammography and gynaecological ultrasound examination in women and chest-abdomen-pelvis computed tomography (CT) examination in all patients.

[^{18}F] fluorodeoxyglucose PET/CT has been proposed as a good screening test for cancer in patients with dermatomyositis and polymyositis [22]. Recent studies support this idea: Li *et al.* [23] found that PET/CT correctly detected the presence of malignancy in seven (18.4%) of 38 patients with IIM, several case reports and small series have suggested its utility [24–26], and studies addressing the cost-effectiveness of the test are now in progress.

Whole-body MRI is another effective radiologic examination for IIM patients, not only as an objective measure of the distribution of muscle activity and damage but also to increase the diagnostic yield of muscle biopsies. Whole-body MRI may also be useful for the diagnosis of cancer in this population. In a retrospective study, conducted by Huang *et al.* [27▪] whole-body MRI was performed in 129 patients, 30 with polymyositis and 99 with dermatomyositis. Nearly 10% of the total patients were found to have a neoplastic lesion, which was malignant in five of the 12 patients. Significantly, three patients had nasopharyngeal carcinoma, a type of cancer that is usually difficult to diagnose, and the two remaining patients had thyroid and ovarian cancer, respectively. In IIM patients with severe dysphagia, real-time MRI may be an excellent tool to ascertain whether the dysphagia is due the inflammatory muscle disease or to an oesophageal neoplasm [28].

EVIDENCE FOR SCREENING IN PATIENTS WITH MYOSITIS

In the last decades, cancer screening programmes have been implemented around the world, with recommendations that often vary between and within countries regarding the tests to use, the intervals between testing, the age to begin and stop,

Table 1. General recommendations and guidelines for cancer screening

Recommendations and guidelines for cancer screening in the general population [35▪]	Target signs
Breast cancer screening: mammography every 2 years in women 50–69 years old	A lump in the breast (mammography) Lymphadenopathy (chest–abdominal–pelvic CT)
Cervical cancer screening: cytology and HPV testing. Both tests every 3–5 years in women 18–29 and 60–70 years of age	Vaginal discharge in women (gynaecologic ultrasound)
Colorectal cancer screening: stool-based tests or a visual (structural) exam of the colon and rectum in patients 45–75 years of age[a]	Iron deficiency anaemia (gastroscopy and colonoscopy)
Prostate cancer screening: not recommended in most countries	Haematuria (urinary cytology)
Skin cancer screening: not recommended in most countries (only the USA, Germany, Austria, and France have addressed this issue, with self-examination or clinical examination for screening)	Change in colour, size, or itching of a mole (evaluation by a dermatologist)
Lung cancer screening: not recommended in most countries (only the USA, Canada, Japan, the United Kingdom, and Australia have addressed this issue, with low-dose CT or chest radiography for screening)	Persistent cough and/or sputum in a heavy smoker (lung CT)

CT, computed tomography; HPV, human papillomavirus.
[a]https://www.cancer.org/cancer/colon-rectal-cancer/detection-diagnosis-staging/acs-recommendations.html.

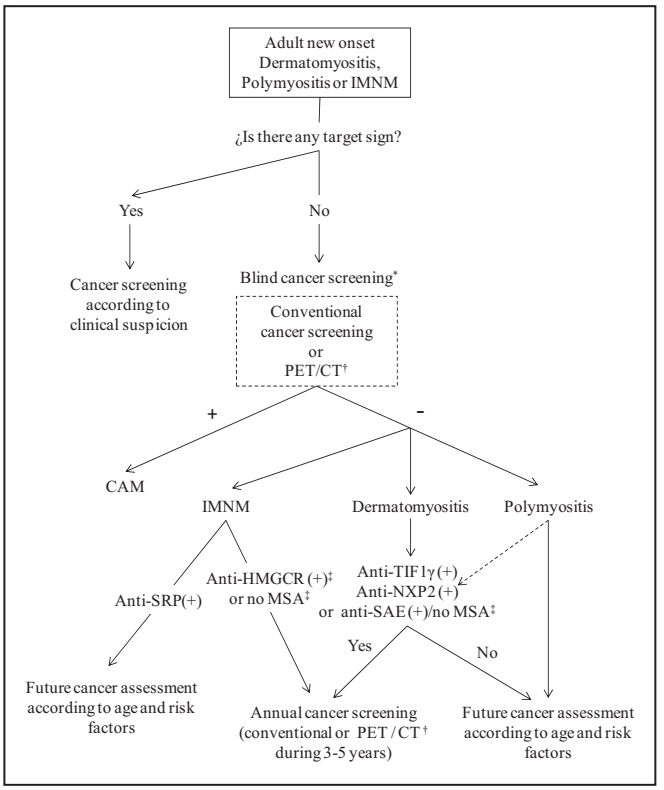

FIGURE 1. Algorithm for cancer screening in patients with idiopathic inflammatory myopathies. Conventional cancer screening: mammography and gynaecological ultrasound exam in women and whole (chest/abdomen/pelvis) computed tomography scan in all the patients. *Whole-body MRI could assist in cancer screening. †If available. In patients with high risk of cancer (i.e. patients with anti-TIF1γ or anti-NXP2 antibodies or high values of serum soluble programmed death ligand 1), a PET/computed tomography can be considered after a normal conventional screening. ‡Anti-HMGCR, anti-SAE, and 'no myositis specific antibodies' association with cancer need further investigation. CAM, cancer-associated myositis; IMNM, immune-mediated necrotizing myopathy; MSA, myositis specific antibodies; PET/computed tomography, [18F] fluorodeoxyglucose PET/computed tomography. HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; SAE, small ubiquitin-like modifier activating enzyme.

and the populations to include. The screening protocols of particular interest for IIM patients are summarized in Table 1.

Most physicians agree that cancer screening is mandatory in patients with IIM, especially those with dermatomyositis, polymyositis, or IMNM [29]. However, as there are no official guidelines or accepted consensus recommendations, the type of screening varies considerably between sites.

Evidence has recently emerged that blind screening for malignancy is warranted in IIM. Leatham *et al.* [30▪▪] studied the yield of cancer screening in patients with no signs of malignancy (i.e. blind screening), and in patients with suspected malignancy based on signs or symptoms in the clinical examination and history taking. In an analysis of 400 patients from two large dermatomyositis cohorts in the United States, the authors found that 48 patients had 53 dermatomyositis-associated neoplasms, and 27 of these patients had an occult malignancy at the time of the dermatomyositis diagnosis. These asymptomatic cancer-associated dermatomyositis patients were mainly diagnosed (58%) on CT findings. In addition, a second blind screening disclosed two new cancers, and two others would have been found if screening had been repeated within a year.

In studies addressing specific cancer types, such as lymphoma, breast cancer, and colorectal carcinoma, no particular issues have been reported beyond the

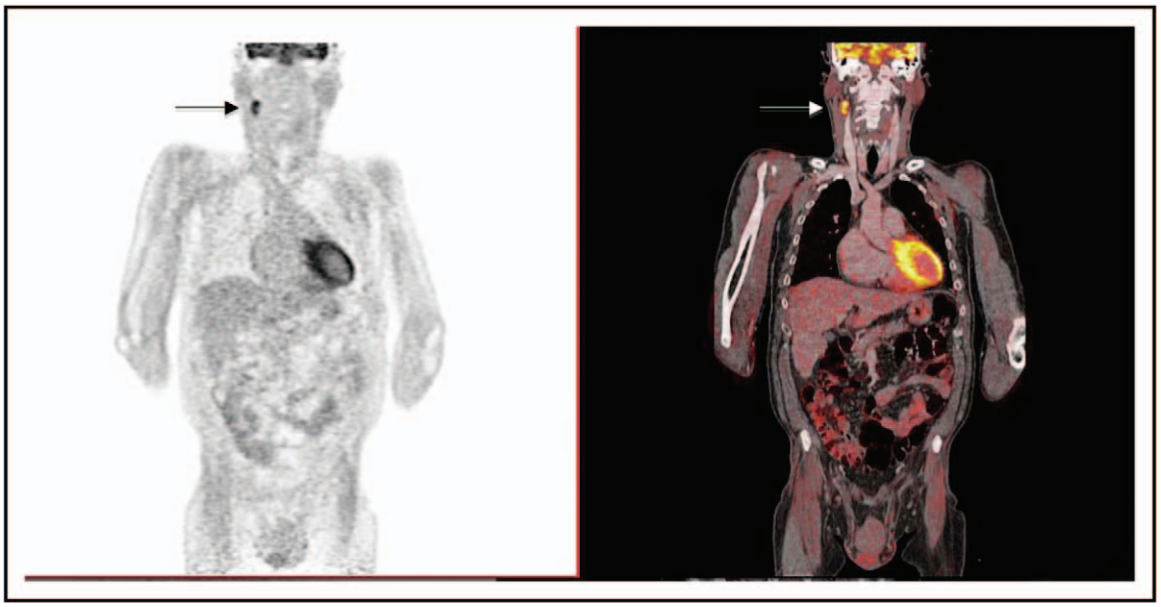

FIGURE 2. A 57-year-old man was diagnosed with dermatomyositis. Initial screening included a through clinical examination that showed no target signs. Blind screening with [^{18}F] PET/computed tomography disclosed a laterocervical lymphadenopathy with a high-standardized uptake value (SUV max = 8.7). Surgical biopsy of the node showed metastasis from a squamous cell type carcinoma of nasopharyngeal origin.

general recommendations in these patients [31–33]. Although optimal guidelines for cancer screening in myositis patients are not available, some authors have proposed recommendations [34].

Physicians should be aware of the prevalence of certain types of cancer in the geographical area where they are working. Age, sex, and ethnicity are important factors in appropriate cancer screening and can be considered the first step in the diagnostic work-up of cancer in myositis patients [35▪]. In accordance with these factors, other published data and our experience, we suggest a comprehensive algorithm for cancer screening in IIM (Fig. 1).

DIAGNOSTIC WORK-UP, THE "EUCLIDES" APPROACH

Here, a step-by-step diagnostic work-up to detect cancer in myositis patients is reported. It is named EUCLIDES (Epidemiological Useful Clinical-Laboratory-Imaging Development Screening) after a great Greek mathematician from the third century BC. Although it is reported as a sequence, the steps can be carried out in any order. This strategy is intended to empower clinicians facing cancer screening in a patient with myositis.

First step – *Epidemiological:* Clinicians should take into account the prevalence of the various types of cancer in their population. For example, the incidence of nasopharyngeal cancer is not the same in Asia as in Europe. Furthermore, it would be of interest to consult the guidelines and recommendations for screening of the general population from the task forces of global scientific societies (Table 1).

Second step – *Phenotype:* Meticulously evaluate the myositis phenotype. The risk of cancer is similar to that of the general population in patients with sIBM, but it is much higher, especially at disease onset, in patients with dermatomyositis.

Third step – *Clinical and complementary targeted exams:* In a specific patient, a complete general physical examination and through history taking is of paramount relevance. When a target sign is found (Table 1), go forward to achieve a prompt diagnosis.

Fourth step – *Laboratory:* Perform a broad autoantibody profile, including anti-TIF1γ and anti-NXP2. Other autoantibodies such as anti-HMGCR or anti-SAE may also be included. Serum determination of sPD-L1 may also be considered, if possible. In the future, early detection of cancer by means of 'liquid biopsy' will make the clinician's work easier when occult cancer is suspected, as in CAM.

Fifth step – *Imaging*: Mammography and gynaecological ultrasound is recommended in women. Moreover, a 'double check' with whole-body MRI and PET/CT would be extremely useful (Fig. 2). In addition to their utility for determining the diagnosis, evaluating disease activity, and identifying the appropriate site for biopsy, both techniques can be useful for detecting occult malignancy.

CONCLUSION

Despite the critical association between myositis and cancer, consensus statements or guidelines establishing the best strategy and frequency for cancer screening in the different types of myositis are still lacking. An International Myositis Assessment & Clinical Studies Group (IMACS) project coordinated by Dr Albert Selva-O'Callaghan and Dr Roith Aggarwal entitled 'Cancer-Associated Myositis Guidelines' is now ongoing with the aim of reaching a consensus in this line. In the interim, a thorough history-taking and clinical examination complemented with a complete autoantibody profile and appropriate imaging techniques remain the key to establishing an early diagnosis and improving the prognosis of patients with CAM.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Qiang JK, Kim WB, Baibergenova A, Alhusayen R. Risk of malignancy in dermatomyositis and polymyositis. J Cutan Med Surg 2017; 21:131–136.
2. Lundberg IE, Tjarnlund A, Bottai M, *et al.* 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis 2017; 76:1955–1964.
3. Trallero-Araguás E, Rodrigo-Pendás JA. Selva-O'Callaghan A, *et al.* Usefulness of antip155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. Arthritis Rheum 2012; 64:523–532.
4. ■ Albayda J, Pinal-Fernandez I, Huang W, *et al.* Antinuclear matrix protein 2 autoantibodies and edema, muscle disease, and malignancy risk in dermatomyositis patients. Arthritis Care Res 2017; 69:1771–1776.

In a large cohort of patients with dermatomyositis, the authors confirmed an association between cancer-associated myositis and anti-NXP2 antibody. Presence of anti-NXP2 confers a 3.68-fold increased risk of associated malignancy.

5. Greenberg SA, Pinkus JL, Amato AA, *et al.* Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. Brain 2016; 139:1348–1360.
6. Dardis C, Antezana A, Tanji K, Maccabee PJ. Inclusion body myositis: a case presenting with respiratory failure and autopsy findings leading to the hypothesis of a paraneoplastic cause. Am J Case Rep 2017; 18:700–706.
7. Allenbach Y, Keraen J, Bouvier AM, *et al.* High risk of cancer in autoimmune necrotizing myopathies: usefulness of myositis specific antibody. Brain 2016; 139:2131–2135.
8. Kadoya M, Hida A, Hashimoto Maeda M, *et al.* Cancer association as a risk factor for anti-HMGCR antibody-positive myopathy. Neurol Neuroimmunol Neuroinflamm 2016; 3:e290.
9. Tiniakou E, Pinal-Fernandez I, Lloyd TE, *et al.* More severe disease and slower recovery in younger patients with anti3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. Rheumatology 2017; 56: 787–794.
10. Suzuki S, Nishikawa A, Kuwana M, *et al.* Inflammatory myopathy with anti-signal recognition particle antibodies: case series of 100 patients. Orphanet J Rare Dis 2015; 10:61.
11. Pinal-Fernandez I, Parks C, Werner JL, *et al.* Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. Arthritis Care Res 2017; 69: 263–270.
12. Pinal-Fernandez I, Casal-Dominguez M, Huapaya JA, *et al.* A longitudinal cohort study of the antisynthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology 2017; 56:999–1007.
13. Minami S, Nakanishi T, Tanaka T, *et al.* A case of antisynthetase syndrome with elevated serum CA19-9 associated with interstitial lung disease. Eur J Dermatol 2017; 27:78–80.
14. Venalis P, Selickaja S, Lundberg K, *et al.* Association of anti-transcription intermediary factor 1γ antibodies with paraneoplastic rheumatic syndromes other than dermatomyositis. Arthritis Care Res 2018; 70: 648–651.
15. ■ Pinal-Fernandez I, Ferrer-Fabregas B, Trallero-Araguas E, *et al.* Tumour TIF1 mutations and loss of heterozygosity related to cancer-associated myositis. Rheumatology 2018; 57:388–396.

In this study, the authors found a higher number of genetic abnormalities in tumours of patients with paraneoplastic dermatomyositis with anti-TIF1γ antibodies than in those without. These findings support the hypothesis that a strong adaptive immune response against neoplastic cells is behind the association of cancer and myositis.

16. Joseph CG, Darrah E, Shah AA, *et al.* Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science 2014; 343:152–157.
17. Scholtissek B, Ferring-Schmitt S, Maier J, Wenzel J. Expression of the autoantigen TRIM33/TIF1γ in skin and muscle of patients with dermatomyositis is upregulated, together with markers of cellular stress. Clin Exp Dermatol 2017; 42:659–662.
18. ■ Yang H, Peng Q, Yin L, *et al.* Identification of multiple cancer-associated myositis-specific autoantibodies in idiopathic inflammatory myopathies: a large longitudinal cohort study. Arthritis Res Ther 2017; 19:259; Erratum in: Arthritis Res Ther. 2018; 20: 71.

The risk of cancer in patients with idiopathic inflammatory myopathies (IIM) can be stratified using the autoantibody profile. The standardized incidence ratio for several myositis autoantibodies is examined in this study. Anti-TIF1γ, anti-NXP2, and anti-SAE antibodies were associated with an increased risk of cancer in a large cohort of Chinese patients with myositis.

19. ■■ Chen H, Peng Q, Yang H, *et al.* Increased levels of soluble programmed death ligand 1 associate with malignancy in patients with dermatomyositis. J Rheumatol 2018; 45:835–840.

A new molecule from the checkpoint pathway, soluble programmed death ligand 1, used together with the well recognized antiTIF1γ antibodies, increases the specificity and positive predictive value for diagnosing occult malignancy in dermatomyositis patients.

20. Santacatterina F, Sánchez-Aragó M, Catalán-García M, *et al.* Pyruvate kinase M2 and the mitochondrial ATPase inhibitory factor 1 provide novel biomarkers of dermatomyositis: a metabolic link to oncogenesis. J Transl Med 2017; 15:29.
21. ■■ Cohen JD, Li L, Wang Y, *et al.* Detection and localization of surgically resectable cancers with a multianalyte blood test. Science 2018; 359: 926–930.

Eight cancer types could be detected at an earlier stage with use of a multianalyte blood test focused on cell-free DNA. Although more data in this line is needed, this may be a valuable approach to identify occult malignancy in myositis patients.

22. Selva-O'Callaghan A, Grau JM, Gámez-Cenzano C, *et al.* Conventional cancer screening versus PET/CT in dermatomyositis/polymyositis. Am J Med 2010; 123:558–562.
23. Li Y, Zhou Y, Wang Q. Multiple values of (18)F-FDG PET/CT in idiopathic inflammatory myopathy. Clin Rheumatol 2017; 36:2297–2305.
24. Sun L, Dong Y, Zhang N, *et al.* [(18)F]Fluorodeoxyglucose positron emission tomography/computed tomography for diagnosing polymyositis/dermatomyositis. Exp Ther Med 2018; 15:5023–5028.
25. Bai X, Tie N, Wang X, Zhao J. Intense muscle activity due to polymyositis incidentally detected in a patient evaluated for possible malignancy by FDG PET/CT imaging. Clin Nucl Med 2017; 42:647–648.
26. Tanaka S, Ikeda K, Uchiyama K, *et al.* [18F]FDG uptake in proximal muscles assessed by PET/CT reflects both global and local muscular inflammation and provides useful information in the management of patients with polymyositis/dermatomyositis. Rheumatology 2013; 52: 1271–1278.
27. ■ Huang ZG, Gao BX, Chen H, *et al.* An efficacy analysis of whole-body magnetic resonance imaging in the diagnosis and follow-up of polymyositis and dermatomyositis. PLoS One 2017; 12:e0181069.

A study in 129 IIM patients showed that whole-body MRI, a noninvasive imaging technique, would be of interest as a screening method for diagnosing systemic malignancies in myositis patients.

28. Olthoff A, Carstens PO, Zhang S, *et al.* Evaluation of dysphagia by novel real-time MRI. Neurology 2016; 87:2132–2138.

29. Dutton K, Soden M. Malignancy screening in autoimmune myositis among Australian rheumatologists. Intern Med J 2017; 47:1367–1375.
30. Leatham H, Schadt C, Chisolm S, *et al*. Evidence supports blind screening for
■■ internal malignancy in dermatomyositis: data from 2 large US dermatology cohorts. Medicine (Baltimore) 2018; 97:e9639.
The study performed in two large cohorts including 400 dermatomyositis patients in the United States is the first to address the use and effectiveness of screening practice in these patients. Blind screening at onset and perhaps repeated screening along time in some patients seems warranted.
31. Hendren E, Vinik O, Faragalla H, Haq R. Breast cancer and dermatomyositis: a case study and literature review. Curr Oncol 2017; 24: e429–e433.
32. Gkegkes ID, Minis EE, Iavazzo C. Dermatomyositis and colorectal cancer: a systematic review. Ir J Med Sci 2017; doi: 10.1007/s11845-017-1716-7. [Epub ahead of print]
33. Stübgen JP. Inflammatory myopathies and lymphoma. J Neurol Sci 2016; 369:377–389.
34. Tiniakou E, Mammen AL. Idiopathic inflammatory myopathies and malignancy: a comprehensive review. Clin Rev Allergy Immunol 2017; 52:20–33.
35. Ebell MH, Thai TN, Royalty KJ. Cancer screening recommendations: an
■ international comparison of high income countries. Public Health Rev 2018; 39:7.
The study uses a comprehensive approach to summarize the main recommendations for cancer screening in different countries.

The clinico-serological spectrum of overlap myositis

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Purpose of review
To provide the most recent evidence on the overlap myositis.

Recent findings
Several new evidences on the overlap myositides have recently emerged. Regarding the classical myositis associated antibodies, several contributions focused on a better definition of the clinical associations and the disease course associated with these autoantibodies. Moreover, in the last years, new autoantibodies in idiopathic inflammatory myositis or other connective tissue diseases have been identified [namely anti-RuvBL1/2, poly-U-binding factor 60 kDa protein (PUF-60) and cytosolic 5′-nucleotidase 1A (NT5C1A)], and an increasing number of publications allow now to consider them as new myositis-associated antibodies with probably their own peculiar clinical profile.

Summary
Overlap myositis is probably the largest subgroup within the idiopathic inflammatory myositis, with a prevalence that can reach 50% of all adult patients. The serological spectrum of overlap myositis has recently been enriched by the discovery of new autoantibodies. The spread of multiparametric methods has facilitated the identification of the autoantibody marker of overlap myositis and the better definition of the clinical profiles associated with them.

Keywords
clinical profile, myositis-associated antibodies, overlap myositis

INTRODUCTION

Overlap syndrome could be defined as a disease in which clinical features of two or more connective tissue diseases (CTDs) in the same patient are detectable [1]. In particular, the overlap myositides includes idiopathic inflammatory myositis (IIM) with extramuscular symptoms, peculiar of systemic lupus erythematosus (SLE), systemic sclerosis (SSc) or Sjögren's syndrome, frequently associated with specific autoantibodies [2]. The so-called myositis-associated autoantibodies (MAA), mostly represented by anti-Ku, anti-PM/Scl, anti-Ro/Sjögren's syndrome antibodies (SSA) and anti-U1-RNP [3], typically characterized overlap myositises in which they can be found alone or associated with other autoantibodies. Additional autoantibodies have recently been reported as potential markers of overlap myositis [RuvBL1/2, poly-U-binding factor 60 kDa protein (PUF-60), anti-fibrillarin (anti-U3-RNP), anticytosolic 5′-nucleotidase 1A (NT5C1A)]. Furthermore, the classical myositis specific autoantibodies (MSAs) can be found when clinical overlap from different diseases are observed [i.e. melanoma differentiation-associated protein 5 (MDA5), glycyl tRNA synthetases (EJ)].

The concept of overlap myositis, as a distinct clinical entity with associated antibodies, was previously proposed in 2005 by Troyanov *et al.* [4]. Overlap myositis is defined by the association of myositis with CTD features, such as Raynaud's phenomenon, arthritis and interstitial lung disease (ILD), as well as features of SSc and SLE, which most commonly are present at the time of diagnosis. In addition, about 15% of patients without overlap clinical features, show an overlap antibody (15 overlap antibodies, including antisynthetase antibodies, are listed) often with suggestive biopsy findings while overlap features developed at follow-up [5]. In such a view, more than 50% of myositis should be accounted as overlap myositis, whereas 'pure' polymyositis (PM) accounts for only 5% of patients and remains a diagnosis of exclusion because its nonspecific phenotype is at high risk for mimickers.

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KEY POINTS

- New autoantibodies (anti-PU60, RuvBL1/2, anti-NT5C1A) present in overlap myositis have been described in recent months.
- Thanks to the publication of extensive case series or reporting of clinical cases, it is currently possible to better define the clinical associations with myositis-associated antibodies (anti-PM/Scl, anti-Ku, anti-U3-RNP).
- Additional autoantibodies have recently been reported as potential markers of overlap myositis (RuvBL1/2, PUF-60, U3-RNP, anti-NT5C1A).
- The spread of multiparametric methods (i.e. lineblot assay) has facilitated the identification of the autoantibody marker of overlap myositis and the better definition of the clinical profiles associated with them.

The antisynthetase syndrome (ASS) is a group of overlapping diseases with distinct phenotypes and clinical course [6▪]. However, for the historical attribution to the subset of IIM clinical forms, for the extensive recent literature and for the inclusion of anti-Jo-1 as the sole autoantibody criterion and with the highest score, in the recently published classification criteria for IIM [7▪▪], it deserves a separate discussion and is therefore not considered in this article.

The main purpose of this review is the presentation of all the recent clinical and immunological acquisitions relevant for a better clinical–serological definition of overlap myositis syndromes.

OVERLAP MYOSITIS WITH SYSTEMIC SCLEROSIS

The most frequent overlap myositis is represented by overlap polydermatomyositis and SSc (PDM/SSc) or scleromyositis, representing more than 44% of all the overlap syndrome with SSc [1]. The most relevant clinical features of PDM/SSc include Raynaud's phenomenon, myositis and arthritis, whereas lung and esophageal involvement prevalence ranges from 32 to 78% of cases [1]. Muscle involvement is usually mild, with a modest increase of muscle enzymes. Serological markers of PDM/SSc are mostly represented by anti-Ku and PM/Scl antibodies. Less common is the presence of other autoantibodies, namely anti-U3-RNP.

Anti-Ku antibodies

Anti-Ku antibodies were originally described in 1981, as serological markers of PM/SSc, but they later could be found in different CTDs, as SLE, IIMs, Sjögren's syndrome and undifferentiated connective tissue diseases [8]. In SSc, they are detected with a prevalence ranging from 2 to 6.8% and are significantly associated with a scleroderma–myositis overlap syndrome [9,10].

Anti-Ku-associated myositis is usually mild, with monophasic clinical course without relapse, and good response to treatment [11]. No specific histological features have been described in anti-Ku myositis; however, myofiber necrosis/regeneration and inflammatory cell infiltrates were reported in some isolated cases of anti-Ku positive PDM/SSc [11]. Other clinical features are represented by inflammatory arthritis, limited cutaneous SSc, esophageal involvement, Raynaud's phenomenon, usually without ulcers complications [9]. By contrast, ILD associated with anti-Ku-positive myositis (with or without SSc overlap) has been reported to be more severe and refractory to corticosteroid therapy in 75% of patients [11].

Dropped head syndrome followed by camptocormia and proximal-dominant extremity weakness has been recently reported in PDM/SSc associated with anti-Ku antibody and histopathological finding of rimmed vacuoles [12▪]. In this case, the presence of anti-Ku antibody is important, together with the distribution of axial weakness, to differentiate overlap myositis from inclusion body myositis (IBM) and ensure the adoption of appropriate treatment.

An overview of immunofluorescence patterns, target antigens, antigens function of anti-Ku and other MAA autoantibodies is reported in Table 1.

Anti-PM/Scl antibodies

Anti-PM/Scl antibodies recognize a nucleolar exosome: the major epitopes are called PM100 and PM75. Anti-PM/Scl antibodies are found in about 33% of PDM/SSc overlap cases [14] and in 4–12% of IIMs [15] with a lower prevalence in Asiatic cohorts [16]. They are associated with limited cutaneous SSc, Raynaud's phenomenon [15], arthritis, mechanic's hands and ILD [17] and rare occurrence of esophageal dysmotility [1], which can be very severe and require feeding with percutaneous endoscopic gastrostomy [18]. In addition, palmoplantar hyperkeratosis has recently been proposed as a distinct dermatological manifestation, described almost exclusively in anti-PM/Scl positive patients [19]. Some authors reported different clinical features associated either with single or both PM/Scl antigens reactivity: isolated anti-PM75 seems more frequently associated with joint contractures and SSc; isolated anti-PM100 with more severe myositis, but no differences were reported between patients

Table 1. An overview of immunofluorescence patterns [13], target antigens, antigens function and main clinical associations of autoantibodies mostly associated with overlap myositis

Autoantibody	ANA IIF pattern	Antigenic target	Principal antigen function	Assays mainly used for detection	Clinical associations in overlap myositis
Ku	Nuclear speckled	70/80-kDa DNA-PK regulatory subunit	DNA repair	IP, CIE, ELISA, IB, LB	IIM/SSc: ILD, arthritis, limited cutaneous SSc, esophageal involvement, Raynaud's phenomenon
PM/Scl	Nucleolar homogeneous and nuclear speckled	Nucleolar exosome (Pm/Scl complex of 11–16 proteins), main antigens 75 and 100 kDa	mRNA degradation	IP, CIE, ELISA, LB	IIM/SSc: mechanic's hands, calcinosis, Raynaud's phenomenon, sclerodactily and ILD; rare severe occurrence of esophageal dysmotility
U3-RNP	Clumpy nucleolar	34-kDa protein (fibrillarin)	Cleavage of pre-rRNA	IP, LB	IIM/SSc with diffuse cutaneous scleroderma
RuvBL1/2	Nuclear speckled	RuvBL1/2 complex	Modulation of transcriptional activation and protein assembly	IP, ELISA	IIM/SSc with diffuse cutaneous scleroderma
Ro52/TRIM21	Nuclear fine speckled or negative	TRIM21 located in nucleus and cytoplasm	E3 ubiquitin ligase activity	ELISA, IB LB	Associated with ASS, possible risk factor for severe ILD, arthritis, mechanic's hands
U1nsRNP	Nuclear large/ coarse speckled	U1 RNA + 70 kDa and A, C proteins of the RNP/Sm complex	RNA splicing	IP, CIE, ELISA, LB,	MCTD
NT5C1A	Cytoplasmic	Cytosolic 5′-nucleotidase 1A	Dephosphorylation of nucleoside monophosphates	IP, ELISA	More severe disease in juvenile myositis
PUF-60	Not reported	Poly (U)-binding-splicing factor 60-kDa protein	RNA splicing	IP, ELISA with recombinant full length human PUF-60 protein	IIM/RA and IIM/SS

ANA, antinuclear antibody; antifibrillarin, anti-U3-RNP; ASS, antisynthetase syndrome; CIE, counterimmunoelectrophoresis; DNA-PK, desoxyribonucleic acid dependent protein kinase; IB, immunoblot; IIF, indirect immuno-fluorescence; IIM, idiopathic inflammatory myositis; ILD, interstitial lung disease; IP, immunoprecipitation; LB, lineblot; MCTD, mixed connective tissue disease; NT5C1A, cytosolic 5′-nucleotidase 1A; PUF-60, poly-U-binding factor 60 kDa protein; RA, rheumatoid arthritis; rRNA, ribosomal ribonucleic acid; SSc, systemic sclerosis; TRIM21, tripartite motif 21; U1snRNP, U1 small nuclear ribonucleoprotein.

with only anti-PM100 or only anti-PM75 antibodies in a recent article [20▪]. The reactivity to both the PM75 and PM100 epitopes is more frequent in the complete overlap syndrome, characterized by myositis, ILD, digital ulcers and rare occurrence of pulmonary hypertension. Myositis associated with anti-PM/Scl showed no peculiar histological features [21], except for muscle fibrosis reported as strongly associated by some authors [22]. It is important to underline that there were no significant differences in the type and severity of pulmonary involvement, as well as the survival rate among patients with anti-PM/Scl overlap syndrome and ASS [23]. A very recent American study [20▪] analyzed the clinical features and disease course of 41 IIM PM/Scl positive patients. The study has confirmed previous reports regarding the prevalence of overlap with SSc, found in 30% of PM/Scl patients, and also the high prevalence of extramuscular symptoms (i.e. mechanic's hands, calcinosis, Raynaud's phenomenon, sclerodactily, subcutaneous edema) compared with other forms of myositis. It is also showed how muscle weakness may develop during the course of the disease after a mean follow-up of 6 years, from 37% at disease onset to 93% at the end of follow-up. Muscle weakness was more common in arm abductors than in hip flexors.

In our experience, calcinosis was detected in 25% of adult patients with IIM. Anti-PM/Scl antibodies were significantly associated with both overlap myositis between SSc/PDM, and with calcinosis. These data suggest that calcinosis should be considered a prominent feature of overlap myositis associated with PM–Scl [24▪].

Although rare, anti-PM–Scl antibody has been reported in a small cohort of juvenile overlap myositis followed by 1.6–15 years, with a good response to treatment with corticosteroids and a benign evolution [25].

Antifibrillarin antibodies

The prevalence of antifibrillarin antibodies, also known as anti-U3-RNP, and their association with myositis has been confirmed in a recent French collaborative work [26▪]: antifibrillarin are detected in 4–14% of patients with SSc, more often non-white and in 25–31.4% of overlap myositis, this rate

can reach 50% when considering diffuse cutaneous SSc [26▪,27]. Few data are available on muscle biopsy and no distinctive microscopic findings were reported [27].

Anti-RuvBL1/2 antibodies

In the original description [28], anti-RuvBL1/2 were reported in 1.8% of a cohort of patients with SSc, and were associated with SSc-overlap syndrome in about 60% of patients. Analyzing antinuclear antibody negative sera from patients with SSc were found in two cases of overlap myositis [29].

MYOSITIS IN MIXED CONNECTIVE TISSUE DISEASE

The term 'mixed connective tissue disease' (MCTD) concerns a systemic autoimmune disease typified by overlapping features between two or more systemic autoimmune diseases associated with antibodies against the U1 small nuclear ribonucleoprotein autoantigen (U1snRNP).

In MCTD a high titer of anti-U1snRNP antibodies is usually found, particularly when the 70-kD protein is targeted, whereas low titers can be found in other CTDs [30]. Myositis is included as one of the main criteria in all four sets of classification criteria proposed for MCTD [31], but recent data on muscle involvement are quite limited [32]. Approximately 35–79% of the MCTD patients will develop signs of myositis during the course of the disease, usually in the earlier phases of the disease. However, myositis is rarely present at disease onset [32] and in a recent study that evaluated 118 MCTDs, none of the patients received a diagnosis of IIM [31] during follow-up.

Detailed clinical descriptions of myositis in MCTD are sparse: it is generally believed that MCTD-related myositis has the same muscular involvement of PM; however, myositis is usually steroid-responsive and does not appear to lead to permanent damage [32].

Data on muscle histology in MCTD are limited and did not allow us to draw a definite conclusion. However, the histopathological features are closer to that of dermatomyositis (DM) than PM [32]. Less data are available regarding other antismall nuclear ribonucleoprotein antibodies, but they have been described in patients with an overlap IIM/SSc.

OVERLAP MYOSITIS/SYSTEMIC LUPUS ERYTHEMATOSUS

A true myositis/SLE overlap is a rare condition characterized by classical histological signs of myositis (i.e. perivascular and perimysial inflammation) and type 2 fibers atrophy as a peculiar feature [33], usually without evidence of vasculitis or neuropathy on muscle biopsy [34▪]. Conflicting data have been published regarding whether the occurrence of SLE could influence the prognosis of these patients [35]. No peculiar serological marker has been associated with myositis/SLE overlap [1]. A recent Chinese article analyzed [34▪] the occurrence of myositis in 1701 patients with SLE, with a point prevalence of 2.6% (44 cases). Myositis was more common in younger patients and with shorter disease duration than SLE without muscular involvement. Myositis was also associated with alopecia, leukopenia and active lupus disease [34▪].

Recently, a rare case of anti-MDA5 DM that precedes the onset of SLE has been published [36]. Within 1 year of the diagnosis of DM, the patient developed a complete clinical and serological SLE with glomerulonephritis and SLE cerebritis.

OTHER OVERLAP MYOSITIS

Sjögren's syndrome in overlap with IIM has been originally reported in about 3% of cases [35], whereas the subclinical signs of myositis could be frequently found during the course of Sjögren's syndrome. A recent nationwide retrospective study in Taiwan reported a higher risk of Sjögren's syndrome in DM patients than in a control population [37], with a incidence rate of 18.37 cases per 1000 person-years. In addition, the authors underlined a differential association between sex, higher in male populations (incidence rate of 12.37 for males versus 5.42 for females). However, another study of more than 1300 Sjögren's syndrome patients reported a 'true' IIM, according to Bohan and Peter's criteria, in 10 cases [38].

Few data are available on the histological muscular features, characterized by interstitial myositis and perivascular inflammation. In the Italian work most biopsies reported nonspecific signs of miopathy; however, the authors identified a possible specific pattern in patients with PM/Sjögren's syndrome, with a peculiar decrease in cytochrome C oxidase staining [38].

The overlap between rheumatoid arthritis (RA) and IIM is difficult to define. Because of the known possibility of muscle involvement in patients with RA, this inflammatory complication has been named 'rheumatoid myositis'. Erosive arthritis with rheumatoid factor and/or anticitrullinated antibodies is frequently reported as a part of ASS. It is difficult to estimate the real prevalence of the overlap RA/IIM which still remain a rare complication, even though a prevalence of 8% has recently been

reported [39]. These authors suggest that rheumatoid myositis usually occurs early during the course of RA, in patients with active disease and with increased acute-phase reactants [39]. Some authors suggest that the histological picture of rheumatoid myositis is typically moderate and patchy, affecting mainly proximal muscle in a symmetrical and bilateral manner.

ANTI-RO/SJÖGREN'S SYNDROME ANTIBODIES IN MYOSITIS

The Ro/SSA antigen, consists of two polypeptide components of 52 and 60 kDa associated with hyRNA; Ro52 (also called TRIM21) is biochemically and immunologically distinct from Ro60, with the highest immunogenicity [40].

Antibodies against Ro52 are one of the most common autoantibodies of the MAA group, occurring in 20–30% of IIM patients [41]. In IIM, they are mostly detected without anti-Ro60 positivity [42] and are frequently associated with other MSAs, such as antisynthetase autoantibodies in which it is particularly prevalent in anti-Jo-1 compared with anti-PL-7 or anti PL-12 [43].

Anti-Ro52 antibodies have been attributed a possible negative prognostic role in IIM, mainly ASS. In fact, in ASS Ro-52 appears to be associated with a more severe ILD and arthritis [44,45] but these data are still being investigated [43].

A recent case report described the association of anti-EJ and anti-Ro in a male patient with acute fibrinous and organizing pneumonia and necrotizing myopathy [46]. Although lung manifestations are frequently found in anti-EJ antibodies, necrotizing myopathy is rarely described in these patients.

OTHER AUTOANTIBODIES

Anti-poly-U-binding factor 60-kDa antibodies

Anti-PUF-60 antibodies are directed to PUF-60 protein and have recently been found in a cohort of patients with dermatomyositis and Sjögren's syndrome, and associated with transcription intermediary factor 1γ autoantibodies [47▪]. A further study from China [48▪] reported a frequency of 10.6% anti-PUF-60 antibodies in IIM patients. Subgrouping analysis revealed a prevalence of 26.5% in overlap myositis mainly represented by myositis-Sjögren's syndrome (37.6%) and myositis-RA (25%). These antibodies were also detected in SLE but not in SSc. Significantly, anti-PUF-60 antibodies could be a potential serum biomarker for overlap myositis and preferentially with Sjögren's syndrome and RA, whereas the anti-PM–Scl and anti-Ku antibodies are preferentially found in association with SSc or SLE.

Anticytosolic 5′-nucleotidase 1A antibodies

Antibodies against NT5C1A, also called anticN1A antibody, have been described as associated with sporadic IBM in the last decade. In the last 2 years, however, several article have reported the presence of these antibodies in other IIMs [49], several other CTDs (mainly Sjögren's syndrome) and juvenile myositis [50], with a prevalence that may differ according to the assays used and the ethnic groups. Therefore, this antibody can be more appropriately included in MAA group. In most cases reported in non-IBM, anti-NT5C1A were found in associations with MSA or Ro-52. Many issues still need to be clarified: the significance of anti-NT5C1A autoantibodies in patients with non-IBM autoimmune diseases remains unclear and fewer data on muscle biopsy is available.

TECHNICAL ISSUES

Although not always satisfactory, the spread of multiparametric assays, such as immunoline blot, for antibodies specific for myositis or SSc or associated antibodies, allows a higher sensitivity [51,52]. Furthermore, some antigens are now divided into major epitopes of different molecular weight that can be detected alone or together. Although not yet defined, it is possible that the recognition of a single epitope may be associated with a different clinical profile of the disease. One of the tasks of the upcoming years will be to consider each new result to redesign the clinical–serological picture of myositis overlap.

CONCLUSION

The serological spectrum of overlap myositis has recently been enriched by the discovery of new autoantibodies. The spread of multiparametric methods has facilitated the identification of the autoantibody marker of overlap myositis and the better definition of the clinical profiles associated with them. Further studies are necessary to confirm the new findings.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Iaccarino L, Gatto M, Bettio S, *et al.* Overlap connective tissue disease syndromes. Autoimmun Rev 2013; 12:363–373.
2. Benveniste O, Stenzel W, Allenbach Y. Advances in serological diagnostics of inflammatory myopathies. Curr Opin Neurol 2016; 29:662–673.
3. Ghirardello A, Borella E, Beggio M, *et al.* Myositis autoantibodies and clinical phenotypes. Auto Immun Highlights 2014; 5:69–75.
4. Troyanov Y, Targoff IN, Tremblay JL, *et al.* Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine (Baltimore) 2005; 84:231–249.
5. Senécal JL, Raynauld JP, Troyanov Y. Editorial: a new classification of adult autoimmune myositis. Arthritis Rheumatol 2017; 69:878–884.
6. ■ Bartoloni E, Gonzalez-Gay MA, Scirè C, *et al.* Clinical follow-up predictors of disease pattern change in anti-Jo1 positive antisynthetase syndrome: results from a multicenter, international and retrospective study. Autoimmun Rev 2017; 16:253–257.

Data from a retrospective cohorts of antisynthetase syndrome (ASS) patients that showed how specific accompanying features may be considered predictors of subsequent appearance of at least one of the classic manifestations in ASS Jo-1-positive patients.

7. ■■ Lundberg IE, Tjärnlund A, Bottai M, *et al.* EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis 2017; 69:2271–2282.

The study identified new classification criteria to be used for research and clinical trials in both adults and children. They show high sensitivity and specificity, but they still need to be fully validated.

8. Hoa S, Hudson M, Troyanov Y, *et al.* Single-specificity anti-Ku antibodies in an international cohort of 2140 systemic sclerosis subjects: clinical associations. Medicine (Baltimore) 2016; 95:e4713.
9. Cavazzana I, Fredi M, Taraborelli M, *et al.* A subset of systemic sclerosis but not of systemic lupus erythematosus is defined by isolated anti-Ku autoantibodies. Clin Exp Rheumatol 2013; 31:2–5.
10. Foocharoen C, Watcharenwong P, Netwijitpan S, *et al.* Relevance of clinical and autoantibody profiles in systemic sclerosis among Thais. Int J Rheum Dis 2017; 20:1572–1581.
11. Rigolet A, Musset L, Dubourg O, *et al.* Inflammatory myopathies with anti-Ku antibodies: a prognosis dependent on associated lung disease. Medicine (Baltimore) 2012; 91:95–102.
12. ■ Yoshida T, Yoshida M, Mitsuyo K, *et al.* Dropped head syndrome and the presence of rimmed vacuoles in a muscle biopsy in scleroderma–polymyositis overlap syndrome associated with anti-Ku antibody. Intern Med 2018; 57:887–891.

In this case, the presence of anti-Ku antibody is important, together with the distribution of axial weakness, to differentiate overlap myositis from inclusion body myositis and ensure the adoption of appropriate treatment.

13. Chan EKL, Damoiseaux J, Carballo OG, *et al.* Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns (ICAP) 2014–2015. Front Immunol 2015; 6:1–13.
14. Pakozdi A, Nihtyanova S, Moinzadeh P, *et al.* Clinical and serological hallmarks of systemic sclerosis overlap syndromes. J Rheumatol 2011; 38:2406–2409.
15. Lega JC, Fabien N, Reynaud Q, *et al.* The clinical phenotype associated with myositis-specific and associated autoantibodies: a meta-analysis revisiting the so-called antisynthetase syndrome. Autoimmun Rev 2014; 13:883–891.
16. Muro Y, Hosono Y, Sugiura K, *et al.* Anti-PM/Scl antibodies are found in Japanese patients with various systemic autoimmune conditions besides myositis and scleroderma. Arthritis Res Ther 2015; 17:1–10.
17. Aguila LA, Lopes MRU, Pretti FZ, *et al.* Clinical and laboratory features of overlap syndromes of idiopathic inflammatory myopathies associated with systemic lupus erythematosus, systemic sclerosis, or rheumatoid arthritis. Clin Rheumatol 2014; 33:1093–1098.
18. Chinniah KJ, Mody GM. Recovery from severe dysphagia in systemic sclerosis – myositis overlap: a case report. Afr Health Sci 2017; 17:593–596.
19. Taki T, Muro Y, Ogawa Y, Akiyama M. Image gallery: palmoplantar hyperkeratosis in dermatomyositis with anti-PM/Scl antibodies. Br J Dermatol 2017; 176:e94.
20. ■ De Lorenzo R, Pinal-fernandez I, Huang W, *et al.* Muscular and extramuscular clinical features of patients with anti-PM/Scl autoantibodies. Neurology 2018; 90:e2068–e2076.

In this article, overlap with systemic sclerosis (SSc) is found in 30% of PM/Scl patients with high prevalence of extramuscular symptoms. Muscle weakness was more common in arm abductors than in hip flexors and may develop during the course of the disease.

21. Lepreux S, Hainfellner JA, Vital A. Idiopathic inflammatory myopathies overlapping with systemic diseases. Clin Neuropathol 2018; 37:6–15.
22. Paik JJ. Myopathy in scleroderma and in other connective tissue diseases. Curr Opin Rheumatol 2016; 28:631–635.
23. Mejía M, Herrera-Bringas D, Pérez-Román DI, *et al.* Interstitial lung disease and myositis-specific and associated autoantibodies: clinical manifestations, survival and the performance of the new ATS/ERS criteria for interstitial pneumonia with autoimmune features (IPAF). Respir Med 2017; 123:79–86.
24. ■ Fredi M, Bartoli F, Cavazzana I, *et al.* Calcinosis in poly-dermatomyositis: clinical and laboratory predictors and treatment options. Clin Exp Rheumatol 2017; 35:303–308.

The study suggests that calcinosis should be considered a prominent feature of overlap myositis associated to PM–Scl.

25. Fotis L, Baszis KW, White AJ, French AR. Four cases of anti-PM/Scl antibody-positive juvenile overlap syndrome with features of myositis and systemic sclerosis. J Rheumatol 2016; 43:1786–1789.
26. ■ Tall F, Dechomet M, Riviere S, *et al.* The clinical relevance of antifibrillarin (anti-U3-RNP) autoantibodies in systemic sclerosis. Scand J Immunol 2017; 85:73–79.

The study confirmed the clinical relevance of antifibrillarin antibodies as marker of overlap myositis in patients with diffuse cutaneous SSc.

27. Aggarwal R, Lucas M, Fertig N, *et al.* Anti-U3 RNP autoantibodies in systemic sclerosis. Arthritis Rheum 2009; 60:1112–1118.
28. Kaji K, Fertig N, Medsger TA, *et al.* Autoantibodies to RuvBL1 and RuvBL2: a novel systemic sclerosis-related antibody associated with diffuse cutaneous and skeletal muscle involvement. Arthritis Care Res 2014; 66:575–584.
29. Pauling JD, Salazar G, Lu H, *et al.* Presence of antieukaryotic initiation factor-2B, anti-RuvBL1/2 and antisynthetase antibodies in patients with antinuclear antibody negative systemic sclerosis. Rheumatol 2018; 57:712–717.
30. Migliorini P, Baldini C, Rocchi V, Bombardieri S. Anti-Sm and anti-RNP antibodies. Autoimmunity 2005; 38:47–54.
31. Reiseter S, Gunnarsson R, Corander J, *et al.* Disease evolution in mixed connective tissue disease: results from a long-term nationwide prospective cohort study. Arthritis Res Ther 2017; 19:284.
32. Hall S, Hanrahan P. Muscle involvement in mixed connective tissue disease. Rheum Dis Clin North Am 2005; 31:509–517.
33. Jakati S, Rajasekhar L, Uppin M, Challa S. SLE myopathy: a clinicopathological study. Int J Rheum Dis 2015; 18:886–891.
34. ■ Liang Y, Leng RX, Pan HF, Ye DQ. Associated variables of myositis in systemic lupus erythematosus: a cross-sectional study. Med Sci Monit 2017; 23:2543–2549.

The study described the overlap idiopathic inflammatory myositis/systemic lupus erythematosus in the Chinese populations; myositis was more common in younger patients, with shorter disease duration and was also associated with alopecia, leukopenia and active lupus disease.

35. Colafrancesco S, Priori R, Valesini G. Inflammatory myopathies and overlap syndromes: update on histological and serological profile. Best Pract Res Clin Rheumatol 2015; 29:810–825.
36. Milam EC, Futran J, Franks AG Jr. Anti-MDA5 antibody dermatomyositis overlap with systemic lupus erythematosus: a case report and review of the literature. Open Rheumatol J 2016; 10:122–128.
37. Tseng CC, Chang SJ, Tsai WC, *et al.* Sex differential association of dermatomyositis with Sjögren syndrome. CMAJ 2017; 189:E187–E193.
38. Colafrancesco S, Priori R, Gattamelata A, *et al.* Myositis in primary Sjögren's syndrome: data from a multicentre cohort. Clin Exp Rheumatol 2015; 33:457–464.
39. Ancuţa C, Pomîrleanu DC, Anton CR, *et al.* Rheumatoid myositis, myth or reality? A clinical, Imaging and histological study. Rom J Morphol Embryol 2014; 55:781–785.
40. Franceschini F, Cavazzana I. Anti-Ro/SSA and La/SSB antibodies. Autoimmunity 2005; 38:55–63.
41. Koenig M, Fritzler MJ, Targoff IN, *et al.* Heterogeneity of autoantibodies in 100 patients with autoimmune myositis: insights into clinical features and outcomes. Arthritis Res Ther 2007; 9:R78.
42. Schulte-Pelkum J, Fritzler M, Mahler M. Latest update on the Ro/SS-A autoantibody system. Autoimmun Rev 2009; 8:632–637.
43. Pinal-Fernandez I, Casal-Dominguez M, Huapaya JA, *et al.* A longitudinal cohort study of the antisynthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology (Oxford) 2017; 56:999–1007.
44. Shi J, Li S, Yang H, *et al.* Clinical profiles and prognosis of patients with distinct antisynthetase autoantibodies. J Rheumatol 2017; 44:1051–1057.
45. Bauhammer J, Blank N, Max R, *et al.* Rituximab in the treatment of Jo1 antibody-associated antisynthetase syndrome: anti-Ro52 positivity as a marker for severity and treatment response. J Rheumatol 2016; 43:1566–1574.
46. Kashif M, Arya D, Niazi M, Khaja M. A rare case of necrotizing myopathy and fibrinous and organizing pneumonia with anti-EJ antisynthetase syndrome and SSA antibodies. Am J Case Rep 2017; 18:448–453.
47. ■ Fiorentino DF, Presby M, Baer AN, *et al.* PUF60: a prominent new target of the autoimmune response in dermatomyositis and Sjögren's syndrome. Ann Rheum Dis 2016; 75:1145–1151.

The study reported for the first time anti-poly-U-binding factor 60-kDa (PUF-60) antibodies, found in a cohort of patients with dermatomyositis and Sjögren's syndrome and associated to TIF1γ autoantibodies.

48. Zhang YM, Yang HB, Shi JL, *et al.* The prevalence and clinical significance of ■ anti-PUF60 antibodies in patients with idiopathic inflammatory myopathy. Clin Rheumatol 2018; 37:1573–1580.
The study highlights anti-PUF-60 antibodies as a potential serum biomarker for overlap myositis and preferentially with Sjögren's syndrome and rheumatoid arthritis.
49. Muro Y, Nakanishi H, Katsuno M, *et al.* Prevalence of anti-NT5C1A antibodies in Japanese patients with autoimmune rheumatic diseases in comparison with other patient cohorts. Clin Chim Acta 2017; 472:1–4.
50. Yeker RM, Pinal-Fernandez I, Kishi T, *et al.* Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis. Ann Rheum Dis 2018; 77:714–719.
51. Cavazzana I, Fredi M, Ceribelli A, *et al.* Testing for myositis specific autoantibodies: comparison between line blot and immunoprecipitation assays in 57 myositis sera. J Immunol Methods 2016; 433:1–5.
52. Ghirardello A, Bettio S, Bassi N, *et al.* Autoantibody testing in patients with myositis: clinical accuracy of a multiparametric line immunoassay. Clin Exp Rheumatol 2017; 35:176–177.

Advances in the early diagnosis and therapy of inclusion body myositis

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Purpose of review
To describe recent advancements in diagnostic and therapeutic approaches to inclusion body myositis (IBM).

Recent findings
Our understanding of the implications of anti-cytosolic 5′-nucleotidase 1A autoantibody status in IBM and other diseases is increasing. Muscle imaging using magnetic resonance techniques and ultrasound is increasingly being performed and characteristic patterns of muscle involvement may help with diagnosis. Longitudinal imaging studies are likely to help with monitoring and as an outcome measure in clinical trials. Recent small-scale studies of Arimoclomol and Rapamycin have shown promising results and further investigation of these medications is ongoing. Exercise is likely to form an increasingly important facet of management of patients with IBM, but the optimal type of exercise programme to enrol patients in is not yet determined.

Summary
Antibody testing and muscle imaging results may improve our ability to diagnose IBM and the availability of effective disease modifying treatments targeting novel non-inflammatory pathways could soon become a reality. It remains the duty of those involved in the management of patients with IBM to facilitate involvement in clinical trials and other research studies.

Keywords
diagnostics, imaging, inclusion body myositis, serology, therapeutics

INTRODUCTION

Inclusion body myositis (IBM) is an acquired myopathy usually occurring in those aged over 50 years. IBM is conventionally grouped with the idiopathic inflammatory myopathies, but for several reasons can be seen as the 'odd one out'. Despite muscle inflammation being a prominent feature, the disease is resistant to treatment with immunosuppressive therapies, none of which lead to convincing or sustained therapeutic benefits. Recognition of this issue, the pathognomonic pattern of weakness and the characteristic histopathological features eventually allowed separation of IBM from polymyositis and other muscle disorders [1].

Disease progression in IBM is characterized by damage to selected skeletal muscles, particularly those of the volar aspect of the forearms and the anterior thigh, which are gradually replaced by fatty–fibrous tissue, leading to increasing weakness of grip and knee extension. This clinical change is mirrored by increasingly conspicuous degenerative features on muscle biopsy, including aggregation of misfolded proteins and rimmed vacuoles [2]. The exact sequence of events that eventually culminates in the severe disability seen is the subject of intense debate.

The current review will describe recent advancements in diagnostic and therapeutic approaches to IBM, focussing on publications since 2016. Detailed discussion of genetic and aetiopathological aspects of the disease are not discussed.

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KEY POINTS

- Anti-cN-1A autoantibodies may prove useful in the diagnosis and stratification of IBM.
- Muscle imaging, including MRI and ultrasound, is playing an increasingly important role in the diagnosis and monitoring of patients with IBM.
- Muscle MRI is also increasingly being used as outcome measures in clinical trials.
- Novel therapeutics targeting nonimmune disease pathways are currently being investigated in IBM.

TOOLS TO ENABLE AN EARLIER DIAGNOSIS?

Many patients with IBM continue to be initially misdiagnosed and are given treatments, including potent immunosuppressive medications, without any prospect of benefit. It is now acknowledged that early iterations of the IBM diagnostic criteria had relatively low sensitivity for those with early disease, in part because of a requirement to demonstrate the histopathological hallmarks associated with established disease which may be absent in earlier stages [3]. Although more recent diagnostic criteria have shifted towards identification of the highly specific pattern of muscle weakness seen in IBM, this potentially remains problematic in the sense that for clinical detection of such weakness, significant muscle damage must have already occurred. This may in part explain the failure to demonstrate the benefits of treatment in IBM, in which the likelihood of response is probably low if skeletal muscle tissue has already been extensively replaced by fat. Identification of a highly specific biomarker of *early* IBM is a key unmet need for patients with this condition.

Serology

Much excitement followed the description of anti-cytosolic 5′-nucleotidase 1A (anti-cN-1A; also referred to as NT5C1A) autoantibodies in IBM by European and American researchers [4,5]. Recent work has demonstrated a relatively poor specificity of antibody positivity for IBM, with relatively large proportions of patients with Sjögren's syndrome and systemic lupus erythematosus also seropositive, highlighting that interpretation of the result must take place in full view of the clinical context [6–8]. However, the power of anti-cN-1A autoantibody status to distinguish between polymyositis and IBM is high, potentially providing a useful tool for early confirmation of IBM, before the appearance of the pathognomonic pattern of weakness. This could help prevent patients with IBM being unnecessarily exposed to potentially harmful immunosuppressive therapies and allow earlier recruitment in to clinical trials.

The identification of anti-cN-1A autoantibodies has also offered clues regarding disease pathogenesis, potentially helping to explain the link between the inflammatory and degenerative processes evident in the disease [9]. It is noted however that anti-cN-1A autoantibody status does not appear to associate independently with a specific human leukocyte antigen genotype, in contrast to some of the established myositis-specific autoantibodies [10,11]. Taken together, anti-cN-1A autoantibodies are generally thought of as myositis-associated rather than myositis-specific autoantibodies.

Separate groups have demonstrated a more severe disease phenotype in anti-cN-1A autoantibody-positive IBM [12,13]. Stratification of the IBM population according the autoantibody status could thus prove useful in guiding patient monitoring and in understanding the response to experimental treatments. Furthermore, a recent publication has highlighted additional complexity, demonstrating the presence of anti-cN-1A autoantibodies in 27% patients with juvenile myositis, compared with 12% of healthy control children ($P = 0.002$) [14]. These autoantibody-positive patients also appeared to have a more severe phenotype exemplified by a higher proportion with pulmonary symptoms at diagnosis, more frequent hospitalizations and requirement for a greater number of medications compared with antibody negative juvenile myositis patients. These findings clearly require further explanation, but it is possible that anti-cN-1A autoantibody status is more useful as a biomarker of disease severity, rather than a diagnostic biomarker for IBM *per se*.

In attempting to understand the pathogenic relevance of anti-cN-1A autoantibodies, a recent publication described in-vitro and in-vivo passive immunization models in which p62 aggregates significantly increased in anti-cN-1A-positive IBM IgG fraction supplemented cells and mice, respectively [15▪]. However, it is possible that some other constituent within the IgG fractions used (which were derived from patients with IBM) is the truly pathogenic entity [16]. One issue of ongoing concern is the lack of standardization of antibody detection methodologies and the consequent difficulties in comparing results from different studies. The future is likely to see anti-cN-1A autoantibodies added to existing commercial myositis antibody multiplex assays.

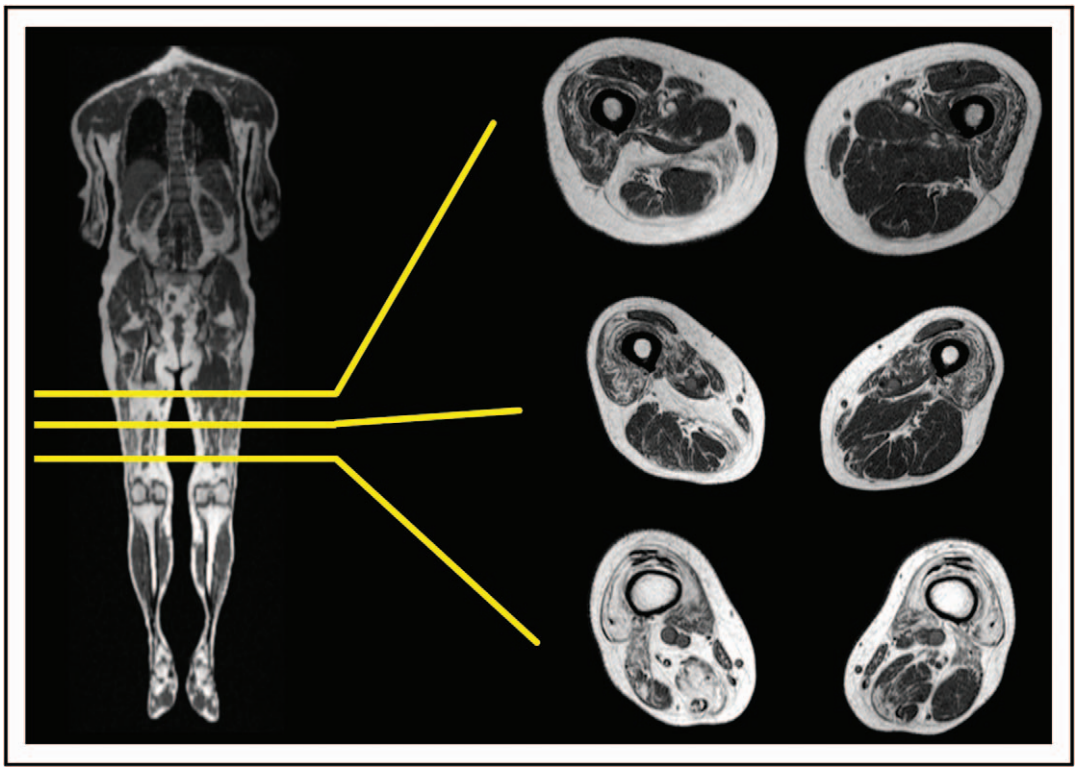

FIGURE 1. Sample images from whole body T1-weighted MRI of muscle with three axial sections through the thigh (yellow line indicates level) in a patient with inclusion body myositis. Typical fatty replacement of muscle, predominantly of the anterior thigh musculature is evident, with a gradient of increasing severity from proximal to distal musculature.

Imaging

Muscle imaging, particularly MRI and ultrasound is increasingly being performed as part of the diagnostic workup and monitoring of patients with muscle disease. Muscle MRI provides high-resolution visualization of soft tissues and can sensitively demonstrate myo-oedematous change (usually using short tau inversion recovery sequences) and fatty replacement (usually using T1-weighted sequences) (Fig. 1).

In IBM, several imaging studies have demonstrated characteristic patterns of muscle involvement, largely mirroring what is observed clinically [17]. A recent study also demonstrated some correlation between the extent of fatty infiltration and functional outcome measures [muscle strength ($r = -0.60$; $P = 0.04$) and the Modified Rankin Scale ($r = 0.48$; $P = 0.03$)] [18]. It is usually observed that fatty replacement of muscle is much more prominent in IBM than in the other inflammatory myopathies, although such changes overlap significantly, limiting usefulness as a diagnostic biomarker in clinical practice. Furthermore, conventional image analysis techniques rely on somewhat subjective, and very laborious, scoring of muscles damage using various grading systems. In future, this is likely to improve with more widespread use of quantitative magnetic resonance techniques that are amenable to automation [19].

In a recent longitudinal study, Morrow *et al.* [20▪] were able to demonstrate that intramuscular fat accumulation determined by MRI in patients with IBM was highly responsive to change and correlated with conventional functional measures, highlighting the potential usefulness as an outcome measure in clinical trials. Validation of sensitive outcome measures is a key priority in a rare disease that progresses relatively slowly. In the future, it is hoped that smaller, shorter and more efficient clinical trials could be facilitated with the use of such techniques. However, issues relating to standardization of magnetic resonance parameters between different scanner systems and complex data analysis pipelines are hurdles to overcome.

An interesting novel application of MRI was described by Olthoff *et al.* [21]. Here, a real-time MRI technique was used to assess dysphagia in patients with IBM and was compared with conventional assessment by flexible endoscopic evaluation of swallowing and videofluoroscopy. Real-time MRI was as capable at demonstrating dysphagia and the lack of radiation exposure and enhanced visualization of soft tissues were highlighted as potential

advantages. Whether these findings will be translated in to more widespread clinical use is yet to be seen, but as with other applications of MRI, issues relating to scanner availability and patient factors such as claustrophobia or implanted metal may be barriers.

Muscle ultrasound is an alternative imaging modality that has some potential advantages over MRI, particularly as it is often more easily accessible and can even be performed at the bedside during an outpatient consultation. Characteristic patterns of ultrasound abnormalities have been demonstrated in IBM, including increased echointensity in flexor digitorum profundus, gastrocnemius and rectus femoris [22]. In addition, ultrasound equipment can be used to perform shear wave elastography, giving an indication of tissue solidity. Bachasson *et al.* [23] were able to use this technique to demonstrate correlations between the muscle shear modulus and strength in patients with IBM, although the clinical value of such change is yet to be determined.

Ultrasound is prone to inter-rater reliability issues due to subjective interpretation of the images produced and technical factors related to probe positioning. Machine learning and deep learning techniques have recently been applied to ultrasound data in IBM in an attempt to overcome some of these issues. Burlina *et al.* [24▪] describe a fully automated deep convolutional neural network technique that was able to correctly distinguish IBM ($n = 19$) from polymyositis ($n = 14$) and dermatomyositis ($n = 14$) with an accuracy of 75%. It is likely that similar techniques will continue to be developed may in the future be integrated in to ultrasound equipment for use in clinical practice with minimal user intervention or training.

TREATMENT HORIZON

Pharmacological treatments

Recent times have seen a shift away from targeting immune pathways and towards targeting alternative damage-inducing mechanisms. A recently completed large phase 2b/3 multicentre study of Bimagrumab (an activin receptor inhibitor) did not meet its primary endpoint [change in 6-min walking distance (6MWD)] [25]. Arimoclomol coinduces the heat shock response by prolonging activation of heat shock factor-1 and may promote normalization of protein handling within muscle. In a recent double-blind, placebo-controlled phase 2a study of Arimoclomol, safety and promising therapeutic signals were demonstrated [26▪▪]. A larger scale phase 2/3 study has recently commenced (ClinicalTrials.gov identifier: NCT02753530). Rapamycin (sirolimus) could restore aberrant autophagic (protein degradation) pathways evident in IBM muscle by inhibiting mammalian target of rapamycin and has immunosuppressive effects mediated via inhibition of IL-2 signalling. A recent abstract described a prospective, randomized, double blind, placebo-controlled phase 2b trial conducted in France. In the study, 22 patients received oral Rapamycin and 22 received placebo over a 12-month period [27]. No difference in the primary outcome (quadriceps strength) was identified at 12 months, but significantly less fatty replacement in quadriceps and hamstrings, beneficial effects on 6MWD, the IBM weakness composite index and the forced vital capacity were observed in the actively treated arm. An open continuation of this study is ongoing to evaluate these findings further (ClinicalTrials.gov identifier: NCT02481453).

A recent study by Mendell *et al.* [28] regarding the use of Follistatin (an antagonist of myostatin) gene therapy in IBM has generated debate. This trial involved injection of the quadriceps with a Follistatin gene therapy in six patients with IBM and with outcomes compared with data obtained from a separate untreated group of eight patients from a neuromuscular clinic. The authors describe a significant improvement in annualized 6MWD and decreased fibrosis and regeneration on muscle biopsy in those receiving treatment. However, although this study potentially opens up an exciting new avenue of treatment for IBM, Greenberg [29] has highlighted several issues with the trial design, including the fact that patients (but not those in the other group) also undertook an exercise programme, the use of an unvalidated *post hoc* created outcome measure and the lack of randomization. Overall, these initial results should probably be viewed with caution and a formal randomized controlled trial would be required to investigate them further.

Of those pursuing immune treatments for IBM, Schmidt *et al.* [30,31] recently reported an additional analysis from a previous open-label study of Alemtuzumab, a mAb binding to CD52 used to treat chronic lymphocytic leukaemia, T-cell lymphoma and multiple sclerosis. The authors obtained pretreatment and posttreatment muscle biopsies and performed quantitative PCR and immunohistochemistry analysis and examined differences in markers of inflammation and degeneration. The expression levels of IL-1β and major histocompatibility complex-I correlated with positive clinical effects, but other important markers of cell stress and degeneration did not change significantly, potentially explaining the only transient effects of Alemtuzumab in the original trial. It remains to be seen whether this potential therapeutic avenue will be explored further, but it is of interest that clonal

lymphocyte expansions meeting diagnostic criteria for large granular lymphocytic leukaemia were identified in 58% (22/38) of patients screened in a recent study, potentially shedding new light on disease pathogenesis [32▪].

Other treatment modalities

Two European groups have recently described their experience in using botulinum toxin injections for the management of dysphagia in patients with IBM. Schrey *et al.* [33] performed a retrospective analysis of 12 patients with IBM that had received botulinum neurotoxin A injections to the cricopharyngeus muscle and highlighted that the rate of aspirations and aspiration pneumonia seemed to decrease after intervention. Di Pede *et al.* [34] describe their experience treating four patients with IBM receiving a multidisciplinary treatment consisting of rehabilitation combined with botulinum toxin injection to the cricopharyngeus muscle in four subjects, three of whom appeared to derive benefit. Such observational data are encouraging, although robust clinical trials will be required to confirm the optimal strategy for managing dysphagia in IBM.

The potential beneficial disease modifying effects of exercise in patients with muscle disease have long been discussed. It is now acknowledged that exertion is safe and should not be avoided in those with myositis [35]. Intriguingly, molecular studies in those without muscle disease has identified correlations between increasing levels of leisure-time physical activity and reduced C-reactive protein and IL-6 levels, potentially indicating activation of systemic anti-inflammatory pathways [36]. The effects of a seven-week resistance exercise programme on gene expression in eight patients (no controls) with polymyositis and dermatomyositis (but not IBM) has been performed to investigate molecular explanations for the effect of exercise [37]. Postintervention analysis demonstrated increased muscle strength, reduced serum muscle enzyme levels and improved disease activity scores, in addition to gene expression profiling showing reduction in proinflammatory and profibrotic gene networks compared with baseline.

However, exercise regimens come in various shapes and sizes, with little to guide the clinician as to which type of intervention might be most helpful in IBM. Blood flow restricted exercise can induce muscle hypertrophy and has previously been investigated in those with IBM [38]. Jørgensen *et al.* [39] sought to investigate this further by performing a 12-week randomized controlled trial of blood-flow restricted training versus nonexercise in a group of 22 IBM patients. Although the primary outcome measure (change in the physical function domain of the Short Form 36) was not met, between-group differences were seen in leg muscle strength favouring the intervention. Exercise is likely to increasingly become a key facet in the management of IBM and may require reorganization of clinics to ensure the regular engagement of the relevant physical therapists.

CONCLUSION

Recent years have seen expansion in the availability of diagnostic tools that may assist clinicians in the workup of patients with muscle disease. Anti-cN-1A autoantibody testing and muscle imaging could be particularly useful in the diagnosis of IBM and may become integrated in to diagnostic and/or classification criteria in the future. Once diagnosis is established, the availability of effective disease modifying treatment strategies may soon become a reality. It remains the duty of those involved in the management of patients with IBM to offer involvement in clinical trials and other research studies. Through such means, it is hoped that an effective treatment for IBM can soon be found.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Griggs RC, Askanas V, DiMauro S, *et al.* Inclusion body myositis and myopathies. Ann Neurol 1995; 38:705–713.
2. Brady S, Squier W, Sewry C, *et al.* A retrospective cohort study identifying the principal pathological features useful in the diagnosis of inclusion body myositis. BMJ Open 2014; 4:e004552.
3. Brady S, Squier W, Hilton-Jones D. Clinical assessment determines the diagnosis of inclusion body myositis independently of pathological features. J Neurol Neurosurg Psychiatry 2013; 84:1240–1246.
4. Pluk H, van Hoeve BJA, van Dooren SHJ, *et al.* Autoantibodies to cytosolic 5′-nucleotidase 1A in inclusion body myositis. Ann Neurol 2013; 73:397–407.
5. Larman HB, Salajegheh M, Nazareno R, *et al.* Cytosolic 5′-nucleotidase 1A autoimmunity in sporadic inclusion body myositis. Ann Neurol 2013; 73:408–418.
6. Herbert MK, Stammen-Vogelzangs J, Verbeek MM, *et al.* Disease specificity of autoantibodies to cytosolic 5′-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. Ann Rheum Dis 2016; 75:696–701.

7. Lloyd TE, Christopher-Stine L, Pinal-Fernandez I, *et al.* Cytosolic 5′-nucleotidase 1A as a target of circulating autoantibodies in autoimmune diseases. Arthritis Care Res 2016; 68:66–71.
8. Muro Y, Nakanishi H, Katsuno M, *et al.* Prevalence of anti-NT5C1A antibodies in Japanese patients with autoimmune rheumatic diseases in comparison with other patient cohorts. Clin Chim Acta 2017; 472:1–4.
9. Greenberg SA. Biomarkers of inclusion body myositis. Curr Opin Rheumatol 2013; 25:753–762.
10. Rothwell S, Cooper RG, Lundberg IE, *et al.* Immune-array analysis in sporadic inclusion body myositis reveals HLA-DRB1 amino acid heterogeneity across the myositis spectrum. Arthritis Rheumatol 2017; 69: 1090–1099.
11. O'Hanlon TP, Carrick DM, Targoff IN, *et al.* Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. Medicine (Baltimore) 2006; 85: 111–127.
12. Lilleker JB, Rietveld A, Pye SR, *et al.* Cytosolic 5′-nucleotidase 1A autoantibody profile and clinical characteristics in inclusion body myositis. Ann Rheum Dis 2017; 76:862–868.
13. Goyal NA, Cash TM, Alam U, *et al.* Seropositivity for NT5c1A antibody in sporadic inclusion body myositis predicts more severe motor, bulbar and respiratory involvement. J Neurol Neurosurg Psychiatry 2016; 87: 373–378.
14. Yeker RM, Pinal-Fernandez I, Kishi T, *et al.* Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis. Ann Rheum Dis 2018; 77:714–719.
15. ■ Tawara N, Yamashita S, Zhang X, *et al.* Pathomechanisms of anticytosolic 5′-nucleotidase 1A autoantibodies in sporadic inclusion body myositis. Ann Neurol 2017; 81:512–525.

Demonstration of the potentially direct pathogenic effects of anti-cN-1A autoantibodies using in-vitro and in-vivo passive immunization models.

16. Greenberg SA. Inclusion body myositis pathogenesis: steady progress. Ann Neurol 2017; 81:498–501.
17. Cox FM, Reijnierse M, van Rijswijk CSP, *et al.* Magnetic resonance imaging of skeletal muscles in sporadic inclusion body myositis. Rheumatology (Oxford) 2011; 50:1153–1161.
18. Guimaraes JB, Zanoteli E, Link TM, *et al.* Sporadic inclusion body myositis: MRI findings and correlation with clinical and functional parameters. AJR Am J Roentgenol 2017; 209:1340–1347.
19. Yao L, Yip AL, Shrader JA, *et al.* Magnetic resonance measurement of muscle T2, fat-corrected T2 and fat fraction in the assessment of idiopathic inflammatory myopathies. Rheumatology (Oxford) 2016; 55:441–449.
20. ■ Morrow JM, Sinclair CDJ, Fischmann A, *et al.* MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. Lancet Neurol 2016; 15:65–77.

Study demonstrating potential for MRI biomarkers in inclusion body myositis (IBM) to be used outcome measure in clinical trials.

21. Olthoff A, Carstens PO, Zhang S, *et al.* Evaluation of dysphagia by novel real-time MRI. Neurology 2016; 87:2132–2138.
22. Albayda J, Christopher-Stine L, Bingham Iii CO, *et al.* Pattern of muscle involvement in inclusion body myositis: a sonographic study. Clin Exp Rheumatol 2018. [Epub ahead of print]
23. Bachasson D, Dubois GJR, Allenbach Y, *et al.* Muscle shear wave elastography in inclusion body myositis: feasibility, reliability and relationships with muscle impairments. Ultrasound Med Biol 2018; 44:1423–1432.
24. ■ Burlina P, Billings S, Joshi N, Albayda J. Automated diagnosis of myositis from muscle ultrasound: exploring the use of machine learning and deep learning methods. PLoS One 2017; 12:e0184059.

Study of a novel machine learning technique applied to muscle ultrasound data in inflammatory muscle disease. Ability of the technique to classify different disease subtypes is described.

25. Amato AA, Badrising U, Benveniste O, *et al.* A randomized, double-blind, placebo-controlled study of bimagrumab in patients with sporadic inclusion body myositis. Arthritis Rheumatol 2016; 68(Suppl 10). [Abstract]
26. ■■ Ahmed M, Machado PM, Miller A, *et al.* Targeting protein homeostasis in sporadic inclusion body myositis. Sci Transl Med 2016; 8:331ra41.

In this study, the in-vitro and in-vivo benefits of Arimoclomol are demonstrated before description of a proof of concept patient trial demonstrating safety and tolerability in IBM.

27. Benveniste O, Hogrel J, Annoussamy M, *et al.* Rapamycin vs. placebo for the treatment of inclusion body myositis: improvement of the 6 min walking distance, a functional scale, the FVC and muscle quantitative MRI. Arthritis Rheumatol 2017; 69. [Abstract]
28. Mendell JR, Sahenk Z, Al-Zaidy S, *et al.* Follistatin gene therapy for sporadic inclusion body myositis improves functional outcomes. Mol Ther 2017; 25:870–879.
29. Greenberg SA. Unfounded claims of improved functional outcomes attributed to follistatin gene therapy in inclusion body myositis. Mol Ther 2017; 25:2235–2237.
30. Schmidt K, Kleinschnitz K, Rakocevic G, *et al.* Molecular treatment effects of alemtuzumab in skeletal muscles of patients with IBM. BMC Neurol 2016; 16:48.
31. Dalakas MC, Rakocevic G, Schmidt J, *et al.* Effect of Alemtuzumab (CAMPATH 1-H) in patients with inclusion-body myositis. Brain 2009; 132: 1536–1544.
32. ■ Greenberg SA, Pinkus JL, Amato AA, *et al.* Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. Brain 2016; 139:1348–1360.

Study describing association between IBM and T-cell large granular lymphocytic leukaemia, providing potential explanation for the lack of response seen to immunosuppression in IBM.

33. Schrey A, Airas L, Jokela M, Pulkkinen J. Botulinum toxin alleviates dysphagia of patients with inclusion body myositis. J Neurol Sci 2017; 380:142–147.
34. Di Pede C, Masiero S, Bonsangue V, *et al.* Botulinum toxin and rehabilitation treatment in inclusion body myositis for severe oropharyngeal dysphagia. Neurol Sci 2016; 37:1743–1745.
35. Arnardottir S, Alexanderson H, Lundberg IE, Borg K. Sporadic inclusion body myositis: pilot study on the effects of a home exercise program on muscle function, histopathology and inflammatory reaction. J Rehabil Med 2003; 35:31–35.
36. Fischer CP, Berntsen A, Perstrup LB, *et al.* Plasma levels of interleukin-6 and C-reactive protein are associated with physical inactivity independent of obesity. Scand J Med Sci Sports 2007; 17:580–587.
37. Nader GA, Dastmalchi M, Alexanderson H, *et al.* A longitudinal, integrated, clinical, histological and mRNA profiling study of resistance exercise in myositis. Mol Med 2010; 16:455–464.
38. Gualano B, Neves M, Lima FR, *et al.* Resistance training with vascular occlusion in inclusion body myositis: a case study. Med Sci Sports Exerc 2010; 42:250–254.
39. Jørgensen A, Aagaard P, Frandsen U, *et al.* Blood-flow restricted resistance training in patients with sporadic inclusion body myositis: a randomized controlled trial. Scand J Rheumatol 2018; 1–10. [Epub ahead of print]

Juvenile dermatomyositis: novel treatment approaches and outcomes

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Purpose of review
The aim of this article is to provide a summary of the recent therapeutic advances and the latest research on outcome measures for juvenile dermatomyositis (JDM).

Recent findings
Several new international studies have developed consensus-based guidelines on diagnosis, outcome measures and treatment of JDM to standardize and improve patient care. Myositis-specific antibodies together with muscle biopsy histopathology may help the clinician to predict disease outcome. A newly developed MRI-based scoring system has been developed to standardize the use of MRI in assessing disease activity in JDM. New data regarding the efficacy and safety of rituximab, especially for skin disease, and cyclophosphamide in JDM support the use of these medications for severe refractory cases.

Summary
International network studies, new biomarkers and outcome measures have led to significant progress in understanding and managing the rare inflammatory myositis conditions such as JDM.

Keywords
advanced treatment, juvenile dermatomyositis, outcome measures

INTRODUCTION

Juvenile dermatomyositis (JDM) is a rare systemic autoimmune disease characterized by a vasculopathy that primarily affects muscle and skin, but may involve the lung, bowel, heart and other organs [1,2]. JDM is the most common inflammatory myopathy of childhood, affecting 1.9 cases per million children in the United Kingdom [3] and 2.4–4.1 cases per million children in USA [4]. In this review, we will summarize the recent developments in the clinical assessment, treatments and outcomes in JDM.

CLINICAL OUTCOMES AND CORE SET CRITERIA

International collaborations have been undertaken to unify and standardize assessments and treatments of rare diseases such as idiopathic inflammatory myositis (IIM). The Paediatric Rheumatology International Trials Organization (PRINTO) and the International Myositis Assessment & Clinical Studies Group (IMACS) initial preliminary response criteria considerably improved clinical assessment and therapeutic response of JDM patients, but were lacking in sensitivity and still presented several differences in the individual core set measurement [5–7]. To overcome these issues, these two international organizations joined forces and developed a new set of consensus-driven response criteria for adult dermatomyositis/polymyositis and children with JDM. This new tool is based on a continuous model, with a total improvement score of 0–100, and with different thresholds for minimal (≥30), moderate (≥45) and major (≥70) clinical response based on weighted scores applied to an absolute percentage improvement [8▪▪]. The core set measures were identified by consensus among expert paediatric and adult rheumatologists, neurologists and dermatologists, using the Delphi method. The agreed measures were the following: Physician global activity; Parent or Patient global activity; Manual Muscle testing

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KEY POINTS

- European and American study groups proposed a consensus for optimal dataset and criteria of minimal, moderate and major response to treatment in JDM.
- New consensus-based guidelines are available for diagnosis and management of children with JDM, and particularly with predominant skin disease and persistent skin disease.
- New autoantibody association, especially combined with muscle biopsy histopathology, and a new MRI scoring system may help the clinician with treatment choice and disease prognosis.

(MMT) or Childhood Myositis Assessment Scale (CMAS); Childhood Health Assessment Questionnaire; Muscle enzymes (creatine kinase, aldolase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase) or Physical Summary Score of the Child Health Questionnaire-Parent Form 50 and Extramuscular activity or Disease Activity Score (DAS). These new response criteria provide a quantitative measurement of disease improvement and resolve the differences between PRINTO and IMACS criteria, enabling an easier comparison between different datasets and facilitating future trials.

Another important step towards effective communication between different study groups by using standardized clinical data has been created by International Group of Experts (McCann *et al.* [9▪]), who have defined an optimal dataset for JDM to capture disease subphenotype, activity, comorbidity and damage over time. Both an international panel of experts took part in a Delphi process, but also parents and patients with JDM participated in the survey, enabling the group to highlight what patients and families feel are essential items of the clinical assessment in JDM, with good agreement with the healthcare professionals.

A recent large analysis of the EuroMyositis registry, which includes both adult and paediatric onset cases of all types of IIM has highlighted the differences between JDM and adult dermatomyositis and polymyositis, the former being less associated with interstitial lung disease and malignancy and having different skin disease characteristics [10].

Little is known regarding long-term outcome in JDM, but two recent studies shed some light on this extremely important aspect of care. Silverberg *et al.* [11] evaluated over 14 million hospitalization of patients with JDM over a 10-year period and showed significantly higher odds for cardiovascular and cerebrovascular comorbidities in this US cohort of patients, especially for girls and ethnic minorities. Ethnicity and lower family income were found to be associated with worse outcome, increased morbidity and decreased function in another large American cohort study [12]. A further study showed worse cardiovascular outcome in JDM patients (tested with a 6-min walk test, timed 'up and go' test, CMAS, echocardiography, lung function test, thoracic high resolution computed tomography scan and MRI and health-related quality of life questionnaire) with a mean of 17 years of disease history when compared with sex-match and age-match controls, especially those with active disease [13].

One of the ongoing challenges of the management of JDM has been identifying a reliable, practical tool to measure the skin disease. A prospective study tested the PRINTO proposed criteria for clinically inactive disease, which stated that at least three of four conditions should be met: creatinine kinase 150 U/l or less, CMAS at least 48, MMT of eight groups at least 78 and physician global assessment of overall disease activity 0.2 or less [14]. This analysis by Almeida *et al.* [15] showed the importance of incorporating the physician global assessment of overall disease activity as an essential criterion of clinically inactive disease, as this helps prevent the misclassification of patients with active skin disease. Subsequent to this study, the same study group tested three different skin scoring tools in JDM, the Myositis Intention to Treat Activity Index, abbreviated Cutaneous Assessment Tool and DAS and correlated them with the physician's 10-cm skin visual analogue scale (VAS). All three tools were easy and quick to use, and this study showed that the DAS best correlated with the physician VAS. However, all three skin tools had limitations, suggesting that future studies should design a new tool with all the strengths of the existing ones [16].

ANTIBODIES

Juvenile myositis is a highly heterogeneous disease ranging from profound muscle weakness and visceral involvement to normal muscle strength. In recent years, autoantibodies have been identified in 60–70% children with myositis and have been able to identify clinically homogeneous groups [17–21]. This concept has been recently further validated in a large study including 379 juvenile myositis patients, which confirmed that the myositis-specific autoantibodies (MSA) are exclusively found in children with IIM, and not in healthy children or patients with other autoimmune diseases (including arthritis or lupus) or muscular dystrophy. Therefore, this study suggested that the presence of MSA should be considered highly suggestive of the

diagnosis of myositis [19]. In this study-specific MSA such as anti-transcription intermediary factor 1-gamma (TIF1-γ) was shown to be associated with the use of more powerful medication; in addition, anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and antisignal recognition particle (SRP) antibodies were also found in patients with profound muscle weakness and slow/poor response to treatment.

These findings will help the clinician to predict disease features and outcome and to guide the treatment. Furthermore, Deakin *et al.* showed that the severity of the muscle biopsy (defined using a standardized score tool), in combination with MSA subtype can predict the risk of remaining on treatment in patients with JDM. Surprisingly, children with anti-Mi2 antibody were associated with a better prognosis, despite the severity of the muscle biopsy in these cases, whereas in patients with anti-nuclear matrix protein (NXP-2), anti-TIF-1g, or no detectable antibodies, the biopsy score was predictive of the probability of remaining on treatment over time [22▪▪].

IMAGING

The use of MRI has played an increasingly important role to help clinicians with diagnosis and follow-up of children with inflammatory myositis, especially as it does not involve ionizing radiation. It helps with selection of the muscle biopsy site, and it is not invasive, unlike electromyography or muscle biopsy [23,24]. A recent study showed that where a flare was questioned, if the MRI showed active myositis, the physician would change or escalate treatment. This biomarker can be useful especially as up to 75% of patients suspected of having a flare had no abnormal muscle enzymes [25].

To date the use of MRI is not standardized and might differ significantly in different centres, for example in terms of which part of the body is assessed, which planes to perform, the protocol used, and the usefulness of intravenous contrast. To overcome these limitations, Thyoka *et al.* recently improved the previously published MRI-based scoring system for JDM initially developed by Davis *et al.* [26]. Nine paediatric radiologists with an interest in musculoskeletal imaging and two paediatric rheumatologists reviewed and modified the previously developed criteria and tested it on a set of MRI scans from 20 patients with JDM. The resulting new scoring system showed good interobserver reliability with no significant difference when using either the coronal or the axial planes. The study showed that various combinations of techniques can be useful, T1-weighted to assess muscle atrophy and T2-weighted/fat suppression or short TI inversion recovery (STIR) to visualize inflammatory changes of the skin and soft tissue oedema. The panel considered MRI of gluteal and thigh muscle optimal to assess disease activity and severity and, also, was more easily available than whole body MRI, and gadolinium contrast was not needed [27▪].

CONSENSUS TREATMENT PLANS

In recent years, several international efforts have been undertaken to achieve evidence-based guidelines with the aim to standardize outcome measures and management of children with JDM. The Single Hub and Access point for paediatric Rheumatology group has been working on harmonizing the care of paediatric rheumatology patients in Europe since 2012 and have recently published consensus-based recommendations for the management of JDM developed by an evidence-informed consensus process involving systematic literature review, online survey and final consensus meeting among 21 experts in paediatric rheumatology and physical therapy [28▪▪].

In parallel, the Childhood Arthritis and Rheumatology Research Alliance (CARRA) has developed consensus treatment plans for several paediatric rheumatologic diseases including juvenile localized scleroderma, systemic juvenile idiopathic arthritis (JIA), polyarticular JIA, lupus nephritis and JDM [29]. With respect to JDM, the CARRA group has recently proposed a consensus-based treatment plan for JDM with predominant skin disease consisting of three different options for the clinician: option A included hydroxycloroquine alone, option B included hydroxycloroquine and methotrexate and option C consisted of hydroxycloroquine, methotrexate and corticosteroids [30]. The same study group also proposed a consensus treatment plan for JDM with persistent skin disease despite the resolution of the muscle disease with three different plans: Plan A to add intravenous immunoglobulin (IVIG), Plan B to add mycophenolate mofetil and Plan C to add cyclosporine [31]. Continuation of previous treatments including corticosteroids, methotrexate and IVIG was allowed in Plans B and C. The next step in both studies will be to collect prospective data to understand which treatment option is the most effective.

ADVANCES IN TREATMENT

To date, only two randomized controlled trials were performed including JDM patients. These were the PRINTO trial which showed that corticosteroids and methotrexate were the most effective and safest treatment option in new-onset JDM when compared with prednisolone alone and prednisolone

and cyclosporine [32▪▪], and the Rituximab in Myositis trial which, although it did not meet its primary endpoint, showed an overall good response rate and ability to taper corticosteroids in adult and JDM [33]. In the same cohort of patients, the efficacy of rituximab in treating the cutaneous disease was subsequently assessed. The disease activity was evaluated using the cutaneous assessment of the Myositis Disease Activity Assessment tool and the damage using the Myositis Damage Index. In JDM, Rituximab treatment significantly improved skin disease activity, especially cutaneous ulcerations, erythroderma, heliotrope rash and Gottron's sign/papules. No major changes were seen among damage items, including calcinosis [34].

Cyclophosphamide is currently used to treat malignancy, systemic lupus erythematosus and vasculitis. Clinicians may be reluctant to give cyclophosphamide in JDM because of the lack of evidence and its side effects. Recently, the efficacy on skin, muscle and global disease activity of cyclophosphamide has been reported in 56 severe and refractory cases of JDM. The long-term side effects are still unknown but its short-term safety profile in this study is encouraging [35].

A combination of cyclophosphamide, IVIG and Rituximab has proven to be effective in anti-SRP myositis, a very rare inflammatory myopathy characterized by profound muscle weakness, raised creatinine kinase and no skin rash with a much improve outcome compared with the very little literature available [36]. The CARRA group in North America conducted a survey regarding the use of biologic agents in treating JDM which showed that biologics were used only for refractory cases of JDM with the general belief that these were effective in reducing complications, particularly calcinosis, and therefore were an appropriate step when corticosteroids, methotrexate and IVIG fail to control the disease. The most common biologics used were Rituximab, Abatacept, antitumour necrosis factor and Tocilizumab suggesting that these agents could be considered for future studies [37]. An anecdotal report described a successful use of Ustekinumab (human mAb against IL-12/23) in treating a case of juvenile amyopathic dermatomyositis with psoriasis and active skin disease [38]. An analysis of a large number of JDM patients treated with tumour necrosis factor blockade, to date published in abstract form, suggests efficacy of blocking tumour necrosis factor for severe cases of JDM [39].

FUTURE TREATMENT OPTIONS

Promising options are coming from the world of adult dermatomyositis, including a randomized control trial of Infliximab in 12 refractory polymyositis and dermatomyositis which showed some benefit and good safety profile [40]. Another randomized control trial concluded that 50% of patients with adult dermatomyositis and polymyositis treated with Abatacept had lower disease activity [41]. In addition, Rituximab has been successful in improving respiratory symptoms and lung function tests, but also in reducing the daily corticosteroid dose in refractory progressive interstitial lung disease in anti-melanoma differentiation-associated protein 5 (MDA5)-positive amyopathic dermatomyositis, infection was the main side effect reported [42].

CONCLUSION

In conclusion, in the recent years several international efforts have achieved important goals with the ultimate aim to harmonize and standardize the management of children with juvenile inflammatory myopathies. Collaborative networks are essential to facilitate research in rare diseases and provide evidenced-based treatments for JDM.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Rider LG, Lidsley CB, Miller FW. Juvenile dermatomyositis. In: Petty RE, Laxer RM, Lindsey CB, Wedderburn LR, editors. Textbook of pediatric rheumatology, 7th ed. Philadelphia: Elsevier Saunder; 2016. pp. 351–384.
2. Feldman BM, Rider LG, Reed AM, Pachman LM. Juvenile dermatomyositis and other idiopathic inflammatory myopathies of childhood. Lancet 2008; 371:2201–2212.
3. Symmons DP, Sills JA, Davis SM. The incidence of juvenile dermatomyositis: results from a nation-wide study. Br J Rheumatol 1995; 34:732–736.
4. Mendez EP, Lipton R, Ramsey-Goldman R, *et al.*, NIAMS Juvenile DM Registry Physician Referral Group. US incidence of juvenile dermatomyositis, 1995–1998: results from the National Institute of Arthritis and Musculoskeletal and Skin Diseases Registry. Arthritis Rheum 2003; 49:300–305.
5. Rider LG, Giannini EH, Brunner HI, *et al.* International consensus on preliminary definitions of improvement in adult and juvenile myositis. Arthritis Rheum 2004; 50:2281–2290.
6. Ruperto N, Pistorio A, Ravelli A, *et al.* The Pediatric Rheumatology International Trials Organization provisional criteria for the evaluation of response to therapy in juvenile dermatomyositis. Arthritis Care Res 2010; 62:1533–1541.

7. Ruperto N, Pistorio A, Ravelli A, *et al.* Criteria to define response to therapy in paediatric rheumatic diseases. Eur J Clin Pharmacol 2011; 67(Suppl 1): 125–131.
8. ■■ Rider LG, Aggarwal R, Pistorio A, *et al.* 2016 American College of Rheumatology/European League Against Rheumatism Criteria for Minimal, Moderate, and Major Clinical Response in Juvenile Dermatomyositis: an International Myositis Assessment and Clinical Studies Group/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Ann Rheum Dis 2017; 76:782–791.

The report shows the new response criteria for juvenile dermatomyositis (JDM), with thresholds for minimal, moderate and major improvement.

9. ■ McCann LJ, Pilkington CA, Huber AM, *et al.* Development of a consensus core dataset in juvenile dermatomyositis for clinical use to inform research. Ann Rheum Dis 2018; 77:241–250.

The study introduces an internationally agreed dataset for JDM that is designed to capture disease activity and damage over time.

10. Lilleker JB, Vencovsky J, Wang G, *et al.* The EuroMyositis registry: an international collaborative tool to facilitate myositis research. Ann Rheum Dis 2018; 77:30–39.
11. Silverberg JI, Kwa L, Kwa MC, *et al.* Cardiovascular and cerebrovascular comorbidities of juvenile dermatomyositis in US children: an analysis of the National Inpatient Sample. Rheumatology (Oxford) 2018; 57:694–702.
12. Philippi K, Hoeltzel M, Byun Robinson A, *et al.* Race, income and disease outcomes in juvenile dermatomyositis. J Pediatr 2017; 184:38–44.
13. Berntsen KS, Tollisen A, Schwartz T, *et al.* Submaximal exercise capacity in juvenile dermatomyositis after longterm disease: the contribution of muscle, lung, and heart involvement. J Rheumatol 2017; 44:827–834.
14. Lazarevic D, Pistorio A, Palmisani E, *et al.* The PRINTO criteria for clinically inactive disease in juvenile dermatomyositis. Ann Rheum Dis 2013; 72:686–693.
15. Almeida B, Campanilho-Marques R, Arnold K, *et al.* Analysis of published criteria for Clinically Inactive Disease in a Large Juvenile Dermatomyositis cohort shows that skin disease is underestimated. Arthritis Rheumatol 2015; 67:2495–2502.
16. Campanilho-Marques R, Almeida B, Deakin C, *et al.* Comparison of the utility and validity of three scoring tools to measure skin involvement in patients with juvenile dermatomyositis. Arthritis Care Res 2016; 68:1514–1521.
17. Tansley SL, Betteridge ZE, Shaddick G, *et al.* Calcinosis in juvenile dermatomyositis is influenced by both anti-NXP2 autoantibody status and age at disease onset. Rheumatology (Oxford) 2014; 53:2204–2208.
18. Tansley SL, Betteridge ZE, Gunawardena H, *et al.* Anti-MDA5 autoantibodies in juvenile dermatomyositis identify a distinct clinical phenotype: a prospective cohort study. Arthritis Res Ther 2014; 16:R138.
19. Rider LG, Shah M, Mamyrova G, *et al.* The myositis autoantibody phenotypes of the juvenile idiopathic inflammatory myopathies. Medicine (Baltimore) 2013; 92:223–243.
20. Gunawardena H, Wedderburn LR, North J, *et al.* Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis. Rheumatology (Oxford) 2008; 47:324–328.
21. Gunawardena H, Betteridge ZE, McHugh NJ. Myositis-specific autoantibodies: their clinical and pathogenic significance in disease expression. Rheumatology (Oxford) 2009; 48:607–612.
22. ■■ Deakin CT, Yasin SA, Simou S, *et al.* Biopsy findings in combination with myositis-specific autoantibodies aid prediction of outcomes in juvenile dermatomyositis. Arthritis Rheumatol 2016; 68:2806–2816.

This was the first study to demonstrate that the muscle biopsy and the myositis-specific autoantibodies can help the clinician to predict the outcome in JDM.

23. Maillard SM, Jones R, Owens C, *et al.* Quantitative assessment of MRI T2 relaxation time of thigh muscles in juvenile dermatomyositis. Rheumatology (Oxford) 2004; 43:603–608.
24. Park JH, Vansant JP, Kumar NG, *et al.* Dermatomyositis: correlative MR imaging and P-31 MR spectroscopy for quantitative characterization of inflammatory disease. Radiology 1990; 177:473–479.
25. Abdul-Aziz R, Yu CY, Adler B, *et al.* Muscle MRI at the time of questionable disease flares in juvenile dermatomyositis (JDM). Pediatr Rheumatol Online J 2017; 15:25.
26. Davis WR, Halls JE, Offiah AC, *et al.* Assessment of active inflammation in juvenile dermatomyositis: a novel magnetic resonance imaging-based scoring system. Rheumatology (Oxford) 2011; 50:2237–2244.
27. ■ Thyoka M., Adekunle O., Pilkington C., *et al.* Introduction of a novel magnetic resonance imaging-based scoring system for assessing disease activity in children with juvenile dermatomyositis. Rheumatology (Oxford) 2018; doi:10.1093/rheumatology/key144. [Epub ahead of print]

The new scoring system will help standardize the use of MRI in JDM.

28. ■■ Enders FB, Bader-Meunier B, Baildam E, *et al.* Consensus-based recommendations for the management of juvenile dermatomyositis. Ann Rheum Dis 2017; 76:329–340.

The international effort is an important step towards a unified and standardized management of JDM.

29. Ringold S, Nigrovic PA, Feldman BM, *et al.* The Childhood Arthritis and Rheumatology Research Alliance consensus treatment plans. Arthritis Rheumatol 2018; 70:669–678.
30. Kim S, Kahn P, Robinson AB, *et al.* Childhood Arthritis and Rheumatology Research Alliance consensus clinical treatment plans for juvenile dermatomyositis with skin predominant disease. Pediatr Rheumatol Online J 2017; 15:1.
31. Huber AM, Kim S, Reed AM, *et al.* Childhood Arthritis and Rheumatology Research Alliance consensus clinical treatment plans for juvenile dermatomyositis with persistent skin rash. J Rheumatol 2017; 44:110–116.
32. ■■ Ruperto N, Pistorio A, Oliveira S, *et al.* Prednisone versus prednisone plus cyclosporine versus prednisone plus methotrexate in new-onset juvenile dermatomyositis: a randomised trial. Lancet 2016; 387:671–678.

A randomized controlled trial showing the superiority of prednisone and methotrexate over prednisone alone or prednisone and cyclosporine.

33. Oddis CV, Reed AM, Aggarwal R, *et al.* Rituximab in the treatment of refractory adult and juvenile dermatomyositis and adult polymyositis: a randomized, placebo-phase trial. Arthritis Rheum 2013; 65:314–324.
34. Aggarwal R, Loganathan P, Koontz D, *et al.* Cutaneous improvement in refractory adult and juvenile dermatomyositis after treatment with rituximab. Rheumatology (Oxford) 2017; 56:247–254.
35. Deakin CT, Campanilho-Marques R, Simou S, *et al.* Efficacy and safety of cyclophosphamide treatment in severe juvenile dermatomyositis shown by marginal structural modeling. Arthritis Rheumatol 2018; 70:785–793.
36. Binns EL, Moraitis E, Maillard S, *et al.* Effective induction therapy for anti-SRP associated myositis in childhood: a small case series and review of the literature. Pediatr Rheumatol Online J 2017; 15:77.
37. Spencer CH, Rouster-Stevens K, Gewanter H, *et al.* Biologic therapies for refractory juvenile dermatomyositis: five years of experience of the Childhood Arthritis and Rheumatology Research Alliance in North America. Pediatr Rheumatol Online J 2017; 15:50.
38. Montoya CL, Gonzalez ML, Ospina FE, Tobón GJ. A rare case of amyopathic juvenile dermatomyositis associated with psoriasis successfully treated with ustekinumab. J Clin Rheumatol 2017; 23:129–130.
39. Campanilho-Marques R, Deakin C, Simou S, *et al.* OP0221 efficacy and safety of tumour necrosis factor-alpha antagonists in a large cohort of juvenile dermatomyositis patients. Ann Rheum Dis 2016; 75(Suppl 2):140.
40. Shiffenbouer A, Garg M, Castro C, *et al.* A randomised, double blind, placebo-control trial of infliximab in refractory polymyositis and dermatomyositis. Semin Arthritis Rheum 2018; 47:858–864.
41. Tiarnlund A, Tang Q, Wick C, *et al.* Abatacept in the treatment of adult dermatomyositis and polymyositis: a randomised, phase IIb treatment delayed-start trial. Ann Rheum Dis 2018; 77:55–62.
42. So H, Wong VTL, Lao VWN, *et al.* Rituximab for refractory rapidly progressive interstitial lung disease related to anti-MDA5 antibody positive amyophatic dermatomyositis. Clin Rheumatol 2018; 37:1983–1989.

Peculiar clinicopathological features of immune-mediated necrotizing myopathies

Yves Allenbach[a,b] *and Olivier Benveniste*[a,b]

Purpose of review
In the past decade, immune-mediated necrotizing myopathies have emerged as a separate entity in the heterogenous group of autoimmune myopathies. This group is characterized by clinical manifestations restricted to the muscle tissue, and until recently, the definition was based on muscular pathological features.

Recent findings
It was shown that they are the most severe autoimmune myopathies in term of muscle damages. They have been associated with two myositis-specific antibodies: either anti-signal recognition particle (anti-SRP) or anti-hydroxy-3-methylglutaryl-CoA reductase (anti-HMGCR) antibodies. These two antibodies are now considered as immune-mediated necrotizing myopathy (IMNM) diagnostic criteria. Each antibody delineates a homogenous subgroup of IMNM patients in terms of severity and IMNM without myositis-specific antibodies have a high risk of malignancy. In addition, pathological observations as well as in-vitro experiments suggest the pathogenic role of anti-SRP and anti-HMGCR antibodies.

Summary
IMNM are muscle-specific autoimmune diseases associated with a severe weakness and a risk poor muscle strength recovery. Anti-SRP and anti-HMGCR antibodies are specifically associated with this condition and are crucial for the diagnosis and the prognosis. The muscle biopsy remains necessary for IMNM diagnosis in absence of myositis-specific antibodies.

Keywords
autoantibodies, hydroxy-3-methylglutaryl-CoA reductase, myositis, necrotizing myopathies, signal recognition particle

INTRODUCTION

The spectrum of immune myopathies range from muscle-specific autoimmune diseases to systemic autoimmune diseases. Historically, they were classified into two categories, namely, polymyositis and dermatomyositis, based on the presence or absence of the characteristic dermatomyositis skin rash [1]. Nevertheless, both groups are heterogeneous, as patients may present with extramuscular manifestations and/or different types of myositis-specific autoantibodies [2].

Initially, in 1986, antisignal recognition particle (anti-SRP) antibody was identified in a subgroup of polymyositis patients [3]. Later, in 2002, it was shown that muscle biopsies from anti-SRP antibody positive (anti-SRP+) patients showed the presence of necrotic muscle fibre without significant muscle inflammation [4]. One year later, a group of immune-mediated necrotizing myopathies (IMNMs) was recognized for the first time as a separate entity. The definition was based on pathological criteria showing predominant muscle fibre necrosis with no or mild muscle infiltrates [5]. On the basis of this definition, a new myositis-specific antibody targeting the hydroxy-3-methylglutaryl-CoA reductase (HMGCR) protein was discovered in a subset of IMNM patients [6,7].

Now, IMNMs are a homogenous group of severe autoimmune muscle diseases without clinically relevant extramuscular manifestations. Yet, variations can be observed in IMNMs depending on serological status. This is one of the reasons why IMNM diagnosis criteria have been refined recently [8▪] to distinguish anti-SRP+ IMNM, anti-HMGCR+ IMNM and antibody negative IMNM.

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KEY POINTS

- IMNM are the most severe autoimmune myopathies with a risk of poor muscle recovery.
- In presence of either anti-SRP or anti-HMGCR antibodies, the muscle biopsy is not necessary anymore to the diagnosis.
- For IMNM without myositis-specific antibodies, the muscle biopsy remains necessary for the diagnosis and this group has a high risk of malignancy.
- Pathological finding and in-vitro experiment suggest the pathological role of anti-SRP and anti-HMGCR antibody.

In this review, we aim to describe the clinico-pathological features of INMN patients as well as prognosis, pathophysiology and therapeutic approaches, focusing on the recent advances in the field.

MUSCLE CLINICAL PICTURE

IMNMs represent 20–30% of patients suffering from auto-immune myositis [9,10▪,11], and the overall prevalence of myositis is 14 out of 10 000 [12]. It can occur at any age, ranging from juvenile cases to elderly patients [13,14]. Most of the cases occur between 40 and 60 years of age, and women are more frequently affected [7,9,10▪,13].

Anti-HMGCR+ IMNMs may be triggered by a statin exposure in one-half to two-thirds of the cases [7,13]. The statin exposure in IMNM patients varies depending on the age of onset; in patients older than 50 years, a statin exposure is observed in greater than 90% of the cases [7]. Interestingly, Dr. A. Mammen suggests that statins are also present in some foods and dietary supplements (oyster mushroom, red yeast rice or pu-erh tea), and this may trigger the disease, especially in Asian people in whom statin drug exposure in anti-IMNM patients is low [15].

At presentation, patients usually complain of muscle signs. Extramuscle manifestations are uncommon. There are two clinical phenotypes of the disease: patients with a subacute onset and patients with a slowly progressive disease.

Patients with a subacute onset are the most common and account for greater than two-thirds of the cases [13,14]. Typically, patients complain from a rapidly progressive muscle deficit occurring within weeks or months (less than 6 months) before the first hospital visit; they generally visit the Rheumatology department. In addition, myalgia is regularly reported (30–50%) in IMNMs [10▪,13,16]. One-quarter to one-third of anti-SRP+ or anti-HMGCR+ patients suffer from a slowly progressive onset of disease, greater than 6–12 months [13,14]. These later patients may present as dystrophic patients and are seen in Neurology/Myology departments.

Clinical examination shows a severe proximal, bilateral and symmetric muscle weakness. The hip flexor muscles are the most severely affected compared with other muscle groups in anti-SRP+ and anti-HMGCR+ patients [10▪,17▪]. Compared with other autoimmune myopathies, INMN patients more frequently harbour severe muscle weakness [16,18▪]. Nevertheless, there are some variations between anti-SRP+ and anti-HMGCR+ patients. Anti-SRP+ patients suffer from a more severe muscle weakness than anti-HMGCR patients [9,18▪].

With a slowly progressive onset, muscle strength can be clinically considered normal (isolated increased creatine kinase level) for years, but more frequently, in addition to the muscle weakness, a muscle atrophy with some cases of winged scapula is observed [14], mimicking a limb girdle muscle dystrophy.

Life-threatening complications must always be considered. Swallowing troubles are frequent in IMNM patients, especially in anti-SRP+ patients where they occur in more than half of cases [9].

In addition, dyspnoea related to a weakness of the respiratory muscles may be observed. Usually, it occurs in very severely affected patients in whom exercise dyspnoea is difficult to demonstrate. The patients may complain of morning signs related to hypercapnia. This lung insufficiency can be screened for by nocturnal polygraphia, morning arterial gas measurements and pulmonary functional testing, ideally performed in both the sitting and supine position. Indeed, a more than 20% decrease in vital capacity measured in the supine position versus the sitting position may be helpful in detecting severe or predominantly diaphragmatic weakness [19].

According to myopathological findings, the mean creatine kinase level in IMNM is higher than that observed in other myositis cases [9,18▪,20]. Higher creatine kinase levels are also measured in anti-SRP+ patients than anti-HMGCR+ patients [9,18▪].

DIAGNOSTIC TOOLS

Myositis antibody-detection

In the case of autoimmune myositis, screening for myositis auto-antibodies is crucial, as by definition,

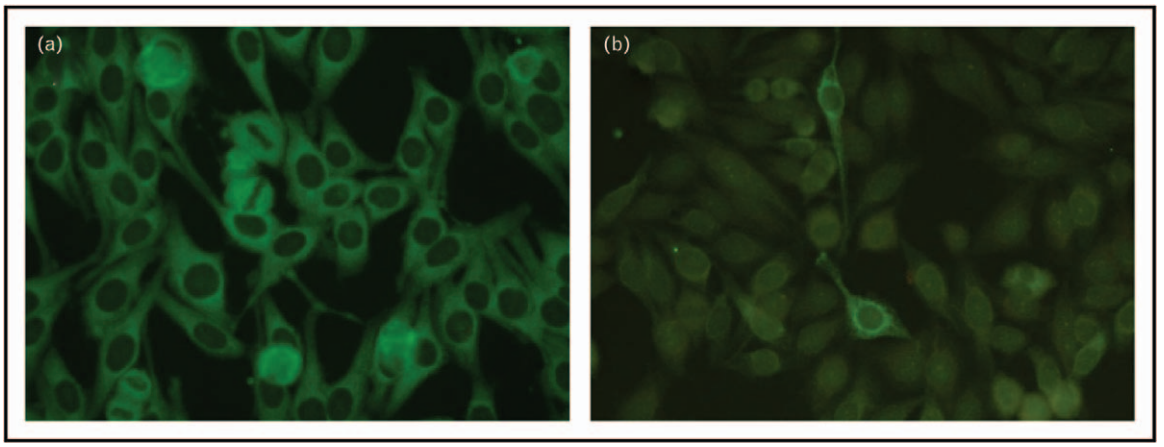

FIGURE 1. Anti-signal recognition particle and anti-hydroxy-3-methylglutaryl-CoA reductase auto-antibodies. (a) Indirect immunofluorescence anti-SRP antibody screening in serum from anti-SRP+ patients on HEp-2 cells shows diffuse positive cytoplasmic immunofluorescence on the majority of the cells. (b) Indirect immunofluorescence anti-HMGCR antibody screening in serum from anti-HMGCR+ patients on HEp-2 cells shows a fine granular positive cytoplasmic immunofluorescence on the minority of the cells.

it is a key diagnostic tool and separates a homogeneous group of patients in terms of phenotype and prognosis [2]. In IMNM, anti-SRP and anti-HMGCR are considered (for the first time in autoimmune myopathies) a key diagnostic criterion [2]. In the case of IMNM, the clinician must test for anti-SRP as well as anti-HMGCR [21,22]. Screening for antinuclear antibodies was not sensitive enough to detect anti-SRP and anti-HMGCR antibodies using a screening test in HEp-2 cells [21,22]. SRP and HMGCR are intracytoplasmic proteins related to the endoplasmic reticulum, and indirect immunofluorescence may be negative (Fig. 1). SRP is key in the delivery of newly synthesized proteins [23], and HMGCR is a key enzyme in cholesterol biosynthesis [24]. There are different commercial kits for myositis-specific auto-antibody detection that have a limited risk of false-positive or false-negative results [2,11,21,22]. Immunoprecipitation remains the gold standard of auto-antibody detection, but it is not routinely feasible. For these reasons, the results of the tests must be challenged with the clinicopathological picture, and a retest can be performed using different methods if doubts exist.

Muscle biopsy

In the case of a typical clinicobiological picture of IMNMs with a positive detection of either anti-SRP or anti-HMGCR antibody, a muscle biopsy is not necessary to diagnose anti-SRP+ or anti-HMGCR+ IMNM [8▪]. In other cases, a muscle biopsy must be performed.

The detection of the elementary lesions (muscle fibre, vascular, connective domains) and their distribution is important to recognize IMNMs (Fig. 2) [25]. Initially, IMNMs were defined on the basis of pathological criteria, including predominant muscle necrosis, whereas inflammatory infiltrates were mild or absent [5]. It has been shown that the percentage of necrotic muscle fibres is low in IMNMs (3.2% in anti-SRP+ patients vs. 1.8% in anti-HMGCR patients, $P < 0.05$) [18▪]. The necrotic muscle fibres are randomly distributed, whereas they are confined in the perifascicular area in antisynthetase syndrome, another condition wherein significant muscle fibre necrosis is observed [18▪]. However, indirect signs of necrosis, such as regenerating muscle fibres, are much more frequent [18▪].

According to the first definition of IMNM, the inflammatory infiltrates are mild and mainly composed of macrophages [9,18▪,26]. Nevertheless, significant inflammatory infiltrates with cell densities reaching the same range as those measured in other myositides are observed in one-quarter of IMNM cases (Fig. 2) [18▪]. A subgroup of IMNM patients may have diffuse sarcolemmal major histocompatibility complex (MHC)–I positive staining. Together, those data showed that a subset of INMN patients have significant signs of muscle inflammation that are correlated with the percentage of necrotic muscle fibres [18▪]. Thus, significant muscle inflammation is not considered an exclusion criterion in the new antibody-positive IMNM definition [8▪].

The pathological criteria for auto-antibody negative IMNM are the presence of necrotic fibres and regenerative fibres (with different stages) with a scattered distribution and the presence of macrophage-predominant, paucilymphocytic infiltrates. Additional consistent features are sarcolemmal

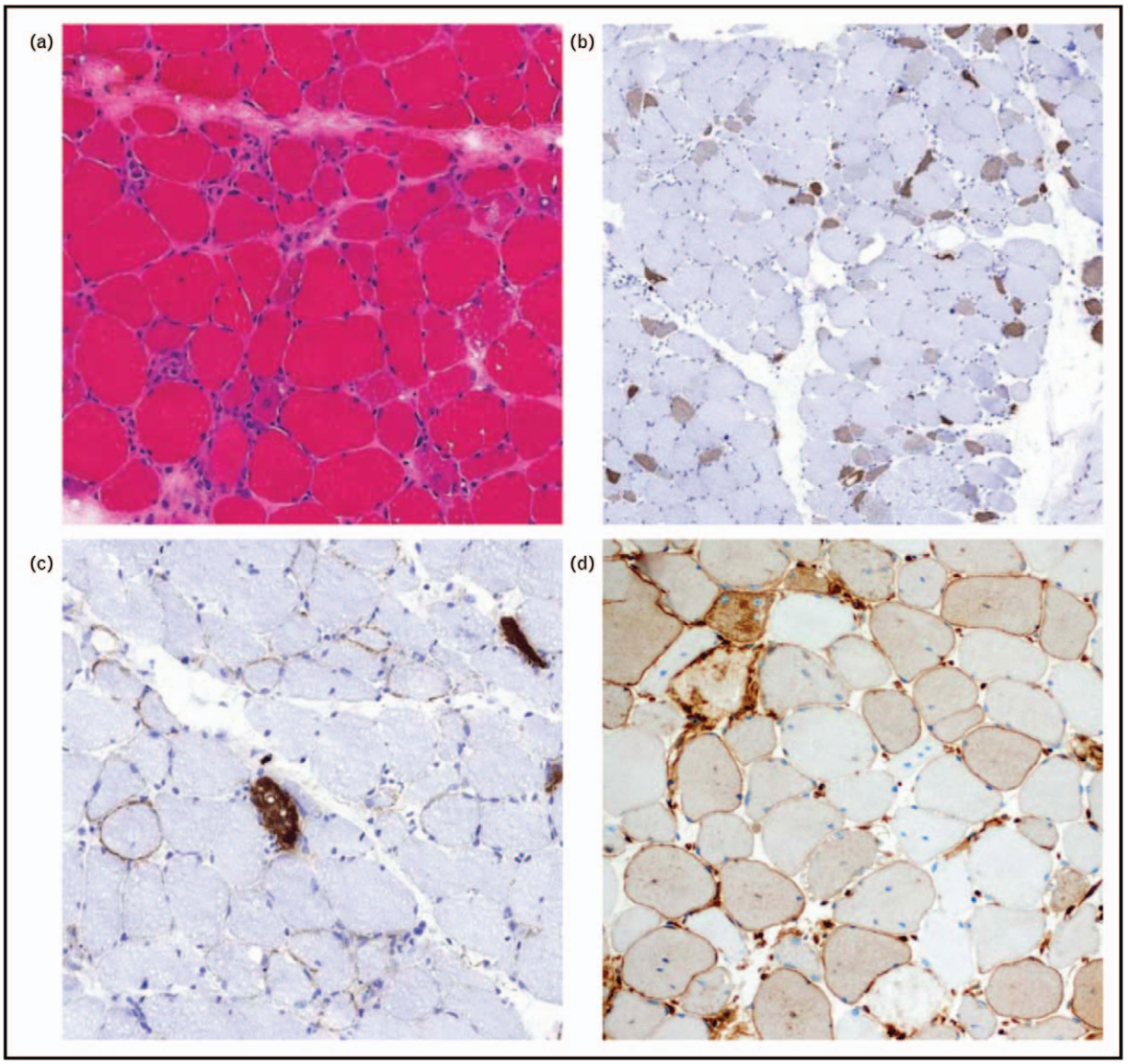

FIGURE 2. Pathological features of immune-mediated necrotizing myopathies. (a) Numerous necrotic muscle fibres randomly distributed in an IMNM muscle biopsy. Necrotic muscle fibres are at different stages of necrosis, ranging from pale hyalinized fibres to myophagocytosis. (HE staining). (b) High frequency of regenerating muscle fibres (positive neonate myosin heavy chain staining) randomly distributed. (c) Sarcolemmal C5b-9 deposits decorate nonnecrotic muscle fibres in INMM patients. Of note, nonspecific homogenous sarcoplasmic C5b-9 staining was found on necrotic muscle fibres. (d) Mild positive major histocompatibility complex I staining with a patchy distribution in IMNM patients on regenerating muscle fibres.

MHC class I expression (seen on nonnecrotic/nonregenerating fibres), sarcolemmal complement deposition, endomysial fibrosis and proliferation, and enlarged capillaries [8▪].

EXTRASKELETAL MUSCLE FEATURES

Cardiac muscle features

Conduction abnormalities, rhythm disorders and left ventricular dysfunction have been reported in 2–40% of anti-SRP cases [14,16,20,27,28]. If clear myocarditis has been reported in anti-SRP+ patients, the significance and the specificity of all of the above-mentioned cardiac manifestations remain to be established. Nevertheless, in our opinion, a systematic cardiac screening is necessary for anti-SRP patients. To date, cardiac involvement has not been clearly associated with anti-HMGCR patients.

Extramuscular autoimmune features

IMNM may be considered a pure muscle autoimmune disease. Specifically, in anti-HMGCR no auto-immune extra-muscle involvement has been

reported. Anti-SRP+ patients do not suffer frequently from extramuscular manifestations, even though arthralgia (0–39%) [14,20] and Raynaud's phenomenon (0–26%) [20,29] have been reported. Anti-SRP IMNM may also result in an interstitial lung disease that is detected when a computed tomography (CT)-scan is performed (0–22%) [20,28]. Again, this finding is not clinically significant and is not associated with pulmonary function test abnormalities.

On the contrary, it must be mentioned that some overlapping myositides may have myopathological features similar to IMNM, with diffuse randomly distributed necrotic/regenerating muscle fibres. This is the case in myositis-associated scleroderma [30].

Malignancy in immune-mediated necrotizing myopathies

An increased risk of malignancy in autoimmune myopathies is mainly observed in dermatomyositis, but it has also been reported in 'polymyositis' [31]. On the basis of this observation and previous case reports describing IMNM and cancer, the risk of malignancy in IMNM patients was compared with that expected in a sex and age-matched population. It was shown that patients with antibody-negative IMNM have a higher risk of malignancy, whereas the risk was similar for anti-SRP patients [32▪]. A mild increase was observed in two studies of anti-HMGCR+ patients [32▪,33], but this was not observed by others [17▪]. In the case of cancer association, malignancy occurs within 3 years before or after the diagnosis and in most cases within 1 year of diagnosis [32▪]. Cancer affects mainly patients older than 50 years. No specific type of cancer is observed in IMNM, and patients with malignancy have a lower survival rate [32▪].

IMMUNE-MEDIATED NECROTIZING MYOPATHY PROGNOSIS

Patients suffering from autoimmune myopathies have a higher mortality rate than the general population [34]. The main causes of death in autoimmune myopathies are malignancy and diseases of the respiratory and circulatory systems [34]. All of these life-threatening complications, except severe interstitial lung disease, may occur in IMNM patients.

One-quarter of IMNM patients suffer from difficulties in their daily living, graded as modified Rankin Scale scores of 3–5 [9]. Only 50% of anti-SRP+ patients reached near-full or full strength after 4 years of treatment, and a younger age at onset is associated with more severe weakness [10▪]. Only 44% of anti-HMGCR+ patients reached full strength with immunosuppressive therapy, and younger patients had more severe disease and a worse prognosis than older patients [17▪]. Of note, statin exposure was not independently associated with the rate of muscle strength improvement [17▪].

These observations are in line with the presence of significant muscle damage in IMNM. Compared with the other myositides, IMNM was characterized by a higher proportion of thigh muscles with oedema, atrophy and fatty replacement (Fig. 3) [35▪▪]. According to the clinicobiological observations, among IMNM, anti-SRP+ patients had more atrophy and fatty replacement than anti-HMGCR+ patients [35▪▪].

Together, the data show that IMNMs are the most severe group of myositides with a poor outcome in terms of muscle strength compared with dermatomyositis or the antisynthetase syndrome.

PATHOPHYSIOLOGY

The pathophysiology of IMNM is not fully understood. Some human leukocyte antigen (HLA) class I and II antigens have been associated with IMNM [36–38]. Statin exposure is another factor associated with anti-HMGCR+ IMNM onset, suggesting that in genetically predisposed patients, the drug may trigger the disease. It has been shown that HMGCR expression is upregulated in regenerating myofibers in anti-HMGCR IMNM patients [7]. Similarly, HMGCR and SRP proteins were detected at the sarcolemmal level in IMNM patients [18▪]. This observation and the presence of membrane attack complexes at the surface of nonnecrotic muscle fibres suggest a pathogenic role of the auto-antibodies. This hypothesis is reinforced by the observation of IgG and C1q at the sarcolemmal level in IMNM patients, suggesting classical pathway activation (antibody-dependant) of complement [18▪]. The titre of the auto-antibodies correlates with the disease activity of anti-SRP+ and anti-HMGCR+ myositis [13,17▪,22]. In addition, it has been shown *in vitro* that anti-SRP and anti-HMGCR induce muscle fibre atrophy and impair muscle regeneration [39▪▪]. Together, these observations suggest a pathogenic role of auto-antibodies in IMNM.

TREATMENT

IMNM treatment remains challenging. There has been no randomized clinical trial specifically designed for IMNM patients. Retrospective studies have shown that anti-SRP and anti-HMGCR patients have a long disease duration with frequent relapses when using several treatments, including

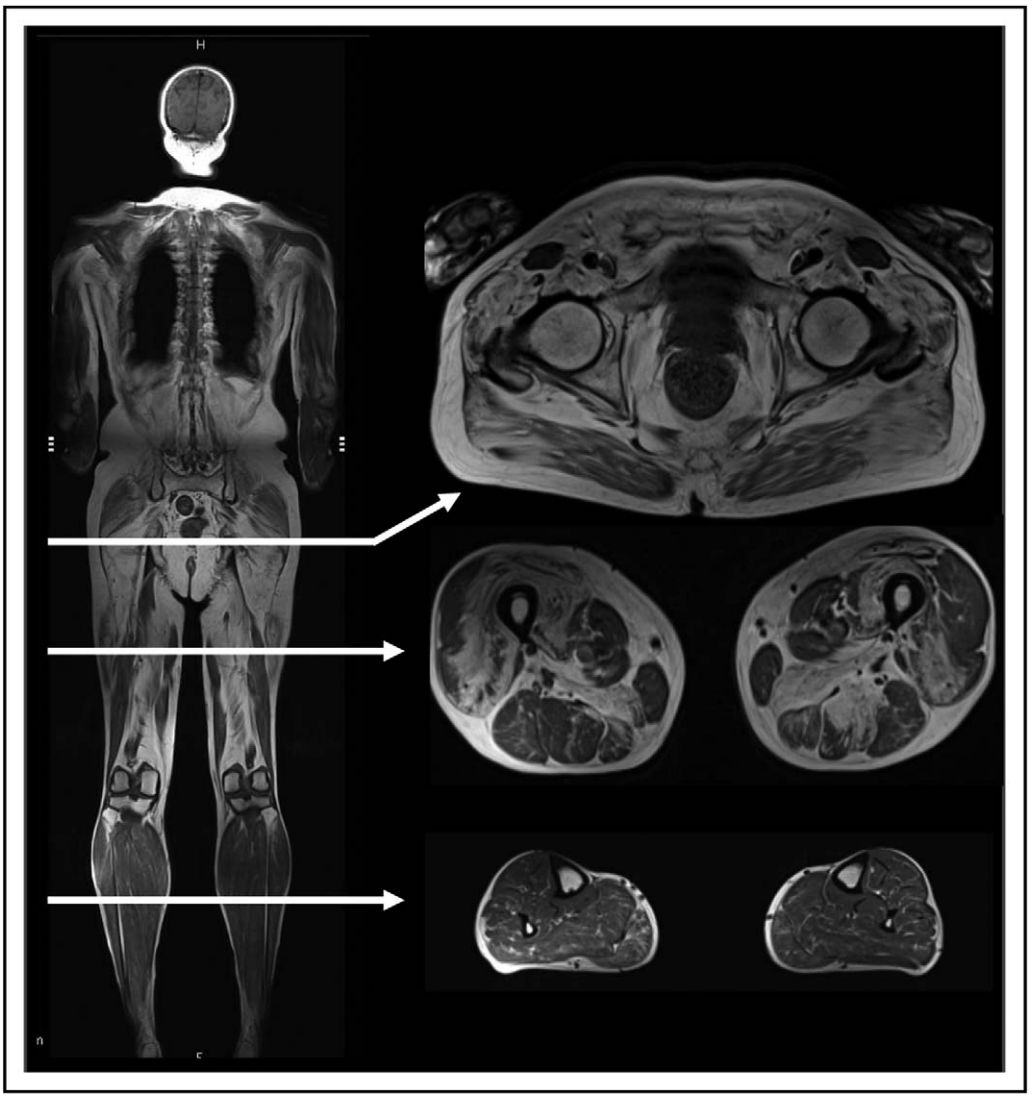

FIGURE 3. Immune-mediated necrotizing myopathy whole-body MRI. Whole-body MRI (T1) of a severely affected IMNM patients show important muscle damages in the lower limbs. The important fatty replacement (T1 hypersignal) is observed in the gluteus muscles as well as in the thigh muscles, whereas the muscles of the lowers limbs are spared.

corticosteroids and immunosuppressants [9,10▪, 13,17▪]. It was shown that rituximab administration was efficacious in 75% of cases [10▪,40], leading to the consideration of its use as a first-line treatment in anti-SRP patients [8▪]. In anti-HMGCR+ patients, intravenous immunoglobulin has been efficacious as a monotherapy [41], showing its importance in the treatment strategy for those patients [8▪].

The first-line treatment strategy must be adapted to each patients' condition and the severity of the muscle disease. The recommendations of the 224th ENMC are shown in Fig. 4.

Disease assessment is challenging in autoimmune myositis. The ACR/EULAR recommendations have been updated to capture a significant improvement in the domains (e.g. muscle, skin, lung or joints) that could be affected in myositis patients [42]. In anti-SRP+ and anti-HMGCR+ IMNM, it was shown that the creatine kinase level is associated with disease activity [13,17▪], as the creatine kinase level mirrors the percentage of necrotic muscle fibres [18▪]. Regarding the severe muscle damage observed in IMNM patients [35▪▪], it seems logical to target normal creatine kinase levels to treat IMNM and determine disease remission.

In addition, a slight increase in creatine kinase levels may be a sign of important and persistent disease activity in severely affected patients, as a normal creatine kinase level is correlated with muscle mass. Indeed, IMNM patients with important clinically defined sarcopenia and low creatinine blood levels may have subnormal creatine kinase levels, whereas persistent disease activity is determined by muscle oedema using MRI.

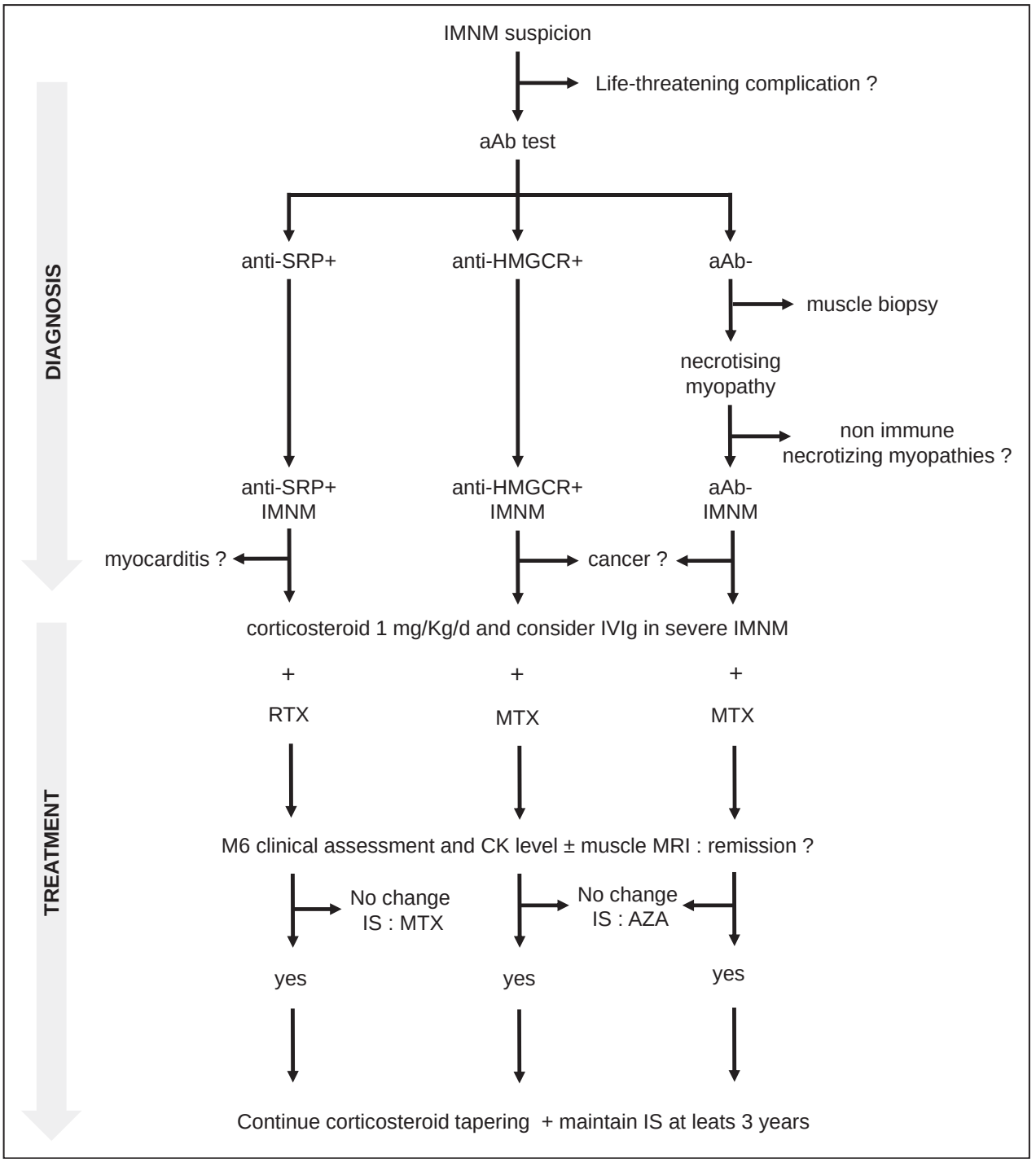

FIGURE 4. Immune-mediated necrotizing myopathy diagnostic and treatment strategy. The life-threatening complications of IMNMs are swallowing trouble and/or dyspnoea. The causes of nonimmune necrotizing myopathies are acquired necrotizing myopathies (drug/toxic, infections and endocrine/metabolic) and genetic myopathies (muscular dystrophy in case of slowly progressive onset and metabolic myopathies in case of acute onset). Severe IMNM cases are patients with dysphagia and/or walking difficulty. aAb, auto-antibodies; AZA, azathioprine; IS, immunosuppressant; MTX, methotrexate; RTX, rituximab.

MRI of the muscle is an important tool to monitor IMNM patients, as MRI permits the measurement of disease activity and also muscle damage. MRI is sometimes used to avoid treatment escalation in patients with definitive fixed muscle damage with fatty replacement.

To conclude, IMNM is the most severe autoimmune muscular disease with regard to patient

impairment and the risk of muscle damage. IMNM can be anti-SRP+, anti-HMGCR+ or auto-antibody negative. This serological distinction allows the determination of a more definitive phenotype in the patient (more severe in anti-SRP patients), the determination of potential cancer association (mainly in antibody-negative IMNM) and the development of treatment strategies.

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We want to acknowledge Drs. Jean-Luc Charuel and Lucile Musset for having provided the pictures of immunofluorescence of myositis-specific antibodies. Similarly, we thank Professor Pierre Carlier and Jean-Marc Boisserie for the MRI pictures of IMNM patients they gave to illustrate the muscle damages.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975; 292:344–347.
2. Benveniste O, Stenzel W, Allenbach Y. Advances in serological diagnostics of inflammatory myopathies. Curr Opin Neurol 2016; 29:662–673.
3. Reeves WH, Nigam SK, Blobel G. Human autoantibodies reactive with the signal-recognition particle. Proc Natl Acad Sci U S A 1986; 83:9507–9511.
4. Miller T, Al-Lozi M, Lopate G, Pestronk A. Myopathy with antibodies to the signal recognition particle: clinical and pathological features. J Neurol Neurosurg Psychiatry 2002; 73:420–428.
5. Hoogendijk JE, Amato AA, Lecky BR, *et al.* 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10-12 October 2003, Naarden, The Netherlands. Neuromuscul Disord NMD 2004; 14:337–345.
6. Christopher-Stine L, Casciola-Rosen LA, Hong G, *et al.* A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy. Arthritis Rheum 2010; 62:2757–2766.
7. Mammen AL, Chung T, Christopher-Stine L, *et al.* Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme a reductase (HMGCR) in patients with statin-associated autoimmune myopathy. Arthritis Rheum 2011; 63:713–721.
8. ■ Allenbach Y, Mammen AL, Stenzel W, Benveniste O. Immune-mediated necrotizing Myopathies Working Group. 224th ENMC International Workshop: Clinico-sero-pathological classification of immune-mediated necrotizing myopathies Zandvoort, The Netherlands, 14–16 October 2016. Neuromuscul Disord 2018; 28:87–99.

In this conference, an international consensus was reached to refine IMNM criteria. For the first time, IMNM definition is based on serological criteria.

9. Watanabe Y, Uruha A, Suzuki S, *et al.* Clinical features and prognosis in anti-SRP and anti-HMGCR necrotising myopathy. J Neurol Neurosurg Psychiatry 2016; 87:1038–1044.
10. ■ Pinal-Fernandez I, Parks C, Werner JL, *et al.* Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. Arthritis Care Res 2017; 69:263–270.

In this article, the authors described for the first time the precise muscle phenotype of anti-SRP+ patients. For the first time, they have clearly demonstrated the poor muscle outcome.

11. Mammen AL, Pak K, Williams EK, *et al.* Rarity of anti3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies in statin users, including those with self-limited musculoskeletal side effects. Arthritis Care Res Hoboken 2012; 64:269–272.
12. Meyer A, Meyer N, Schaeffer M, *et al.* Incidence and prevalence of inflammatory myopathies: a systematic review. Rheumatol Oxf Engl 2015; 54:50–63.
13. Allenbach Y, Drouot L, Rigolet A, *et al.* Anti-HMGCR autoantibodies in European patients with autoimmune necrotizing myopathies: inconstant exposure to statin. Medicine (Baltimore) 2014; 93:150–157.
14. Suzuki S, Hayashi YK, Kuwana M, *et al.* Myopathy associated with antibodies to signal recognition particle: disease progression and neurological outcome. Arch Neurol 2012; 69:728–732.
15. Pinal-Fernandez I, Casal-Dominguez M, Mammen AL. Immune-mediated necrotizing myopathy. Curr Rheumatol Rep 2018; 20:21.
16. Kao AH, Lacomis D, Lucas M, *et al.* Antisignal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. Arthritis Rheum 2004; 50:209–215.
17. ■ Tiniakou E, Pinal-Fernandez I, Lloyd TE, *et al.* More severe disease and slower recovery in younger patients with anti3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. Rheumatol Oxf Engl 2017; 56:787–794.

In this article, the authors described for the first time the precise muscle phenotype of anti-HMGCR+ patients. For the first time, they have clearly demonstrated the poor muscle outcome, stratifying the outcome based on the age of onset.

18. ■ Allenbach Y, Arouche-Delaperche L, Preusse C, *et al.* Necrosis in anti-SRP+ and anti-HMGCR+myopathies: role of autoantibodies and complement. Neurology 2018; 90:e507–e517.

In this article, the authors described myopathological changes IMNM with a quantitative approach. This study shows IgG and complement deposits at the sarcolemmal level and suggest a pathogenic role of auto-antibody in muscle fibre necrosis.

19. Fromageot C, Lofaso F, Annane D, *et al.* Supine fall in lung volumes in the assessment of diaphragmatic weakness in neuromuscular disorders. Arch Phys Med Rehabil 2001; 82:123–128.
20. Hengstman GJD, ter Laak HJ, Egberts WTMV, *et al.* Antisignal recognition particle autoantibodies: marker of a necrotising myopathy. Ann Rheum Dis 2006; 65:1635–1638.
21. Drouot L, Allenbach Y, Jouen F, *et al.* Exploring necrotizing autoimmune myopathies with a novel immunoassay for anti3-hydroxy-3-methyl-glutaryl-CoA reductase autoantibodies. Arthritis Res Ther 2014; 16:R39.
22. Benveniste O, Drouot L, Jouen F, *et al.* Correlation of antisignal recognition particle autoantibody levels with creatine kinase activity in patients with necrotizing myopathy. Arthritis Rheum 2011; 63:1961–1971.
23. Saraogi I, Shan S. Molecular mechanism of co-translational protein targeting by the signal recognition particle. Traffic Cph Den 2011; 12:535–542.
24. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell 2006; 124:35–46.
25. Allenbach Y, Benveniste O, Goebel H-H, Stenzel W. Integrated classification of inflammatory myopathies. Neuropathol Appl Neurobiol 2017; 43:62–81.
26. Chung T, Christopher-Stine L, Paik JJ, *et al.* The composition of cellular infiltrates in anti-HMG-CoA reductase-associated myopathy. Muscle Nerve 2015; 52:189–195.
27. Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. Arthritis Rheum 1990; 33:1361–1370.
28. Basnayake SK, Blumbergs P, Tan JA, *et al.* Inflammatory myopathy with anti-SRP antibodies: case series of a South Australian cohort. Clin Rheumatol 2015; 34:603–608.
29. Wang L, Liu L, Hao H, *et al.* Myopathy with antisignal recognition particle antibodies: clinical and histopathological features in Chinese patients. Neuromuscul Disord 2014; 24:335–341.
30. Ranque B, Authier FJ, Berezne A, *et al.* Systemic sclerosis-associated myopathy. Ann N Acad Sci 2007; 1108:268–282.
31. Zahr ZA, Baer AN. Malignancy in myositis. Curr Rheumatol Rep 2011; 13:208–215.
32. ■ Allenbach Y, Keraen J, Bouvier A-M, *et al.* High risk of cancer in autoimmune necrotizing myopathies: usefulness of myositis specific antibody. Brain J Neurol 2016; 139:2131–2135.

In this study, the authors have compared the prevalence of malignancy in the three IMNM subtypes (anti-SRP+, anti-HMGCR+ and seronegative) to the aged and sex-marched population. They have showed an important increased risk in antibody-negative IMNM, whereas the risk is mild in anti HMGCR+ patients.

33. Kadoya M, Hida A, Hashimoto Maeda M, *et al.* Cancer association as a risk factor for anti-HMGCR antibody-positive myopathy. Neurol Neuroimmunol Neuroinflammation 2016; 3:e290.
34. Dobloug C, Garen T, Bitter H, *et al.* Prevalence and clinical characteristics of adult polymyositis and dermatomyositis; data from a large and unselected Norwegian cohort. Ann Rheum Dis 2015; 74:1551–1556.
35. ■■ Pinal-Fernandez I, Casal-Dominguez M, Carrino JA, *et al.* Thigh muscle MRI in immune-mediated necrotising myopathy: extensive oedema, early muscle damage and role of anti-SRP autoantibodies as a marker of severity. Ann Rheum Dis 2017; 76:681–687.

This study has reported for the first time the muscle damages occurring in a large cohort of IMNM compared with others myositis. According to the poor outcomes of IMNM, the authors showed the important fatty replacement in IMNM patients compared with the other group of IMNM.

36. Mammen AL, Gaudet D, Brisson D, *et al.* Increased frequency of DRB1∗11:01 in anti-HMG-CoA reductase-associated autoimmune myopathy. Arthritis Care Res (Hoboken) 2012; 64:1233–1237.
37. Kishi T, Rider LG, Pak K, *et al.* Association of anti3-hydroxy-3-methylglutaryl-coenzyme A reductase autoantibodies with DRB1∗07:01 and severe myositis in juvenile myositis patients. Arthritis Care Res 2017; 69: 1088–1094.

38. Ohnuki Y, Suzuki S, Shiina T, *et al.* HLA-DRB1 alleles in immune-mediated necrotizing myopathy. Neurology 2016; 87:1954–1955.
39. ■■ Arouche-Delaperche L, Allenbach Y, Amelin D, *et al.* Pathogenic role of antisignal recognition protein and anti3-Hydroxy-3-methylglutaryl-CoA reductase antibodies in necrotizing myopathies: myofiber atrophy and impairment of muscle regeneration in necrotizing autoimmune myopathies. Ann Neurol 2017; 81:538–548.
In this study, the authors test *in vitro* the pathogenic role of the anti-SRP and anti-HMGCR antibodies. For the first time, the authors showed that they can induce muscle atrophy and impair muscle regeneration.
40. Valiyil R, Casciola-Rosen L, Hong G, *et al.* Rituximab therapy for myopathy associated with antisignal recognition particle antibodies: a case series. Arthritis Care Res Hoboken 2010; 62:1328–1334.
41. Mammen AL, Tiniakou E. Intravenous immune globulin for statin-triggered autoimmune myopathy. N Engl J Med 2015; 373:1680–1682.
42. Rider LG, Ruperto N, Pistorio A, *et al.* 2016 ACR-EULAR adult dermatomyositis and polymyositis and juvenile dermatomyositis response criteria: methodological aspects. Rheumatol Oxf Engl 2017; 56:1884–1893.

Antisynthetase syndrome pathogenesis: knowledge and uncertainties

Laure Gallay[a,b], *Catherine Gayed*[c], *and Baptiste Hervier*[c,d]

Purpose of review
Antisynthetase syndrome (ASyS) is an acquired myopathy characterized by the presence of myositis-specific autoantibodies directed against tRNA-synthetases. ASyS is potentially life threatening due to lung involvement and treatment remains a challenge to date. With symptoms not limited to muscles but also involving lung, skin and joints, ASyS appears specific and has a particular pathogenesis, different from the other inflammatory myopathies. This review is intended to discuss the current understanding of ASyS pathogenesis, pointing its current knowledge and also the crucial prospects that may lead to critical improvement of ASyS care.

Recent findings
Regarding ASyS pathogenesis, initiation of the disease seems to arise in a multifactorial context, with first lesions occurring within the lungs. This may lead to aberrant self-antigen exposure and tolerance breakdown. The consequences are abnormal activation of both innate and adaptive immunity, resulting in the patients with favourable genetic background to autoimmune-mediated organ lesions. Immune and nonimmune roles of the antigen, as well as antigen presentation leading to specific T-cell and B-cell activation and to the production of specific autoantibodies belong to the disease process.

Summary
This work aims to detail ASyS pathogenesis understanding, from initiation to the disease propagation and target tissue lesions, in order to considering future treatment directions.

Keywords
antisynthetase syndrome, autoimmunity, inflammatory myopathy

INTRODUCTION

Since the initial classification in 1975 [1], the inflammatory myopathy classification now identified dermatomyositis, sporadic inclusion body myositis, immune-mediated necrotizing myopathy and overlapping myositis [2], among which the antisynthetase syndrome (ASyS) is the most common [3]. The immunological specificity of ASyS is the presence of myositis-specific autoantibodies, which are directed against tRNA-synthetases (ARSs). The most frequent of these autoantibodies (anti-Jo-1) specifically recognizes histidyl-tRNA-synthetase (HisRS). Seven other antitRNA-synthetase autoantibodies (anti-ARSs) have been described to date [3] and are mutually exclusive. Despite lack of validated classification criteria for ASyS, association of these autoantibodies with inflammatory myopathy and interstitial lung disease has been recognized a long time ago [4] and now distinguishes ASyS as an own entity. In addition, the recently described specificities of the ASyS muscular histology – as compared to other inflammatory myopathies – may correspond to specific immuno-pathogenesis [5,6]. However, ASyS pathophysiology remains insufficiently understood.

This review describes the current knowledge of ASyS immuno-pathogenesis and focuses on the initiation of the disease, the multiple roles of antigens, the

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KEY POINTS

- Like rheumatoid arthritis, ASyS starts in the lungs, following aggression by different environmental factors and upper respiratory tract infections.
- These first events lead to unspecific innate immunity activation, resulting in self-antigen cleavage by granzyme B.
- In addition to various nonimmune roles, the cleaved auto-antigen has the capacity to recruit immune cells and stimulate immunity, leading to tolerance breakdown with generation of specific T and B cells.
- By invading other target tissue in which expression of the antigen as well as HLA-I molecules is abnormal, adaptive immune cells are responsible for immune-mediated tissue lesions and disease propagation.

innate and adaptive immunity activation, leading to autoimmunity, the disease propagation and finally discusses the future therapeutic directions.

INITIATION OF THE DISEASE

Immune tolerance and immune breakdown

In contrast to the auto-inflammatory diseases, which result in overactivation of innate immune system induced by a monogenic mutation, autoimmune disorders arise from innate and acquired immune system misdirection. Under particular circumstances, the protection system of the organism can recognize the self as nonself and overdrive the multiple control mechanisms, resulting in self-reactivity. Despite numerous checkpoints, mechanisms of tolerance breakdown may take place in central compartment, involving especially T-cell and B-cell lineages as well as in the peripheral compartments [7–9]. The final activation of the auto-reactive immune cells seems to be favoured by different factors including: association of genetic predispositions, resulting from the additive effects of several common risk variants, [10], estrogens as potential activators of the immune system, explaining the higher incidence of autoimmunity in women [11], microbiome, even for diseases distant to the interface [12], tissue microenvironment changes and self-cell damage, which occur for example after viral exposure and/or radiation and lead to autoantigen exhibition. In addition to these different elements, recent advances have been done in understanding the role of environmental factors as unspecific triggers of autoimmunity.

Environmental aggression of the self and activation of innate immunity

The airway mucosa is permanently exposed to inhaled substances and is a critical place of interaction between environmental factors and immunity. Some examples are found by correlation between different autoimmune diseases and exposition to airborne particles and incidence of after the collapse of World Trade Center in 2001 [13,14], or smoking habit [15–17]. Likewise, international literature reports several pulmonary triggers of autoimmunity in ASyS patients [18,19], including tobacco exposure [20], particularly in association with particular human leukocyte antigen (HLA) genetic background [21]. Interestingly, reflecting a potential other source of immunogenic triggers, a higher frequency of respiratory tract infections at least 1 year before the onset of inflammatory myopathy has been reported in a large population-based case–control study [22▪▪]. These data suggest pathogens act as danger signals, initiating the immune system misdirection.

By aggressing the epithelia [23,24], airborne contaminants as well as pathogens end up at cell stress, cell death and microparticle release. Cytoplasmic and nuclear components of high potential for immune system activation and polarization may be exposed and imply both cell-cell crosstalk and chemical messengers. Among these cell death mechanisms, NETosis of neutrophils might be a contributor of particle release: the levels of some human neutrophil peptides could be increased both in broncho-alveolar lavage fluid (BALF) from ASyS patients [25]. In addition, the in-vitro development of neutrophil extracellular traps is promoted by ASyS patients' sera [26]. Synergistically with an impairment of DNAse–I activity, such events lead to the liberation of many particles (proteins, enzymes, cytokines and nucleic acids), which have the capacity of damaging the tissues, potentially causing the exponential release of antigens.

Sustaining the epidemiological associations with infections in humans, animal models help to understand the way in breaking immune tolerance and inducing tissue damage. Elaboration of murine ASyS model has been possible using intramuscular inductors [27–29]. It has been demonstrated *in vitro* and *in vivo* by using toll-like receptor (TLR)-2$^{-/-}$ and TLR-4$^{-/-}$ double knockout mice, that these danger signal receptors were essential for the activation of innate immunity [30]. In another model without HisRS immunization, a complete autoimmune response was obtained after muscle injury only in the presence of TLR-7 agonists [31▪▪]. These two conditions lead to cell death, enough to increase

HisRS expression and to activate cellular cascades responsible for destructive tissue inflammation in murine ASyS [32].

Generation and involvement of self-antigen in antisynthetase syndrome

Apart from unspecific release of antigens following cell death, the cleavage of self-proteins by serine proteases, such as granzyme B during cytotoxic lymphocyte granule-induced death pathway, has been identified as a trigger of autoimmunity. Such cleavage could generate unique protein fragments, which play an important role in tolerance breakdown [33]. Application of this hypothesis has been done for anti-Jo-1 ASyS: some authors have found a particular expression of granzyme B–cleavable conformation of HisRS in the alveolar epithelium [34]. This unique form of HisRS has been proven to be cleaved by granzyme B *in vitro* in its N-terminal domain, and to generate a 50 kDa-fragment. This fragment is detected in large amounts only in the human lung lysates, and absent or present at much lower amount in the other tissues. It is remarkable that the dominant epitope recognized by the anti-HisRS autoantibodies is also located in this N-terminal fragment.

The production of this granzyme B–cleavable conformation of HisRS *in vivo* remains unclear, and the granzyme B producing cells have not yet been identified. A special attention has, however, been recently paid to the natural killer (NK) cells [35]. NK cells are innate immune cells, activated at least through 'stress-induced recognition', which could for example follow tobacco smocking [36] and lead to rapid and large production of granzyme B. NK cells specifically infiltrate the lungs during Jo-1-positive ASyS [35]. Abnormal expression of NKp30 [35], a natural cytotoxicity activating receptor which has 2 cell-stress-induced ligands [37,38], has been shown in ASyS. Thus, following alveolar epithelial cell stress, NK cells could be activated and involved in the production of granzyme B, leading to intact and/or cleaved HisRS generation.

MULTIPLE ROLES OF THE ANTIGEN

To date, only eight aminoacyl-tRNA-synthetases are targeted by eight identified anti-ARS [3]: All the different ARSs have pleiotropic functions, involved in immunity or not, which could directly or indirectly connect ARS to ASyS symptoms (Table 1) [39–45].

Nonimmune roles of tRNA-synthetase: potential implications in antisynthetase syndrome pathogenesis

ARSs are cytoplasmic and mitochondrial enzymes which are highly conserved in living beings and have essential roles in the RNA translation machinery and protein synthesis. Once the anticodon-related amino acid is attached, the ARS interacts with the ribosome in order to transfer the amino acid from tRNA onto a growing peptide. There is specific ARS for each of the 20 different amino acids of the genetic code. These proteins not only are an essential part of the translation apparatus but also have numerous cytoplasmic, nuclear and extracellular functions, such as triggering or silencing of inflammatory/immune responses, participating in lung development or playing a role in neuromuscular disorders (Table 1) [41,45–48].

HisRS, the most common target of anti-ARS, is responsible for the synthesis of histidyl-transfer RNA, which is essential for the incorporation of histidine into proteins [42]. Histidine is an essential proteinogenic amino acid with an imidazole functional group which has antioxidant, antisecretory and anti-inflammatory properties, suppressing proinflammatory cytokine expression possibly through NF-κB pathway [43]. In addition, histidine is also a precursor for carnosine biosynthesis, a highly concentrated protein in muscle and brain tissues, which exhibit antioxydant effect, through metal ions chelation, superoxide dismutase-like activity and reactive oxygen species [49]. Interestingly, histidine is the axial ligand of haem, a major component of myoglobin and haemoglobin, which has for principal function to carry oxygen molecule to muscle tissues.

Direct pathogenicity of the anti-ARS is sustained in ASyS by several studies demonstrating their capacity to inhibit the ARS activities *in vitro* [40,50–54]: anti-KS autoantibody increases the affinity of the synthetase for its tRNA substrate and prevents aminoacylation [53]. In other circumstances, anti-ARS may be competitive inhibitors of the aminoacylation reaction catalyzed by this enzyme [51]. Thus, further data are needed to understand more precisely how anti-ARSs activate or inhibit their cognate ARS. In particular, the mechanisms through which extracellular autoantigens and/or autoantibodies could enter the cells and dysregulate physiological pathways remain unknown.

Immune roles of the antigen

Apart from these multiple roles, ARSs both in full length and cleaved forms have many functions in immune system activation, leading to tolerance

Table 1. Immune and nonimmune roles of the different tRNA-synthetase targeted by antitRNA-synthetase autoantibody

tRNA-synthetase	Abbreviation	Nonimmune role	Immune role	Auto-antibody
Histidyl-tRNA-synthetase	HisRS	Incorporation of histidine into proteins: antioxidant, anti-inflammatory and antisecretory properties [40]	Chemoattraction: expression of ICAM-1 in endothelial cells [44]. Attraction of CCR5+ cells, including IL2-activated monocytes, immature DCs, $CD4^+$ and $CD8^+$ T cells	anti-Jo-1
Alanyl-tRNA-synthetase	AlaRS	Editing activity, which enhances the accuracy of aminoacylation so as to prevent mistranslation. Mutations of AlaRS have been causally associated with neuromuscular disorders such as Charcot–Marie–Tooth [41]	Antibodies directed against AlaRS are identified in 25% of diabetes mellitus 1 [45]	anti-PL12
Threonyl-tRNA-synthetase	ThrRS			anti-PL7
Isoleucyl-tRNA-synthetase	IleRS	Large molecular weight multitRNA synthetase complex (MSC) composed of nine tRNA synthetases (AsnRS and IleRS) and three scaffold proteins known as MSCp43, MSCp38, MSCp18, involve in lung development [42]		anti-OJ
Glycyl-tRNA-synthetase	GlyRS	Editing activity, which enhances the accuracy of aminoacylation so as to prevent mistranslation. Mutations of AlaRS have been causally associated with neuromuscular disorders such as Charcot–Marie–Tooth [41]. GlyRS is also an active antitumor agent through binding to K-cadherin on ras-activated tumor cells and promotes the dephosphorylation and deactivation of ERK [43]	Antibodies directed against GlyRS identified in 8% of diabetes mellitus 1 [45]	anti-EJ
Asparaginyl-tRNA-synthetase	AsnRS	Large molecular weight multitRNA synthetase complex (MSC) composed of nine tRNA synthetases (AsnRS and IleRS) and three scaffold proteins known as MSCp43, MSCp38, MSCp18, involve in lung development [42]	Antibodies directed against AsnRS identified in 13% of diabetes mellitus 1 [45]	anti-KS
Phenylalanyl-tRNA-synthetase	PheRS			anti-Zo
Tyrosyl-tRNA-synthetase	TyrRS	Involvement of catalytic defect in tyrosyl-tRNA synthetase in dominant intermediate Charcot–Marie–Tooth disorder [43]. Balance of angiogenesis for the vasculature.	IL-8 like cytokine properties, binding of CXC chemokine receptor, mimicking of EMAPII	anti-YRS/Tyr

ARSs are enzymes that catalyze the amino-acid attachment to their corresponding tRNAs. Apart from this role, many immune and nonimmune roles have been described.
CCR5, C-C chemokine receptor 5; DCs, dendritic cells; EMAPII, endothelial-monocyte activating polypeptide 2; ICAM-1, intracellular adhesion molecule 1; IL, interleukine.

breakdown and to immune-mediated tissue damage as observed in ASyS patients [55]. Therefore, ASyS immunopathogenesis is more complex than just a basic antigen-antibody-driven response [55].

ARSs themselves display chemo-attractant properties. Sera from anti-Jo-1 positive patients induce the expression of intracellular adhesion molecule 1 in human endothelial cells, which allows immune cells to reach target tissues. After cleavage by proteases, TyrRS fragments act as interleukin (IL)-8 like cytokine, bind CXC chemokine receptor or mimic endothelial-monocyte activating polypeptide 2 [56]. It has been shown that HisRS, and to a lesser extent its cleaved N-term domain, also has also chemo-attractant abilities through C-C chemokine receptor 5 (CCR5) interaction [57]. This results in attraction of both innate and adaptive cells thereby strengthening their collaboration. Place of the lung during the autoimmune process is sustained by specific properties of pulmonary antigen and

significant innate and adaptive cell infiltration within the patients' lungs. Presence of macrophages, dendritic cells, NK cells, T and B lymphocytes – sometimes organized as tertiary lymphoid structures in the lungs – indeed highlights the strong interactions between all innate and adaptive immunity. Antigen cleavage and liberation may activate immune cascades locally, resulting in autoimmune disease. Indeed, in addition, ARS showed activity in inflammatory response through interferon (IFN)-γ and p53 signalling [48,56–58].

Another argument for these roles has been shown in a mouse model: intramuscular immunization of either C57BL/6 or NOD mice with soluble recombinant murine HisRS fragment leads to an inflammatory myopathy with severe muscle and lung inflammation [28,29]. Mouse immunization leads to strong innate and adaptive immune responses, including antigen-specific T-cell and B-cell responses. Interestingly, when abrogating antigen-specific T-cell and B-cell responses (using $RAG2^{-/-}$ transgenic mice), the observed tissue inflammation was comparable to that of C57BL/6 or NOD mice [29]. These data showed that innate immunity activated by HisRS is a major mechanism for the development of murine ASyS, whereas specific T-cell and B-cell responses seemed less crucial in this model for inflammatory destruction of target tissues.

TOWARD AUTOIMMUNITY

Antigen presentation

Once the immunogenic peptide produced, dendritic cells might be attracted within the lungs, where they further mature and activate the antigen presentation process: the importance of antigen presenting cells in promoting T-cell proliferation in ASyS has been clearly assessed *in vitro*. Studies firstly confirmed that priming dendritic cells with HisRS fragments, especially the amino-terminal 151 amino-acids epitope, might be as efficient as the full length antigen stimulation [59]. Secondly, blocking HLA-DP/DQ/DR with specific antibodies clearly showed this process is major histocompatibility complex (MHC) class II dependent [59]. These data agree with the presence of extra-cellular ARS in plasma, BALF or muscles of ASyS patients [44,60]. It is of major importance to connect these data with the single nucleotide polymorphism genotyping studies performed in patients with inflammatory myopathy. As for other auto-immunes diseases, among the different genetic susceptibilities, the strongest association in inflammatory myopathies – reaching high genome wide significance – concerns the HLA-II alleles. Two studies have identified distinct haplotypes independently associated with the inflammatory myopathy subgroups [61,62]. This included notably the 8.1 ancestral haplotype (HLA-B*08 : 01) with anti-Jo1-positive ASyS. Other HLA-II associations are found with other subgroups [63], correlating with the myositis-specific autoantibody stratification and with patients' ethnicity. These genetic associations are risk factors for developing ASyS. HLA-II plays a crucial role in antigen presentation. The structure of the peptide-binding pocket affects the ability to build an efficient adaptive response, thanks to stronger avidity between antigenic peptide and its corresponding MHC molecule. The different single nucleotide polymorphisms associated with inflammatory myopathy correspond to distinct amino-acids. Some of them might be located into the peptide-binding pocket, increasing accordingly the avidity for the auto-antigen [64] and thus lead to a more efficient autoimmune-specific T-cell stimulation. These genetic susceptibilities shed light on the fact that ASyS is a rare disease. It is conceivable that individuals exposed to the causal environmental factors could produce the immunogenic peptide but are unable to mount an efficient adaptive response and to develop a symptomatic ASyS due to the existence of another genetic background.

Humoral immunity and autoantibody pathogenicity

The implication of B cells in ASyS pathogenesis has been shown both in mice and humans, in which treatments targeting $CD20^{+}$ B cells have been shown to be efficient [65].

In ASyS, the different steps of the humoral response, resulting in the synthesis of specific anti-ARS autoantibodies, have been successively shelled. Alike the other immune cells, B cells are sensitive to HisRS antigenicity. Indeed, the generation of auto-reactive B cells is observed following mouse immunisation with native or fragmented HisRS [29]. From epitope mapping studies in humans, the findings that the anti-Jo-1 autoantibodies could bind multiple HisRS epitopes do not plead for a molecular mimicry mechanism [66]. Other analyses have, however, reported that the N-term portion of HisRS is an immuno-dominant epitope [67], which might be expressed within the lungs. Furthermore, the auto-antigen is responsible for a fully functional humoral response. For instance, the existence of high affinity anti-Jo-1 autoantibodies reflects both class switching (prominence of IgG1 isotype) and somatic mutation phenomenon [68] and stresses out the efficient cooperation between B cells and CD4 T helper cells.

The direct pathogenic role of the anti-ARS in ASyS remains controversial to date, with only one clinical study showing correlation between disease activity and autoantibody titres [69]. It is still unclear whether these autoantibodies could mediate deleterious or even protective effects. Some data suggested *in vitro* that anti-Jo-1 autoantibodies could inhibit the initial HisRS cleavage by granzyme B [34] and could block the different effects of HisRS fragment on the immunity activation. Conversely, by targeting HisRS expressed in different tissues (i.e. lungs or regenerating muscle), autoantibodies could theoretically, through opsonization, phagocytosis or antibody-dependent cell-mediated cytotoxicity, induce tissue lesions.

Increased levels of IFNα-an essential cytokine in autoimmunity – might be observed in anti-Jo-1 positive [70]. By analogy of the role played by immune complexes on plasmacytoid dendritic cells in systemic lupus erythematosus, some hypothesized that complexed anti-Jo-1 themselves might herein explain the IFNα production [70]. This IFNα overproduction is linked with the existence of interstitial lung disease and with the observation of BDCA-2 positive plasmacytoid dendritic cells and type I IFN signature in the muscles from patients. These data, in association with the observation of increased levels of B lymphocyte stimulator (BLyS/B-cell activating factor) [71], could act synergistically on B cells as potent activators and prosurvival factors. This generates a positive feedback loop that sustains humoral immunity and could also favour disease propagation through systemic effects.

Cellular adaptive immunity

Both helper and cytotoxic specific T-cell responses have been identified during ASyS, notably through immunization of mice with recombinant murine antigens (HisRS) [28]. Of note, full length or fragmented HisRSs are by themselves capable of building a complete T-cell response, involving both cytotoxic and helper T cells. Contribution of both $CD4^+$ and $CD8^+$ T cells in human inflammatory myopathy pathogenesis has been studied several times [72]. During ASyS, analysis of BALF, peripheral blood and muscles has identified specific CD4 T cells [73,74]. CD4 T cells activation has been reproduced *in vitro* via HisRS or its N-term fragment interaction [73,74]. Activation of Th1-polarized CD4 T cells led to the secretion of IFNγ and to the generation of specific antibody-producing B cells. Interestingly, CXC chemokine receptor 3 (CXCR3) and CCR5 homing markers have been identified on all CD4 T cells in BALF [73], whereas they are decreased in peripheral blood CD4 T cells of ASyS [74]. This differential expression of activated CD4 T cells surface proteins suggests that $CXCR3^+$ $CCR5^+$ CD4 T cells are attracted in the lung, where they may have specific roles in ASyS.

Considering CD8 T cells, their presence during ASyS in the targeted organs supports their immunogenic role, maybe as effectors of lung and muscle lesions [55]. Part of adaptive cellular immunity, T-cell clonal expansion directed against a particular antigen presented by an HLA molecule can lead to apparition of a restricted T-cell receptor gene usage. Englund *et al.* [72] reported in different inflammatory myopathies a restricted TCR Vβ gene segment usage in lung and muscles when analysing separately the different $CD4^+$ and $CD8^+$ subsets. However, the ARS specificity of such oligoclonal CD8 T cells is not clearly determined yet specifically in the context of ASyS. This should, however, be further investigated by TCR repertoire analyses and/or tetrameric simulation of specific CD8 T cells to give deeper insights on the putative specificity of their involvement [72].

Detailed studies characterizing more precisely all the CD4 subpopulations, including regulatory, $CD28^{null}$ [75] and follicular helper T cells, as well as studies focusing on the respective involvement of naive, effector and/or memory CD8 subsets, would give a more complete picture of T cell immunity.

A SYSTEMIC DISEASE

Despite the ubiquitous expression of ARS, only few organs are affected during the disease course. As the initiation site of the disease, the lung is involved in more than 3/4 of the patients with ASyS [56]. Once activated in the lungs, the immune system has the capacity to spread to all organs, and preferentially the muscles. The reason why immune cells become deleterious in some organs but not in others remained poorly understood.

The mechanisms leading to myositis during ASyS probably result from the conjunction of several events, among which abnormal expresion of ARS in the muscles might be one of the first. The expression of intact HisRS is only slightly detected in normal muscles, whereas it is significantly overexpressed in inflamed muscles from patients with inflammatory myopathy, both in infiltrating immune cells and regenerating myofibers [76]. Furthermore, mRNA expression of intact HisRS and of one alternatively spliced variant is upregulated in patients with inflammatory myopathy [60]. Importantly, the main target of anti-Jo-1 autoantibodies, i.e. the antigen corresponding to the HisRS spliced variant, can be secreted from cell cytoplasm into medium *in vitro*, and appears to be a key factor of antigenicity

through recruitment of $CCR5^+$ T cells [57]. Furthermore, extracellular expression of HisRS also facilitates the humoral immune response, providing important clues for the development of an HLA-II-mediated auto-immunity [6]. However, ASyS is also a disease mediated by HLA-I, as attested by its myofiber overexpression in muscles. This questioned viral infection involvement [55,70], and require further investigations. Indeed, this significant characteristic of the inflamed muscles might explain the tissue destruction by CD8 T cells.

Taken together and by analogy with rheumatoid arthritis development [77], this pleads for the role of a second independent hit as disease propagation factor. Although not proven yet, this could theoretically be either mechanical injury affecting the muscles, vascular disorders (potentially mediated by ARS themselves, Table 1) or infectious diseases, such as unspecific viral infections. Indeed, by amplifying HisRS expression on regenerating myofibers during healing and/or secreting HisRS variants in the extracellular milieu, such hits might allow access to immune machinery. However, in contrast to the muscle breach provoked voluntarily in mice, such a hit has not been identified in humans.

Such events could also take place within other target tissues, such as skin and joints. However, in the absence of dedicated studies, it is not possible to further extrapolate these mechanisms to all ASyS affected organs.

UNDERSTANDING THE SHADOWS TO IMPROVE PATIENTS' CARE

Heterogeneity of antisynthetase syndrome and lack of biomarkers

Although ASyS might be easily distinguished from other inflammatory myopathies, it remains, however, a heterogeneous disease in terms of clinical spectrum, severity and progression of the lesions [3].

As the differential expression of HisRS in blood and tissues has been reported, some firstly suggested the disease spectrum could be linked to the ARS themselves. This, however, requires more precise quantitative and qualitative analyses. Significant associations with single nucleotide polymorphisms affecting ARS could eventually explain variations in clinical features, even if such clear genetic susceptibilities have not been found yet. Distinct HLA–haplotype associations and/or other genetic background regarding patients' ethnicity have been suggested to be correlated with the extent and severity of the disease [78,79].

Quantitative analysis of anti-ARS demonstrated titers variation regarding individual but also technics of quantification [73]. For these reasons, it seems impossible for now to make correlations between antibodies titers and the spectrum or prognosis of ASyS.

Subtypes of anti-ARS have been correlated with disease expression and severity [80]. Qualitative analysis of anti-ARS might be improved noticeably regarding antibody isotype and/or epitopes. Interestingly, autoantibody binding of N-terminal HisRS subfragments could be related to specific clinical features, including joint and vascular involvement [67,81]. However, extended works in large patients' cohorts to answer the question of their clinical significance are needed.

Finally, each step from the causal environmental factor to the autoimmunity might be the occasion of variations, revealing the plasticity of the organisms and the immune system. Despite growing evidence regarding different biomarkers, such as IL-18, KL-6, or α-defensine in predicting inflammatory myopathy phenotype or severity [82,83], data in ASyS are still insufficient yet. Finding and validating an immuno-based, reliable and reproducible prognosis biomarker could allow the early patients' stratification and the development of a personalized medicine of the ASyS patients.

Future treatment's directions

Beyond the knowledge of ASyS pathogenesis, the understanding of all these complex mechanisms is of major importance regarding the development of new immuno-based therapeutic strategies. According to the incriminated pathways, this would encompass treatments targeting IFNα pathways directly or indirectly [84,85], such as anti-IFNα-antibodies and janus kinase (JAK) inhibitors currently under evaluation in different autoimmune diseases; anti-BLyS, which have been validated in systemic lupus erythematosus [86] and Sjögren's syndrome [87] or even other biologics now commonly used in rheumatic diseases, such as anti-CTLA-4-Ig, for which few cases reported some efficiency. Furthermore, development of different therapies blocking TLR-dependent immune cell activation [88] and/or targeting T cells specifically or even NK cells could be investigated in the future.

Apart from these immune-mediated mechanisms leading to target tissue damages – to the best of our knowledge – the repair and healing processes have not been studied in ASyS yet. Regarding interstitial lung disease particularly, mechanisms leading to lung fibrosis have not been the subject of dedicated studies, in contrast to what has been explored in idiopathic pulmonary fibrosis. Depending on the progress in the knowledge of these fibrotic

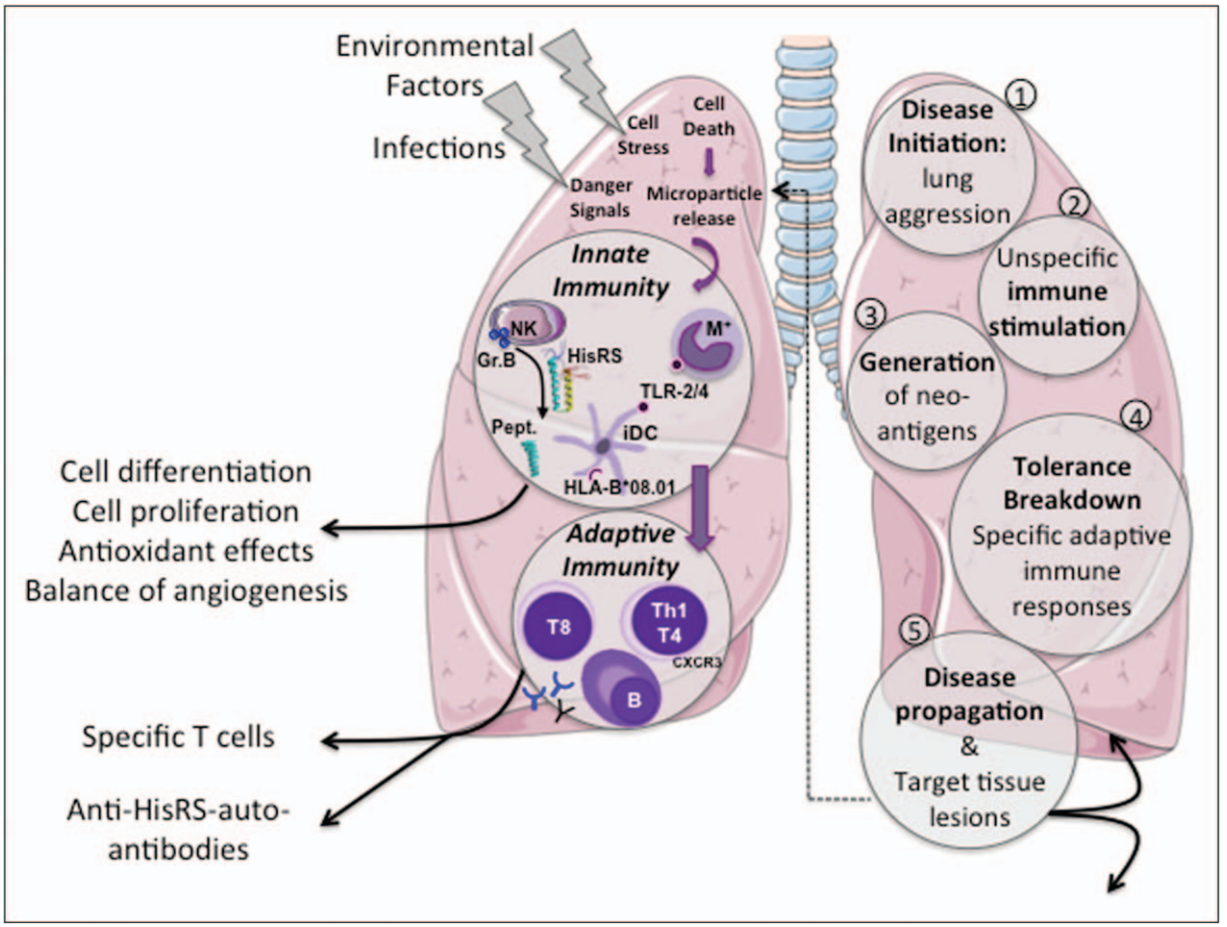

FIGURE 1. Actual hypotheses for ASyS pathogenesis. (1): Disease initiation. ASyS seems to be initiated in the lung, as a consequence of tissue aggression by different environmental factors and infectious agents, a cellular stress occurs. This leads to cell death with microparticle release and danger signal pathway activations. (2): Immune stimulation and (3) generation of neo-antigen. As a result, innate immune cells are unspecifically activated, among which NK cells could split HisRS into an immunogenic peptide. This peptide has many unspecific properties that could be involved in ASyS pathogenesis. In addition, as a first step to tolerance breakdown, the neo-antigen stimulates other immune cells: attracting $CCR5^{+}$ immune cells and activating them, through at least TLR2/4 pathways. (4) Tolerance breakdown. From innate to adaptive immunity, successive events include antigen presentation, efficiently favoured by the HLA-B*08.01 genetic background, CD8 T-cell priming and CD4 helper T-cell–B-cell crosstalk. These phenomena finally lead to autoantibody production, witnessing autoimmunity. (5): Disease propagation. The disease propagation is linked to the circulation of these specific cells, autoantibodies and production of proinflammatory cytokines. In the target tissues, in which the autoantigen HisRS and HLA-I are abnormally expressed, all involved adaptive immune cells cause immune-mediated tissue damages, which in turn may increase cell death and thus further activate the immune system. CXCR3, CXC chemokine receptor 3; Gr. B, granzyme B; HisRS, histidyl-tRNA-synthetase; HLA, human leucocyte antigen; Pept, peptide; TLR, toll-like receptor.

mechanisms at work in the lung of ASyS patients, antifibrotic agents, such as pirfenidone and nintedanib [89], would be of great interest.

Taken together, these pharmaceutical projects aim to improve patients' care, trying to improve morbidity and mortality due to this severe and chronic autoimmune disease.

CONCLUSION

Through the advancement of clinical and immunological knowledge of ASyS, it has been distinguished from others as a particular inflammatory myopathy. Despite numerous in-vivo and in-vitro studies, ASyS pathogenesis remains partially understood, which highlights complexity of the disease process. A global scheme can, however, be drawn (Fig. 1), in light with the previously described data: ASyS starts after pulmonary injury, caused by environmental factors and/or infectious agents; tolerance breakdown might be induced in a permissive context with different genetic and immune susceptibility factors. Strong together, this may lead to spillover effects, finally resulting in global immune system

activation, leading to systemic and characteristic autoimmune-mediated organ lesions. Research goals are now to focus on further understanding in order to identify predictive biomarkers and new target therapies, which fits the future personalized patients' care of this disabling disease.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975; 292:344–347.
2. Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 2016; 280:8–23.
3. Hervier B, Benveniste O. Clinical heterogeneity and outcomes of antisynthetase syndrome. Curr Rheumatol Rep 2013; 15:349.
4. Yoshida S, Akizuki M, Mimori T, *et al.* The precipitating antibody to an acidic nuclear protein antigen, the Jo-1, in connective tissue diseases. A marker for a subset of polymyositis with interstitial pulmonary fibrosis. Arthritis Rheum 1983; 26:604–611.
5. Mescam-Mancini L, Allenbach Y, Hervier B, *et al.* Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis. Brain J Neurol 2015; 138:2485–2492.
6. Aouizerate J, De Antonio M, Bassez G, *et al.* Myofiber HLA-DR expression is a distinctive biomarker for antisynthetase-associated myopathy. Acta Neuropathol Commun 2014; 2:154.
7. Bouneaud C, Kourilsky P, Bousso P. Impact of negative selection on the T cell repertoire reactive to a self-peptide: a large fraction of T cell clones escapes clonal deletion. Immunity 2000; 13:829–840.
8. Avrameas S. Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton.'. Immunol Today 1991; 12:154–159.
9. Theofilopoulos AN, Kono DH, Baccala R. The multiple pathways to autoimmunity. Nat Immunol 2017; 18:716–724.
10. Gutierrez-Arcelus M, Rich SS, Raychaudhuri S. Autoimmune diseases: connecting risk alleles with molecular traits of the immune system. Nat Rev Genet 2016; 17:160–174.
11. Rubtsova K, Marrack P, Rubtsov AV. Sexual dimorphism in autoimmunity. J Clin Invest 2015; 125:2187–2193.
12. Haghikia A, Jörg S, Duscha A, *et al.* Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. Immunity 2015; 43:817–829.
13. Aldrich TK, Vossbrinck M, Zeig-Owens R, *et al.* Lung function trajectories in world trade center-exposed New York City firefighters over 13 years the roles of smoking and smoking cessation. Chest 2016; 149:1419–1427.
14. Webber MP, Moir W, Crowson CS, *et al.* Post-September 11, 2001, incidence of systemic autoimmune diseases in World Trade Center-exposed firefighters and emergency medical service workers. Mayo Clin Proc 2016; 91:23–32.
15. Perricone C, Versini M, Ben-Ami D, *et al.* Smoke and autoimmunity: the fire behind the disease. Autoimmun Rev 2016; 15:354–374.
16. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010; 34:J258–J265.
17. Kearley J, Silver JS, Sanden C, *et al.* Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. Immunity 2015; 42:566–579.
18. Webber MP, Moir W, Zeig-Owens R, *et al.* Nested case-control study of selected systemic autoimmune diseases in World Trade Center rescue/recovery workers. Arthritis Rheumatol 2015; 67:1369–1376.
19. Webber MP, Berman J, Qayyum B, *et al.* Reply: to PMID 25779102. Arthritis Rheumatol 2015; 67:2791.
20. Tillie-Leblond I, Wislez M, Valeyre D, *et al.* Interstitial lung disease and anti-Jo-1 antibodies: difference between acute and gradual onset. Thorax 2008; 63:53–59.
21. Chinoy H, Adimulam S, Marriage F, *et al.* Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. Ann Rheum Dis 2012; 71:961–965.
22. ■■ Svensson J, Holmqvist M, Lundberg IE, Arkema EV. Infections and respiratory tract disease as risk factors for idiopathic inflammatory myopathies: a population-based case-control study. Ann Rheum Dis 2017; 76:1803–1808.

Elegant epidemiological study highlighting the impact of infections on the development of inflammatory myopathies and suggesting that the lungs are involved in the initiation of the disease.

23. Camelo A, Dunmore R, Sleeman MA, Clarke DL. The epithelium in idiopathic pulmonary fibrosis: breaking the barrier. Front Pharmacol 2014; 4:173.
24. Strieter RM. What differentiates normal lung repair and fibrosis? Inflammation, resolution of repair, and fibrosis. Proc Am Thorac Soc 2008; 5:305–310.
25. Sakamoto N, Ishimoto H, Kakugawa T, *et al.* Elevated α-defensin levels in plasma and bronchoalveolar lavage fluid from patients with myositis-associated interstitial lung disease. BMC Pulm Med 2018; 18:44.
26. Zhang S, Shu X, Tian X, *et al.* Enhanced formation and impaired degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential contributor to interstitial lung disease complications. Clin Exp Immunol 2014; 177:134–141.
27. Blechynden LM, Lawson MA, Tabarias H, *et al.* Myositis induced by naked DNA immunization with the gene for histidyl-tRNA synthetase. Hum Gene Ther 1997; 8:1469–1480.
28. Katsumata Y, Ridgway WM, Oriss T, *et al.* Species-specific immune responses generated by histidyl-tRNA synthetase immunization are associated with muscle and lung inflammation. J Autoimmun 2007; 29:174–186.
29. Soejima M, Kang EH, Gu X, *et al.* Role of innate immunity in a murine model of histidyl-transfer RNA synthetase (Jo-1)-mediated myositis. Arthritis Rheum 2011; 63:479–487.
30. Fernandez I, Harlow L, Zang Y, *et al.* Functional redundancy of MyD88-dependent signaling pathways in a murine model of histidyl-transfer RNA synthetase-induced myositis. J Immunol 2013; 191:1865–1872.
31. ■■ Sciorati C, Monno A, Doglio MG, *et al.* Exacerbation of murine experimental autoimmune myositis by toll-like receptor 7/8. Arthritis Rheumatol 2018; 70:1276–1287.

The only murine model available reproducing the human ASyS clearly shows involvement of danger signals (intracellular TLR 7 and 8) and innate immunity in the initial events of the development of the diseases.

32. Sciorati C, Monno A, Ascherman DP, *et al.* Required role of apoptotic myogenic precursors and toll-like receptor stimulation for the establishment of autoimmune myositis in experimental murine models. Arthritis Rheumatol 2015; 67:809–822.
33. Casciola-Rosen L, Andrade F, Ulanet D, *et al.* Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. J Exp Med 1999; 190:815–826.
34. Levine SM, Raben N, Xie D, *et al.* Novel conformation of histidyl-transfer RNA synthetase in the lung: the target tissue in Jo-1 autoantibody-associated myositis. Arthritis Rheum 2007; 56:2729–2739.
35. Hervier B, Perez M, Allenbach Y, *et al.* Involvement of NK cells and NKp30 pathway in antisynthetase syndrome. J Immunol 2016; 197:1621–1630.
36. Wang J, Urbanowicz RA, Tighe PJ, *et al.* Differential activation of killer cells in the circulation and the lung: a study of current smoking status and chronic obstructive pulmonary disease (COPD). PloS One 2013; 8:e58556.
37. Brandt CS, Baratin M, Yi EC, *et al.* The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. J Exp Med 2009; 206:1495–1503.
38. Reiners KS, Topolar D, Henke A, *et al.* Soluble ligands for NK cell receptors promote evasion of chronic lymphocytic leukemia cells from NK cell antitumor activity. Blood 2013; 121:3658–3665.
39. Miller FW, Twitty SA, Biswas T, Plotz PH. Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotype stability, and isotype restriction of anti-Jo-1 autoantibodies. J Clin Invest 1990; 85:468–475.
40. Targoff IN, Reichlin M. Measurement of antibody to Jo-1 by ELISA and comparison to enzyme inhibitory activity. J Immunol 1987; 138:2874–2882.
41. Park MC, Kang T, Jin D, *et al.* Secreted human glycyl-tRNA synthetase implicated in defense against ERK-activated tumorigenesis. Proc Natl Acad Sci U S A 2012; 109:E640–E647.
42. Freist W, Verhey JF, Rühlmann A, *et al.* Histidyl-tRNA synthetase. Biol Chem 1999; 380:623–646.
43. Feng RN, Niu YC, Sun XW, *et al.* Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: a randomised controlled trial. Diabetologia 2013; 56:985–994.
44. Fernandes-Cerqueira C. Extracellular histidyl-tRNA synthetase in myositis. ACR Meeting Abstract. 2016.

45. Jordanova A, Irobi J, Thomas FP, *et al.* Disrupted function and axonal distribution of mutant tyrosyl-tRNA synthetase in dominant intermediate Charcot-Marie-Tooth neuropathy. Nat Genet 2006; 38:197–202.

46. Latour P, Thauvin-Robinet C, Baudelet-Méry C, *et al.* A major determinant for binding and aminoacylation of tRNA(Ala) in cytoplasmic Alanyl-tRNA synthetase is mutated in dominant axonal Charcot-Marie-Tooth disease. Am J Hum Genet 2010; 86:77–82.

47. Park SG, Park HS, Jeong I-K, *et al.* Autoantibodies against aminoacyl-tRNA synthetase: novel diagnostic marker for type 1 diabetes mellitus. Biomark 2010; 15:358–366.

48. Guo M, Schimmel P. Essential nontranslational functions of tRNA synthetases. Nat Chem Biol 2013; 9:145–153.

49. Cuzzocrea S, Genovese T, Failla M, *et al.* Protective effect of orally administered carnosine on bleomycin-induced lung injury. Am J Physiol Lung Cell Mol Physiol 2007; 292:L1095–L1104.

50. Yang DC, Dang CV, Arnett FC. Rat liver histidyl-tRNA synthetase. Purification and inhibition by the myositis-specific anti-Jo-1 autoantibody. Biochem Biophys Res Commun 1984; 120:15–21.

51. Fahoum SK, Yang DC. Purification of mammalian histidyl-tRNA synthetase and its interaction with myositis-specific anti-Jo-1 antibodies. Biochemistry 1987; 26:5871–5877.

52. Targoff IN, Trieu EP, Miller FW. Reaction of anti-OJ autoantibodies with components of the multienzyme complex of aminoacyl-tRNA synthetases in addition to isoleucyl-tRNA synthetase. J Clin Invest 1993; 91:2556–2564.

53. Beaulande M, Tarbouriech N, Härtlein M. Human cytosolic asparaginyl-tRNA synthetase: cDNA sequence, functional expression in Escherichia coli and characterization as human autoantigen. Nucleic Acids Res 1998; 26:521–524.

54. Dang CV, Tan EM, Traugh JA. Myositis autoantibody reactivity and catalytic function of threonyl-tRNA synthetase. FASEB J 1988; 2:2376–2379.

55. Ascherman DP. The role of Jo-1 in the immunopathogenesis of polymyositis: current hypotheses. Curr Rheumatol Rep 2003; 5:425–430.

56. Wakasugi K, Schimmel P. Highly differentiated motifs responsible for two cytokine activities of a split human tRNA synthetase. J Biol Chem 1999; 274:23155–23159.

57. Howard OMZ, Dong HF, Yang D, *et al.* Histidyl-tRNA synthetase and asparaginyl-tRNA synthetase, autoantigens in myositis, activate chemokine receptors on T lymphocytes and immature dendritic cells. J Exp Med 2002; 196:781–791.

58. Lo W-S, Gardiner E, Xu Z, *et al.* Human tRNA synthetase catalytic nulls with diverse functions. Science 2014; 345:328–332.

59. Ascherman DP, Oriss TB, Oddis CV, Wright TM. Critical requirement for professional APCs in eliciting T cell responses to novel fragments of histidyl-tRNA synthetase (Jo-1) in Jo-1 antibody-positive polymyositis. J Immunol 2002; 169:7127–7134.

60. Zhou JJ, Wang F, Xu Z, *et al.* Secreted histidyl-tRNA synthetase splice variants elaborate major epitopes for autoantibodies in inflammatory myositis. J Biol Chem 2014; 289:19269–19275.

61. Rothwell S, Cooper RG, Lundberg IE, *et al.* Dense genotyping of immune-related loci in idiopathic inflammatory myopathies confirms HLA alleles as the strongest genetic risk factor and suggests different genetic background for major clinical subgroups. Ann Rheum Dis 2016; 75:1558–1566.

62. Miller FW, Chen W, O'Hanlon TP, *et al.* Genome-wide association study identifies HLA 8.1 ancestral haplotype alleles as major genetic risk factors for myositis phenotypes. Genes Immun 2015; 16:470–480.

63. Chinoy H, Lamb JA, Ollier WER, Cooper RG. Recent advances in the immunogenetics of idiopathic inflammatory myopathy. Arthritis Res Ther 2011; 13:216.

64. Rothwell S, Lamb JA, Chinoy H. New developments in genetics of myositis. Curr Opin Rheumatol 2016; 28:651–656.

65. Aggarwal R, Bandos A, Reed AM, *et al.* Predictors of clinical improvement in rituximab-treated refractory adult and juvenile dermatomyositis and adult polymyositis. Arthritis Rheumatol 2014; 66:740–749.

66. Miller FW, Waite KA, Biswas T, Plotz PH. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. Proc Natl Acad Sci U S A 1990; 87:9933–9937.

67. LaConti J. Correlations between B cell epitope specificity and clinical features in patients with Jo-1 antibodies and the anti-synthetase syndrome. ACR Meeting Abstract. 2016.

68. Martin A, Shulman MJ, Tsui FW. Epitope studies indicate that histidyl-tRNA synthetase is a stimulating antigen in idiopathic myositis. FASEB J 1995; 9:1226–1233.

69. Stone KB, Oddis CV, Fertig N, *et al.* Anti-Jo-1 antibody levels correlate with disease activity in idiopathic inflammatory myopathy. Arthritis Rheum 2007; 56:3125–3131.

70. Eloranta M-L, Barbasso Helmers S, Ulfgren A-K, *et al.* A possible mechanism for endogenous activation of the type I interferon system in myositis patients with anti-Jo-1 or anti-Ro 52/anti-Ro 60 autoantibodies. Arthritis Rheum 2007; 56:3112–3124.

71. Krystufková O, Vallerskog T, Helmers SB, *et al.* Increased serum levels of B cell activating factor (BAFF) in subsets of patients with idiopathic inflammatory myopathies. Ann Rheum Dis 2009; 68:836–843.

72. Englund P, Wahlström J, Fathi M, *et al.* Restricted T cell receptor BV gene usage in the lungs and muscles of patients with idiopathic inflammatory myopathies. Arthritis Rheum 2007; 56:372–383.

73. Albrecht I. Characterization of histidyl-tRNA synthetase specific Th cell response in blood and broncheoalveolar lavage (BAL) of myositis patients. ACR Meeting Abstract. 2013.

74. Galindo-Feria A. Identification of a novel pro-inflammatory T cell epitope from His-tRNA-synthetase associated with interstitial lung disease in anti-Jo-1 positive patients. ACR Meeting Abstract. [date unknown].

75. Fasth AER, Dastmalchi M, Rahbar A, *et al.* T cell infiltrates in the muscles of patients with dermatomyositis and polymyositis are dominated by CD28null T cells. J Immunol 2009; 183:4792–4799.

76. Casciola-Rosen L, Nagaraju K, Plotz P, *et al.* Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. J Exp Med 2005; 201:591–601.

77. Catrina AI, Svensson CI, Malmström V, *et al.* Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. Nat Rev Rheumatol 2017; 13:79–86.

78. Chinoy H, Salway F, Fertig N, *et al.* In adult onset myositis, the presence of interstitial lung disease and myositis specific/associated antibodies are governed by HLA class II haplotype, rather than by myositis subtype. Arthritis Res Ther 2006; 8:R13.

79. Pinal-Fernandez I, Casal-Dominguez M, Huapaya JA, *et al.* A longitudinal cohort study of the antisynthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology 2017; 56:999–1007.

80. Hervier B, Devilliers H, Stanciu R, *et al.* Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with antitRNA synthetase antibody specificity. Autoimmun Rev 2012; 12:210–217.

81. Chiang K. Characterization of Jo-1 autoantibodies in patients with inflammatory myopathy and interstitial lung disease. ACR Meeting Abstract. 2012.

82. Fathi M, Barbasso Helmers S, Lundberg IE. KL-6: a serological biomarker for interstitial lung disease in patients with polymyositis and dermatomyositis. JIntern Med 2012; 271:589–597.

83. Gono T, Kawaguchi Y, Sugiura T, *et al.* Interleukin-18 is a key mediator in dermatomyositis: potential contribution to development of interstitial lung disease. Rheumatology 2010; 49:1878–1881.

84. Higgs BW, Zhu W, Morehouse C, *et al.* A phase 1b clinical trial evaluating sifalimumab, an anti-IFN-α monoclonal antibody, shows target neutralisation of a type I IFN signature in blood of dermatomyositis and polymyositis patients. Ann Rheum Dis 2014; 73:256–262.

85. Hornung T, Janzen V, Heidgen F-J, *et al.* Remission of recalcitrant dermatomyositis treated with ruxolitinib. N Engl J Med 2014; 371:2537–2538.

86. Navarra SV, Guzmán RM, Gallacher AE, *et al.* Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. Lancet 2011; 377:721–731.

87. Mariette X, Seror R, Quartuccio L, *et al.* Efficacy and safety of belimumab in primary Sjögren's syndrome: results of the BELISS open-label phase II study. Ann Rheum Dis 2015; 74:526–531.

88. Gao W, Xiong Y, Li Q, Yang H. Inhibition of toll-like receptor signaling as a promising therapy for inflammatory diseases: a journey from molecular to nano therapeutics. Front Physiol 2017; 8:508.

89. Raghu G. Idiopathic pulmonary fibrosis: lessons from clinical trials over the past 25 years. Eur Respir J 2017; 50:1–11.