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 Editor in chief and Section Editors for this issue.

EDITOR IN CHIEF

John Varga

Dr Varga was born in Budapest, Hungary, and came to the United States at the age of fifteen as a refugee. He matriculated at Columbia University in New York City, and obtained his medical degree from New York University. Following Rheumatology fellowship in Boston, he pursued research training with Professor Sergio Jimenez at the University of Pennsylvania. He served on the faculty at Jefferson Medical College, the University of Illinois and Feinberg School of Medicine. In 2020 he was recruited as Chief of Rheumatology at the University of Michigan Medical School. Dr Varga's research focuses on the biology and treatment of scleroderma and fibrosis, and bridges clinical and laboratory-based investigation.

Dr Varga has mentored over 20 trainees, several of whom are now independent academic investigators. He is the author of more than 400 original articles and book chapters and four books. His research has been continuously funded by the National Institutes of Health. He is an elected member of the Association of American Physicians, AOA and the Henry Kunkel Society, and is Master of the American College of Rheumatology and member of its Board of Directors.

SECTION EDITORS

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Hasan Yazici, MD, is a retired professor of medicine and the founder of the Division of Rheumatology at Cerrahpaşa Medical Faculty of University of Istanbul - Cerrahpaşa. He currently practices rheumatology at Academic Hospital in Istanbul. His main research interests are Behçet syndrome, clinical research methodology and ethics.

Prof. Yazici has published widely on Behçet syndrome. The dedicated Behçet syndrome multidisciplinary outpatient clinic he has started with a group of colleagues 46 years ago continues to give patient care and conduct clinical research. He has published many original articles in peer reviewed journals (the most quoted author on Behçet syndrome / Web of Science) in addition to his text book contributions, editorials and reviews. He has recieved a number of prestigious awards and has a long list of memberships in the editorial boards and scientific societies which includes being a member of the Science Academy (Turkey) and Academia Europaea; a Master of the American College of Rheumatology and a recipient of the EULAR award for Meritorious Service in Rheumatology.

Yusuf Yazici

Yusuf Yazici, MD, is a Clinical Associate Professor of Medicine at the New York University School of Medicine.

Dr Yazici earned his medical degree from Cerrahpasa Medical Faculty of Istanbul University in Istanbul, Turkey. He completed his internship and residency at Creighton University in Nebraska, USA and his fellowship in rheumatology at the Hospital for Special Surgery of Weill Medical College of Cornell University, USA.

His areas of interest are rheumatoid arthritis, early arthritis, patient reported outcomes, database and registry management and monitoring of arthritis patients in regard to clinical response and adverse events related to treatment and Behcet's syndrome. He has published over 200 articles and presented at various national and international meetings over 100 times.

Dr Yazici divides his time between seeing patients and conducting both industry and investigator-initiated trials, in the areas of RA and Behcet's syndrome.

Mirko Manetti

Dr Manetti, PhD was born in Florence, Italy, and is Associate Professor of Human Anatomy at the Department of Experimental and Clinical Medicine, University of Florence. He received the single-cycle master's degree in Biological Sciences with full marks and honors in 2003, and the PhD degree in Human Morphology and Morphogenesis in 2007 at the University of Florence. In 2006-2007 he was Visiting Scientist at the Department of Internal Medicine of the Justus-Liebig-University of Giessen, Germany. He was Postdoctoral Research Fellow from 2007 to 2017 and Assistant Professor from 2017 to 2021 at the University of Florence, where he currently teaches Human Anatomy to undergraduate students as Associate Professor. In 2018 he obtained the National Scientific Habilitation for Full Professor of Human Anatomy from the Italian Ministry of Education, University and Research. Since 2023 he is member of the Scientific Strategic Committee of the Italian World Scleroderma Foundation.

Dr Manetti is author of numerous scientific publications in peer reviewed journals and proceedings of national and international congresses, and currently serves as reviewer and editorial board member for numerous peer reviewed journals. His main research interests are microscopic anatomy of human organs, morphological and functional aspects of stromal cells, endothelial cell biology and angiogenesis, cellular and molecular mechanisms of tissue fibrosis, and pathogenesis of autoimmune, chronic inflammatory and connective tissue diseases such as scleroderma.

Introduction, vasculitis 2025

Hasan Yazici[a] *and Yusuf Yazici*[b]

The juvenile Behçet syndrome (jBS) manuscript by Yildiz *et al.* opens with the popular classification/diagnosis of vasculitis issue. Many clinicians and scientists are busy in this line of work and the main driver of this enthusiasm, we are afraid, has much to do with our perception of what *others* expect from us [1]. In this line, the authors refer to the 2016 PEDBED (pediatric Behçet disease) criteria which apparently got the blessing of an international group of experts by being the set of criteria with the highest specificity [2] as compared to the 1990 ISG and 2007 ICBD criteria sets [3,4]. The authors also rightfully state that specificity is almost always more useful than sensitivity in correctly diagnosing rare conditions like Behçet syndrome (BS). True, true and related. However, we find the specificity of 100%, as proposed by the authors of the 2023 paper, too optimistic. Perhaps the reason for this extraordinary specificity was simply the fact that all the patients in the comparator group were selected from Eurofever registry, whereas fever is a quite uncommon presenting feature of BS and that only in certain forms of disease expression are associated with some fever [5]. Another issue we seriously consider muddles our correctly recognizing jBS is the attempt to include patients with HAP20 syndrome into jBS clusters or trying to establish biologic ties with PFAPA and BS as discussed by Yildiz *et al.*

In a very informative manuscript, Ozguler and Nowatzky update us on the latest omics studies, in BS. This comprehensive review covers the highlights of practically the bulk of basic research in this condition. We find the observation of genetic differences between the males – the gender usually with higher disease load- and females particularly interesting [6]. To us, the observation of similar expression of chemotaxis-related genes in systemic lupus erythematosus and ANCA associated vasculitis patients being similarly overexpressed alongside the BS patients as compared to healthy controls was also particularly of note. Heightened neutrophil activity has played a central role in the discussions of the hitherto unclarified disease expression in BS and the study by Yu *et al.* [7] once more underlines the necessity of almost always including diseased controls in our work as also emphasized by our reviewers.

VEXAS (vacuoles, enzyme, X-linked, autoinflammatory, somatic) is a newly defined entity. It is proposed that somatic mutations in the ubiquitin gene, present on the X chromosome, and with the main biological function of controlling inflammation, cause heightened pathologic inflammation in many organs, as well as aberrations in the hemopoietic precursor cells. This new entity is curiously associated with a list of well recognized and diverse range of clinical conditions like myelodysplastic syndrome, relapsing polychondritis or Sweet syndrome. Some investigators propose that VEXAS is a new monogenic disease caused by a set of well defined mutations. We propose sticking to the terminology of a syndrome at least for the time being and suggest that the indeed interesting available evidence, reported rather comprehensively in the Kötter and Krusche paper thus far indicate, mainly, important associations. Our contention is primarily backed up by the fact that thus far there are no conclusive data at hand to advise us on the natural history of healthy people who carry the said mutations. Meanwhile [8], we also agree that with the VEXAS syndrome will accentuate the taxonomic debate in what is *disease* in many rheumatologic conditions [9].

The imaging paper by Troum *et al.* expertly takes us through the maze of selecting the most fitting imaging modality we should use to best visualize the diseased vessel wall in a patient with vasculitis. To this end, the authors frequently and rightfully refer to the 2023 EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice [10]. Here, we are reminded of the *simple but important truths* like 'computed tomography angiography (CTA) only allows for assessment of GCA but no other forms of large vessel vasculitis. For Takayasu arteritis, MRI is the preferred imaging modality with FDG-PET, CT or US being alternatives'. Before going to the next paper in this year's collection we also want to make a rather pessimistic note about the imaging paper. Going over this excellent review we are again reminded of the paucity of work in more

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detailed, beyond the US, visualization of the venous wall, apparently still the step-child of vasculitis imaging.

Man Ger Sun and Pope most successfully highlight what we currently know and do not know about giant cell arteritis (GCA) and polymyalgia rheumatica (PR). As practicing rheumatologists our foremost take away messages were that (i) in the light of very recent work imaging of the temporal arteries, preferably along with the axillary arteries, commonly obviates the need for a biopsy in a patient with a typical clinical presentation of PR; (ii) more aggressive treatment of PR at the disease onset is associated with less treatment failures at long term. A further, and we find rather freshening note about this manuscript, is that the authors are generous enough to share a table with us of what they consider are the potential research questions for optimal treatment in GCA and PR. We are tempted to propose that such discussion of potential research questions should be an integral part of all review articles, but this might be too optimistic.

The urticarial vasculitis manuscript by Ergun includes a very useful figure on the differential diagnosis of urticarial vasculitis while in the text the author emphasizes that dermoscopy, a noninvasive tool, is very useful to differentiate this entity from chronic spontaneous urticaria, obviating the need for histopathological examination. We find it curious that no formal studies of this potentially very useful diagnostic tool are available after its initial proposal in 2020 [11]. In this line, we also find it interesting that urticarial vasculitis, a condition that both rheumatologists and dermatologists come across not infrequently in their practice apparently has not recently found much interest among the clinicians and scientists in either discipline. This we deduct by the paucity of highlighted references, the date of publication within 18 months of manuscript submission, in this work.

We like to think that the substance matter of three of the six updates we attempted to comment on in this year's introduction represent one main line of scientific consideration in our attempts to understand/classify/diagnose and manage vasculitides, the personal or precision approach, while that of the remaining three represent the evidence-based approach. Maybe the way to go forward should be to combine precision with evidence.

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There are no conflicts of interest.

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Juvenile Behçet syndrome: a contemporary view and differential diagnosis in pediatric practice

Mehmet Yildiz[a], *Oya Koker*[b] *and Ozgur Kasapcopur*[a]

Purpose of review
This review aims to provide a comprehensive and contemporary overview of juvenile Behçet syndrome (jBS), highlighting its clinical manifestations, diagnostic challenges, and treatment strategies.

Recent findings
Behçet syndrome, with its intricate etiopathogenesis and diverse clinical phenotypes, is more aptly classified as a syndrome than a single disease. Its heterogeneous nature requires a broad diagnostic approach and sophisticated differential diagnosis capabilities. The relatively rare occurrence of Behçet syndrome, combined with incomplete clinical presentations and overlapping differential diagnoses, presents significant diagnostic challenges, particularly in pediatric cases. Nevertheless, substantial progress has been made in treatment, especially in managing inflammatory components and preventing complications. Juvenile patients, given their developmental stage, require distinct therapeutic strategies compared to adults, with careful consideration of treatment side effects on growth and psychosocial development.

Summary
To ensure early identification of jBS, it is imperative to refine and develop diagnostic criteria specifically tailored to pediatric populations. With a deeper understanding of the disease mechanisms, treatment protocols should be designed to address the developmental, psychosocial, and individual needs of patients, aiming to minimize long-term side effects. Additionally, comprehensive studies considering age, sex, and ethnic differences are necessary to fill gaps in the literature and resolve existing inconsistencies.

Keywords
Behçet's disease, children, juvenile Behçet syndrome, pediatric rheumatology, vasculitis

INTRODUCTION

Behçet syndrome, with its complex and incompletely elucidated etiopathogenesis and diverse clinical phenotypes, deserves a comprehensive diagnostic perspective rather than confinement to a singular framework. The mucocutaneous manifestations and ocular involvement, which are primary components of the disease's initial definition, still play a crucial role as 'strong elements' in differentiating Behçet syndrome [1,2]. Additionally, gastrointestinal, neurological, and vascular manifestations, which exhibit varying distribution and severity, are integral components of the disease's clinical spectrum characterized by flares and remissions. The varying distribution and intersection of these symptoms have revealed distinct clusters that reflect the heterogeneous nature of Behçet syndrome. This variability cannot be explained solely by demographic influences or a single immunogenetic mechanism [3,4]. The involvement of blood vessels of various sizes and types has classified the syndrome as a variable vessel vasculitis according to the revised 2012 International Chapel Hill Consensus Conference (CHCC) terminology [5]. Over time, heightened awareness and the identification of both innate and adaptive immune system features in its pathogenesis have placed it at the crossroads of autoimmune diseases and autoinflammatory syndromes [6].

Behçet syndrome typically manifests during young adulthood, between the second and fourth decades of life. Some symptoms of the syndrome may present in childhood and continue into

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KEY POINTS

- Behçet syndrome, with its complex and varied clinical phenotypes influenced by genetic and environmental factors, cannot be confined to a single diagnostic framework. The lack of specific laboratory markers and the diverse presentation of symptoms complicate the development of standardized diagnostic criteria, making early and accurate diagnosis particularly challenging in pediatric cases.
- Although the exact pathogenesis of Behçet syndrome remains unclear, it is thought to involve multiple causal pathways. This complex process is influenced by the interplay of infectious agents and microbiota, genetic and epigenetic factors, immune system dysfunction, and other environmental influences.
- With a more profound understanding of the disease mechanisms, treatment protocols should be tailored to meet the developmental, psychosocial, and individual needs of patients, with a focus on minimizing long-term side effects. Extensive studies that account for age, sex, and ethnic variations are essential to address gaps in the literature and clarify existing inconsistencies.

adulthood, completing the clinical picture over time. Conversely, in some patients, the complete spectrum of symptoms manifests in childhood. This review will discuss such patients under the heading of juvenile Behçet syndrome (jBS), addressing current aspects and insights.

DIAGNOSTIC/CLASSIFICATION CRITERIA

The existence of different clinical phenotypes and the lack of specific laboratory markers have complicated the development of standardized diagnostic criteria and their universal acceptance for the disease. Furthermore, the syndrome's relatively rare occurrence, the long period between its initial signs and the full development of its phenotype, incomplete presentations, and the broad differential diagnoses that can mimic similar symptoms all pose additional challenges in the diagnostic approach of childhood cases [7–10].

For many years, there have been no specific criteria for jBS, and the criteria proposed for adults have remained a topic of debate. The most widely accepted International Study Group (ISG) criteria [11] have demonstrated low performance as they require oral ulcers and include the pathergy test, thus failing to cover all patients [9,12▪]. Moreover, the lack of inclusion of neurological, vascular, and gastrointestinal symptoms in these criteria has raised concerns about the risk of missing diagnoses in patients who present with major organ involvement [13]. Moreover, a major criticism has been the neglect of symptom distribution, with patients being selected from a homogeneous region and the control group not being appropriately chosen for differential diagnosis [2,6,14].

Although the international criteria for Behçet's Disease (ICBD) criteria [15], which include vascular and neurological involvement, offer a higher sensitivity with a scoring system addressing existing limitations, they have been shown to be less specific diagnostically [12▪]. Eventually, in 2015, the pediatric Behçet Disease (PEDBD) criteria were introduced by an international panel of experts, addressing the unique characteristics of the juvenile population and the shortcomings of existing criteria for this age group [16]. When all these criteria are comparatively evaluated in children, they generally show the following pattern: ICBD > PEDBD > ISG in terms of sensitivity, and ISG > ICBD > PEDBD in terms of specificity [17–23]. It is believed that the emphasis placed on oral ulcers is a key factor in these differences. A recent international assessment confirmed that the PEDBD criteria are highly specific, whereas the ICBD criteria are more sensitive [12▪]. The effectiveness of the PEDBD criteria in guiding the diagnosis of jBS exemplifies how, particularly in rare conditions, specificity can be more useful than sensitivity in ensuring diagnostic accuracy.

Another issue is the conceptual confusion regarding whether the criteria should be labeled as diagnostic or classification. Classification criteria need to be highly homogeneous and specific, providing clear guidance for research. Diagnostic criteria, on the other hand, are designed to identify a condition based on a range of symptoms and signs that, while suggestive, are not exclusive to the disease. Given the nature of Behçet syndrome, categorization is challenging due to significant individual variations. Moreover, the absence of a pathognomonic criterion and the fact that findings strongly suggesting the disease are not very specific complicate the clear distinction between these definitions [24,25] Ultimately, diagnosing the disease often relies on expert opinion.

EPIDEMIOLOGY

Behçet syndrome presents notable geographical variations in its prevalence, with higher prevalence particularly among communities living along the historic Silk Road. Global pooled prevalence of Behçet syndrome in adults is estimated as 10.3 per 100 000 people [95% confidence interval (95% CI) 6.1–17.7] [26]. Data regarding the prevalence and incidence of jBS are extremely limited. In a prospective study conducted over a period of 2 years in the

United Kingdom and Ireland, the prevalence of Behçet syndrome among children under the age of 16 were reported to be 0.42 per 100 000 children (95% CI: 0.32–0.54) [27].

Behçet syndrome typically manifests in adulthood, often between the ages of 15 and 45, although it can more rarely present during childhood [14,28]. Zouboulis *et al.* [29] reported that the rate of juvenile disease in their Behçet syndrome cohort was 6.9%. According to the published jBS cohorts, the mean disease onset varies between 4.87 and 12.29 years and no clear gender predilection has been noticed [20,30–33]. Although there are studies reporting conflicting results, many studies suggest that the frequency and severity of clinical manifestations in jBS may differ between the sexes (Fig. 1). Published data suggest that there are higher incidences of familial cases of jBS compared to adult cases, indicating a potentially stronger genetic predisposition [34,35].

ETIOPATHOGENESIS

Although the etiopathogenesis of Behçet syndrome is not yet fully understood, it is thought to occur in genetically predisposed individuals with the stimulation of various trigger factors. The disease exhibits overlapping clinical and laboratory features with both autoimmune and autoinflammatory diseases [6,36]. Some authors suggest Behçet syndrome should be classified within the MHC-I-opathy group due to shared characteristics with seronegative spondyloarthropathies, such as the epistatic interaction between ERAP-1 and HLA, specific ERAP-1 polymorphisms, and activation of the IL23/IL17 pathway [37].

Interleukin-10 (IL-10), an anti-inflammatory cytokine primarily secreted by Th2 and regulatory T cells, plays a role in suppressing Th1-associated immune responses and reducing the antigen-presenting capacity of macrophages [38]. Numerous studies have investigated the relationship between

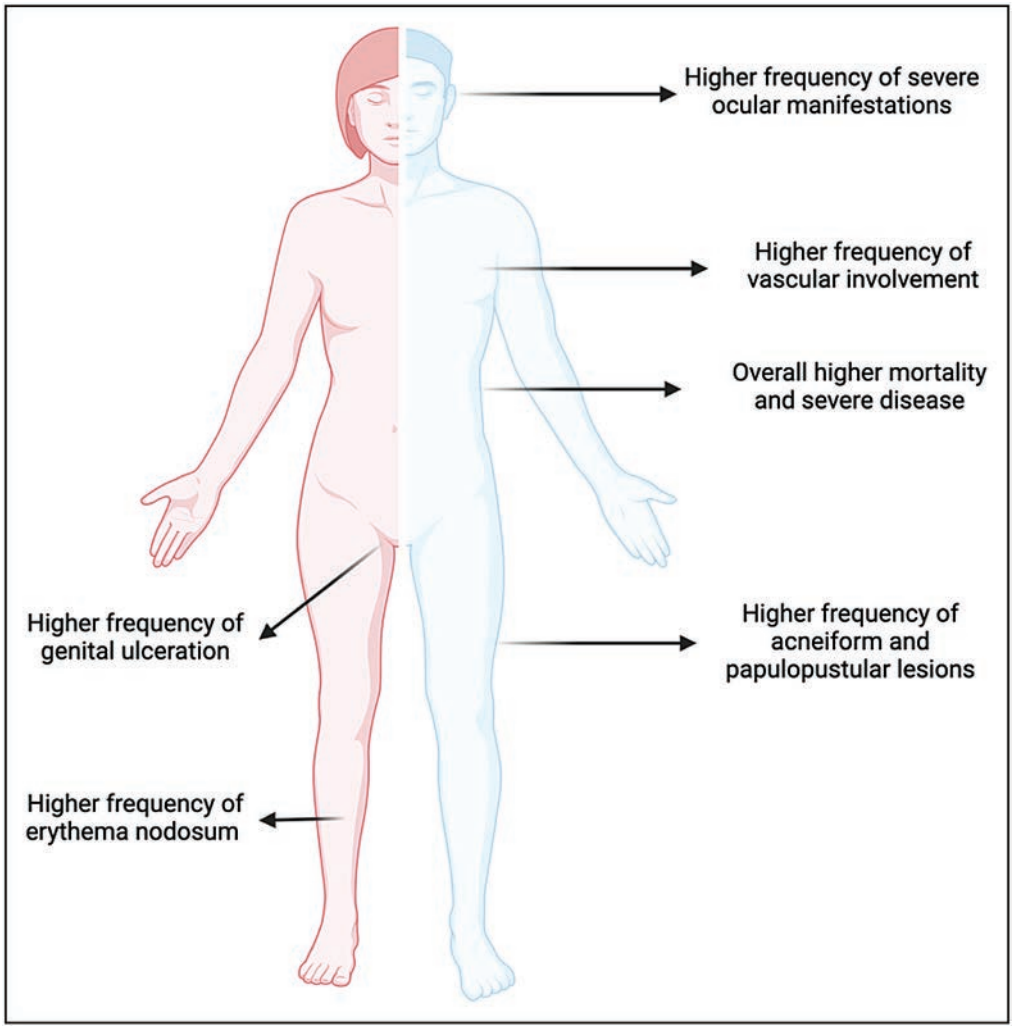

FIGURE 1. Main differences in clinical findings of juvenile Behçet syndrome according to the sex.

IL-10 levels, IL-10 gene polymorphisms, and Behçet syndrome in adult patients. In a study by Ben Ahmed *et al.* [39], a significant increase in the expression of Th1-associated cytokines was detected in biopsy samples from active mucocutaneous lesions of patients diagnosed with Behçet syndrome. The same study reported an approximately 75-fold increase in IL-10 expression, one of the Th2-associated cytokines, but noted that IL-4 and IL-13 were undetectable in most biopsy samples. In a meta-analysis conducted by Li *et al.* [40], which included 5971 Behçet syndrome patients and 8913 controls from a total of 15 studies, a significant association was reported between IL-10 -819T>C polymorphisms and the risk of Behçet syndrome. In addition, IL-10 -592 A>C polymorphism, along with the ACC haplotype, demonstrated a significant protective association with reduced susceptibility to Behçet syndrome. Studies investigating the association between IL-10 gene polymorphisms and jBS are limited and further studies are needed.

Research on both juvenile and adult populations identifies symptom clusters like acne, arthritis, enthesitis, or a vasculitic cluster [4,41]. The significant clinical variability among disease subtypes indicates multiple pathways in its pathogenesis.

Environmental factors, especially infectious triggers, have been linked to the disease. Rare serotypes of *Streptococcus sanguis* cross-react with 65 kD heat shock protein (HSP), similar to human mitochondrial HSP. Behçet syndrome patients have higher antibody levels against *S. sanguinis* and *S. pyogenes* than controls, supporting the infectious trigger hypothesis [42,43]. Furthermore, patients with Behçet syndrome exhibit notable gut microbiota dysbiosis and a significant reduction in butyrate production, which is essential for promoting regulatory T cell differentiation [44].

The distribution of Behçet syndrome along the Silk Road and its prevalence among regional populations indicate a genetic component to the disease. The most significant genetic link to Behçet syndrome is the class I MHC molecule B5, especially subtype B51 [45]. A meta-analysis revealed that having this allele increases Behçet syndrome risk by 5.78 times. Other MHC class I alleles like A24, B15, B27, B56, B56, and Cw1602 also increase Behçet syndrome risk [46–49]. Apart from HLA genes, GWAS studies have identified other genetic predisposition genes such as CCR1-CCR3, STAT4, KLRK1-KLRC4, and ERAP1 [50,51]. Variants in innate immunity-related genes like TLR4 and MEFV are also linked to Behçet syndrome [50,52].

Behçet syndrome is characterized by the prominent activation of neutrophils, which can be clinically demonstrated by the pathergy phenomenon [9,46]. Activated neutrophils contribute to tissue damage and thrombo-inflammation through mechanisms like increased phagocytosis, superoxide production, and NET (neutrophil extracellular trap) formation [48,53–55]. NETs are particularly significant in Behçet syndrome's vascular symptoms and can promote thrombosis in inflammatory conditions [56]. Beyond neutrophils, immune cells such as natural killer (NK) cells and T cell subtypes (Th1, Th17, Tregs) are crucial in Behçet syndrome's immunopathology [57]. NK cells modulate other immune cells and control inflammatory cytokine production, while Th1 and Th17 cells are linked to disease activity through elevated cytokines like IL-17, IL-23, and IFN-γ [46]. Reduced NK cell numbers have been observed in Behçet syndrome patients, and disease activity negatively impacts peripheral blood NK cells [58].

CLINICAL MANIFESTATIONS

Behçet syndrome often begins insidiously, exhibiting a relapsing and remitting course in the process. Recurrent oral ulcers are the most common symptom, but it is difficult to establish specific rates or rankings for other symptoms. The clinical presentation reflects the disease's heterogeneity, influenced by demographic factors, environmental conditions, and genetic background. Although flare-ups may be self-limiting, ocular symptoms can progress to visual loss, and vascular involvement is both highly specific for diagnosis and a significant determinant of morbidity [6]. Current data support that male patients exhibit more severe symptoms across all age groups [12▪,20,59–63], although some argue that sex has a minimal impact on the clinical expression of jBS [30,33]. Some of the cardinal clinical findings from jBS are shown in Fig. 2.

Mucocutaneous lesions

Oral ulcers are both a prominent and primary finding in both children and adults. Although they are not deemed essential in the PEDBD criteria [16], they are a strong indicator suggesting the diagnosis, and their absence may necessitate reconsideration of differential diagnoses [64]. Despite the morphological challenge in distinguishing them, their persistence, recurrence, and painful nature are notable [6,65]. Recent studies have emphasized the role of environmental factors and irregular brushing habits as triggers [66,67]. Genital and perianal ulcers were identified as distinguishing features in children [12▪]. Compared to oral ulcers, they are deeper with irregular borders and may leave a scar during the healing phase. Unlike oral ulcers, they may disappear in the later stages of the disease [68].

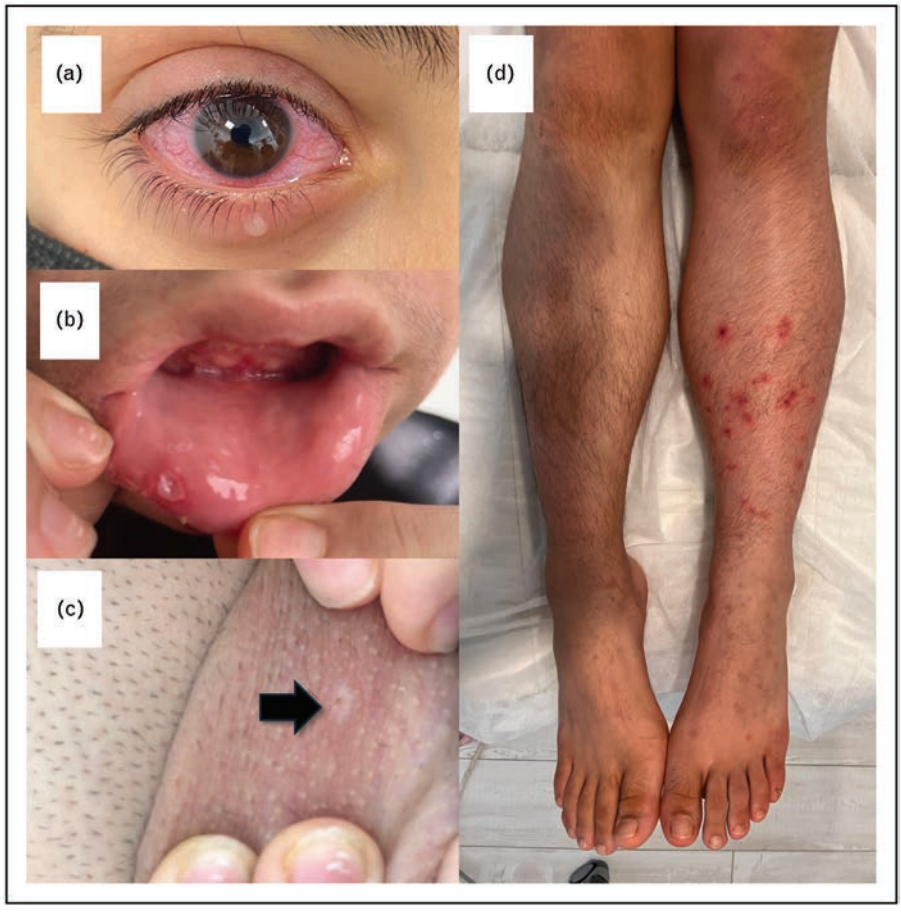

FIGURE 2. (a) Acute uveitis. (b) Oral aphthous lesion. (c) Genital scarring due to genital ulceration. (d) Circumference and color difference between lower extremities due to deep vein thrombosis in a patient with juvenile Behçet syndrome.

Skin manifestations of jBS vary by country but can occur in up to 90% of cases [69,70]. Particularly, the differential diagnosis of papulopustular and erythematous nodular lesions in the juvenile period requires meticulous attention. Unlike adolescent acne, papulopustular or acneiform lesions in jBS are characterized by their atypical locations and widespread distribution [65,71]. Erythema nodosum can appear during childhood, especially in the course of bacterial infections. Its occurrence in atypical locations and recurrent nature can be indicative. In a cross-sectional study, women have exhibited more oral and genital ulcers, while men have been more prone to skin lesions [30,63,72].

Ocular involvement

Recent data on ocular involvement in children with Behçet syndrome indicate similar rates to those in adults, at around 43–45% [69,73,74]. A recent algorithm has been proposed for diagnosing Behçet uveitis in adults using classification and regression tree analysis, highlighting characteristic findings such as the presence of superficial retinal infiltrate, signs of occlusive retinal vasculitis, and diffuse retinal capillary leakage on fluorescein angiography [75]. In juvenile cohorts, ocular involvement manifests as uveitis and retinal vasculitis [9,74,76]. Studies involving different ethnic groups have reported that anterior uveitis is more common in children under ten years old, posterior uveitis is associated with a later onset age and male gender, and panuveitis tends to emerge during adolescence [16,76,77]. Ocular complications such as macular edema, cataracts, and/or posterior synechiae are associated with permanent damage and are major causes of morbidity [78]. Frequent relapses and prolonged corticosteroid treatment are linked to a higher incidence of complications, regardless of early age at disease onset [76].

Vascular involvement

Thrombosis is estimated to occur in 1.8–21% of jBS patients. An international registry found that thrombosis most commonly affects venous and

cerebral sinus locations. Notably, 20% of patients experienced thrombosis at disease onset, and among those who developed it later, fever and pustulosis were the most frequent associated symptoms [79].

Preliminary studies have shown a significant increase in venous wall thickness in the lower extremities of Behçet syndrome patients [80–83], a finding supported in juvenile cases as well. Measuring common femoral vein thickness has been suggested as a diagnostic test due to its high sensitivity and specificity (>80%) [84]. However, while the results presented in adult studies are conflicting, there is also a lack of sufficient research in children to reach a definitive conclusion. Additionally, research by Demir *et al.* [85] indicates that jBS patients with vascular involvement often have more cardiovascular risk factors and should be assessed for cardiovascular comorbidities regardless of other symptoms.

Neurologic involvement

Neurological involvement ranges from 3.6 to 59.6%, often appearing after puberty [86▪,87]. Headache is a common early symptom, seen in 25–40% of cases, but is not a standalone diagnostic criterion [86▪,88]. In children, nonparenchymal involvement, such as cerebral venous sinus thrombosis, is more common, while adults typically have parenchymal lesions affecting the brainstem, basal ganglia, and diencephalon [88,89]. Differentiating neuro-Behçet syndrome from its mimics requires MRI to assess the location and extent of lesions, along with evaluating other systemic signs to complete the diagnostic picture.

Gastrointestinal involvement

Gastrointestinal involvement is commonly reported, particularly in Japan and Korea, where it tends to be more severe [16,33,57]. Symptoms can include diarrhea, abdominal pain, and widespread ulceration throughout the digestive tract. The similarity in both the pattern of involvement and other symptoms makes it challenging to distinguish Behçet syndrome from Crohn's disease across all age groups.

Musculoskeletal involvement

Musculoskeletal system symptoms have been reported as arthralgia, arthritis of mono- or oligoarticular nature, or axial involvement, and myalgia [90]. The limitations of the results are due to the inclusion of only those patients who presented with symptoms at the time of disease presentation and the insufficient number of patients. Additionally, it has been noted that these findings are often associated with recurrent ulcers and pseudofolliculitis. Furthermore, a recent cluster analysis indicated that arthritis represents a specific cluster in jBS, with papulopustular lesions present in 37.1% of cases and enthesitis in 62.9% of cases [41]. These findings support the cluster definition showing the association of acne-arthritis-enthesitis in adult patients.

AGE-RELATED VARIATIONS IN CLINICAL FEATURES

Familial aggregation and geographical distribution serve as evidence for genetic influences in pathogenesis [91]. It has been shown that familial aggregation is significantly higher in pediatric patients compared to adults [35,92▪▪,93]. However, further comparative studies are needed to provide a definitive ratio.

Major organ involvement, such as ocular, vascular, and neurological issues, is often influenced by the age of onset, with these conditions frequently observed before the age of 25 [59,61].

Recent comparative studies indicate that peripheral and axial arthritis, folliculitis, and nonspecific gastrointestinal symptoms such as abdominal pain and diarrhea are more frequent in the juvenile cohort. In contrast, neurological and vascular events are more common in adults. Consequently, this suggests a relatively better prognosis for the juvenile group [62,94,95]. In parallel, the juvenile cohort has been found to have lower disease activity and severity scores [96]. Reviewing various epidemiological studies, the inconsistencies in results likely stem from differences in the scope of representative data, age range, disease duration, and methodological approaches. Moreover, outcome data from studies encompassing a diverse range of ethnic backgrounds and age groups would be particularly valuable. Main differences in clinical findings between juvenile and adult-onset Behçet syndrome are outlined in Fig. 3.

TREATMENT OF JUVENILE BEHÇET SYNDROME

Treatment aims to rapidly suppress and prevent flares while avoiding irreversible organ damage through lifestyle recommendations, balanced diet, physical activity, and appropriate vaccination advice [97–99].

Mucocutaneous involvement

Providing oral hygiene education and encouragement to patients is crucial for oral lesions [99]. The use of chlorhexidine-containing antiseptic mouthwashes/gels may be considered in selected cases [99].

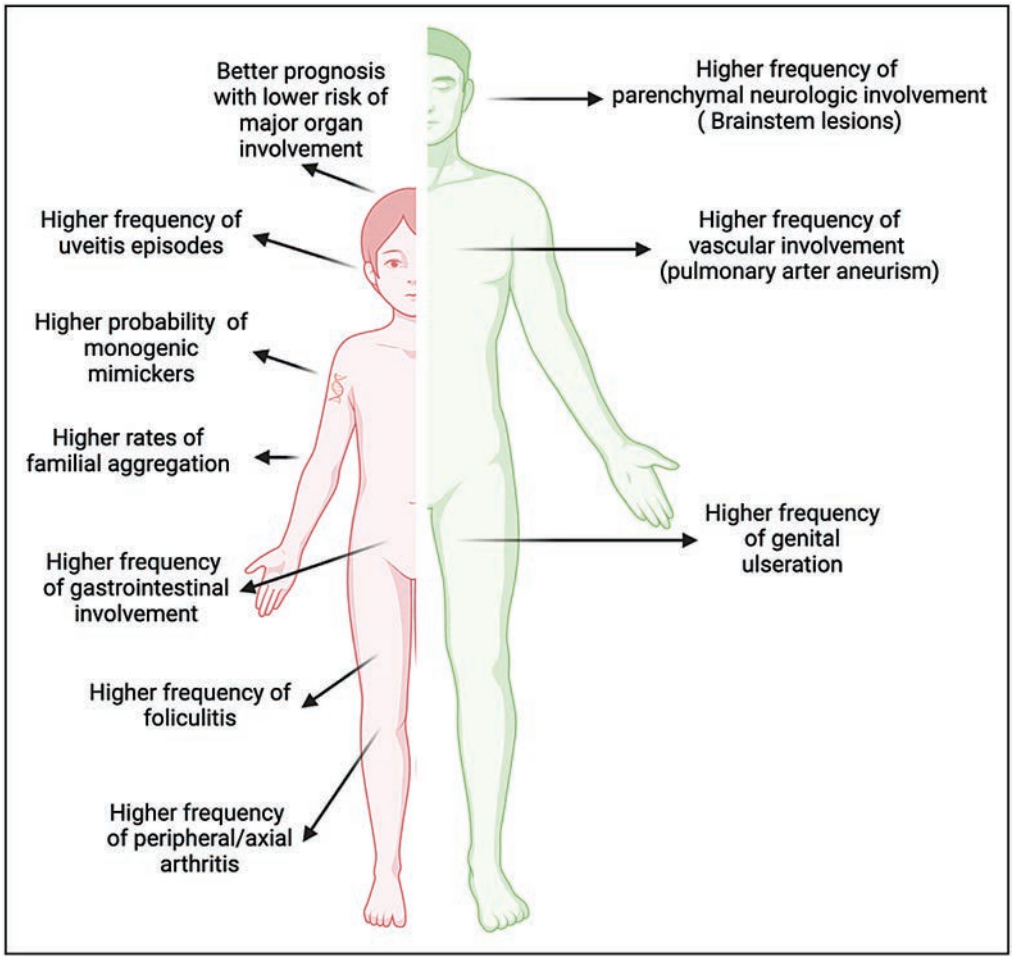

FIGURE 3. Main differences in clinical findings between juvenile and adult-onset Behçet syndrome.

Lidocaine containing sprays/gels are effective for pain management. Topical preparations like hyaluronic acid and sucralfate shorten healing time ulcerative lesions [99–101]. Moreover, topically applied nonsteroidal anti-inflammatory drugs and steroids are also recommended [99].

Colchicine is the primary systemic therapy for treating and preventing mucocutaneous lesions in Behçet syndrome, despite its debated efficacy for oral ulcers and reduced effectiveness for papulopustular lesions [97–99]. Topical or systemic steroids can manage flares, while azathioprine, thalidomide, dapsone, interferon alpha, anti-TNF agents, or apremilast are alternatives for resistant cases [97–99].

The oral phosphodiesterase 4 inhibitor apremilast is effective for oral ulcers in adult Behçet syndrome and is recommended by EULAR for mucocutaneous involvement [97,102]. Apremilast also treats genital ulcers, skin lesions, and arthritis effectively, with the advantages of oral administration, a favorable safety profile, and compatibility with other disease-modifying agents [99]. Research on apremilast's efficacy in pediatric cases is ongoing.

Eye involvement

For uncomplicated anterior uveitis, steroid eye drops are recommended [97,99]. Mydriatic and cycloplegic agents are also used to relieve pain and prevent adhesions [98,99]. Systemic treatment is required for posterior uveitis, panuveitis, and relapsed/refractory cases, including hypopyon uveitis, which commonly necessitates systemic steroids [98]. However, using systemic steroids alone for posterior uveitis treatment is not recommended [97–99].

Azathioprine, cyclosporin-A, interferon-alpha, and anti-TNF agents are systemic treatments for uveitis in Behçet syndrome [97–99]. Azathioprine and cyclosporin-A prevent relapses and improve visual acuity [98,99,103]. For severe or unresponsive uveitis, decreased visual acuity, macular edema, and/or retinal vasculitis, interferon-alpha or anti-TNF drugs are recommended [97–99]. The choice between interferon-alpha and anti-TNF agents depends on patient characteristics [97]. Infliximab and adalimumab effectively treat recurring, refractory, anterior, and posterior uveitis [37,50–52]. EULAR suggests switching between these agents in

cases of unresponsiveness or side effects [97]. Etanercept is not indicated for uveitis treatment [98]. Interferon-alpha is an effective alternative for anti-TNF-resistant uveitis [99,104], more effective and better tolerated than cyclosporin, and has similar efficacy to infliximab in treating Behçet syndrome-associated uveitis [99,105,106].

Vascular involvement

Thrombosis in Behçet syndrome primarily results from vascular inflammation, making immunosuppression the main treatment for acute venous thrombosis [97,99]. Steroids and immunosuppressive agents like azathioprine, cyclosporin-A, and cyclophosphamide are used in treatment [97]. No data or guidelines favor one agent over another, but cyclophosphamide may be reserved for severe cases due to its side effects [97]. Studies indicate that monoclonal anti-TNF agents are effective for refractory venous thrombosis [107,108]. Recently, tocilizumab has been suggested as an effective and tolerable option for severe or refractory vascular Behçet syndrome [109].

The effectiveness of anticoagulant therapy for thrombosis in Behçet syndrome is debated. A meta-analysis of adult patient studies showed that combining anticoagulant and immunosuppressive treatments did not significantly lower relapse rates. [97]. A retrospective study indicated that avoiding anticoagulants heightened the risk of postthrombotic syndrome. The French guidelines for Behçet syndrome management, which offer recommendations for both adult and pediatric cases, suggest beginning curative anticoagulant therapy for deep vein thrombosis [98].

For arterial involvement, high-dose steroids and cyclophosphamide are the primary treatments. Anti-TNF agents are advised in refractory cases [97,98]. Steroid and immunosuppressive treatments should precede interventions to minimize postoperative complications. For symptomatic cases, invasive radiological procedures are preferred over open surgery [97,98].

Neurological involvement

Acute parenchymal neuro-Behçet generally responds to high-dose steroids, beginning with a 3–7-day course and then tapering methylprednisolone [97,98]. Treatment should incorporate systemic DMARDs such as azathioprine or methotrexate [98]. Severe or refractory cases may require additional anti-TNF agents or cyclophosphamide [97,98]. Limited studies suggest tocilizumab for refractory cases [97]. Isolated meningitis can be managed with steroids alone [98]. Cyclosporine is not recommended due to neurotoxicity risks, and anti-TNF agents may increase the risk of demyelinating events [97–99,110].

Acute cerebral thrombophlebitis in Behçet syndrome is treated similarly to parenchymal disease with high-dose steroids. Immunosuppressive drugs do not show additional benefits in the first cerebral vein thrombosis attack but should be added in relapsed cases [97,98]. Anticoagulants are also recommended in managing cerebral thrombophlebitis [98].

Gastrointestinal involvement

Despite the risk of gastrointestinal bleeding, steroids are recommended for acute attacks, early induction, and severe disease [97,98]. For mild to moderate gastrointestinal involvement without small bowel issues, 5-aminosalicylates are suitable [97,98]. Azathioprine is recommended for moderate to severe cases [97,98]. Anti-TNF agents, such as infliximab and adalimumab, have proven efficacy in azathioprine-refractory cases [111,112] and have become primary treatments for severe cases. Switching between anti-TNF agents can be beneficial when the initial agent fails [113].

Articular involvement

Colchicine is typically the first-line treatment for Behçet syndrome-related arthritis. Intra-articular steroids are beneficial for a few affected large joints. For refractory and recurrent cases, methotrexate, azathioprine, or anti-TNF agents are generally preferred [98].

DIFFERANTIAL DIAGNOSIS AND MONOGENIC MIMICKERS

The disease's multisystemic nature, lack of a definitive diagnostic test, and incomplete Behçet syndrome phenotype in childhood cases, leading to overlapping clinical findings with other pediatric diseases, complicate the differential diagnosis. Monogenic mimickers should be considered, especially in cases with parental consanguinity, early onset, atypical treatment response or clinical features, predominant autoimmune characteristics, or signs of primary immunodeficiencies. This section will address diseases to consider in the differential diagnosis of Behçet syndrome, particularly in pediatric patients.

NF-KB pathway and related diseases

The NF-KB pathway is linked to innate and adaptive immunity, inflammation, and cell proliferation.

Pathogenic variants in the NF-KB pathway can cause a Behçet syndrome like clinical picture [114,115].

A20 haploinsufficiency (HA20)

A new autoinflammatory disease was described in 2016 that displayed similarities to Behçet syndrome and resulted from the loss of function mutations in TNFAIP3 causing A20 haploinsufficiency [57,115,116]. In a recently published systematic literature review, 177 patients with HA20; the median age at the beginning of treatment was 3 years (0–17) and 109 (61.5%) of the patients were female [117▪▪]. A total of 129 (72.8%) patients had mucosal ulcer [123 (69%) oral ulcers and 63 (36%) genital ulcers], 93 (52.5%) had fever, 81 (45.7%) had gastrointestinal involvement, 76 (42.9%) had skin involvement [29(16%) pseudofolliculitis/acne/pustulosis, 17 (10%) rash, 10 (6%) panniculitis], 61 (34.4%) had autoimmunity and 54 (30.5%) had joint involvement. Moreover, four (2%) of patients had venous thromboembolism [117▪▪].

A study comparing patients with Behçet syndrome and HA20 revealed that the latter experienced an earlier onset of symptoms, a higher prevalence of family history, more recurrent fever episodes, higher rates of gastrointestinal involvement, and lower frequency of ocular involvement [118].

Diseases mimicking Behçet syndrome caused by variants related to proteins such as otulin and NEMO, which are negative regulators in this pathway, have also been reported [117▪▪,119].

Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis syndrome

PFAPA syndrome is a polygenic periodic fever syndrome, characterized by recurrent fever, aphthous stomatitis, pharyngitis, and cervical lymphadenopathy, usually at regular intervals (3–8 weeks) lasting 3–7 days [120]. Some researchers suggest that there are clinical and immunologic similarities between PFAPA syndrome and Behçet syndrome. In a study of Cantarini *et al.* [121], 80 adult patients with Behçet syndrome were contacted by a rheumatologist, a pediatrician, and an internist and posed questions to the patients about their symptom history. The rheumatologist identified 30%, the pediatrician 10%, and the internist 7.5% of the patients as meeting the diagnostic criteria for PFAPA syndrome during their childhood [121]. Manthiram *et al.* [122]. reported that PFAPA syndrome, Behçet syndrome, and recurrent aphthous stomatitis possess common genetic susceptibility loci, particularly in the IL12A, STAT4, IL10, and CCR1-CCR3 regions, and suggested that these three conditions might be a part of the Behçet spectrum disorders, implying a shared pathogenesis among them. Contrarily to this perspective, Ben-Chetrit and Yazici [123] critically examine the evidence supporting this connection. The authors argue that similar genotype-phenotype associations are not sufficient to cluster diseases, as such associations might have opposite implications, exemplified by TLR4 and NOD2 variants being protective in Behçet syndrome but associated with increased risk in Crohn's disease. The authors also note that completely different genetic backgrounds can result in very similar phenotypes, as evidenced by MKD, which resembles PFAPA syndrome, and by A20 haplotype insufficiency, which leads to a clinical presentation closely mimicking Behçet syndrome. However, these similarities are not sufficient grounds to cluster these diseases together.

In addition, early-onset inflammatory bowel disease, adenosine deaminase-2 deficiency, and primary immunodeficiencies such as chronic granulomatous disease should be kept in mind in the differential diagnosis of Behçet syndrome.

CONCLUSION

Juvenile Behçet syndrome poses significant diagnostic and management challenges due to its complex and variable presentation. The disease's heterogeneous nature, influenced by genetic, environmental, and immunological factors, requires a multifaceted approach to diagnosis and treatment. Pediatric cases exhibit unique characteristics that necessitate tailored approaches to management and care. Continued work and individualized treatment strategies are essential to address the unique needs of pediatric patients and improve their quality of life.

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Omics studies in Behçet's disease

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Purpose of review
In this review, we aimed to highlight recent findings from "-omics" studies in Behçet's disease.

Recent findings
Recent genomic studies in Behçet's disease identified possible risk loci associated with Behçet's disease related uveitis, neurologic involvement and gastrointestinal involvement. Additionally, sex-specific genetic effects were determined in Behçet's disease. Transcriptomic analyses of immune cells in Behçet's disease revealed that key inflammatory pathways such as NF-κB and MAPK have roles in Behçet's disease pathogenesis. Proteomic studies have highlighted the role of immune cell derived extracellular vesicles and identified potential biomarkers for vascular involvement and examined HLA I-bound immunopeptidomes. Metabolomics studies are still limited, but recent research has pointed to alterations in fatty acid metabolism and lipid profiles in Behçet's disease patient.

Summary
Omics studies have gained importance in the field of Behçet's disease through the generation of large data sets and efforts to extend their application are intensifying. These studies can provide opportunities for understanding Behçet's disease pathogenesis when they lead to testable hypotheses. Current challenges include the choice of appropriately homogeneous patient and control groups, effective data management and sharing, high cost and a rapidly increasing gap between the wealth of observational data generated and the relative paucity of controlled experimental efforts that could lead to mechanistic understanding.

Keywords
Behçet's disease, epigenomics, genomics, metabolomics, proteomics, transcriptomics

INTRODUCTION

Behçet's disease is a complex-polygenic systemic vasculitis with heterogeneous clinical features. Its pathogenesis has not yet been fully elucidated. Ethnicity, age, sex, disease duration may contribute to the expression of its disease phenotypes. The strongest genetic association is with *HLA-B51* and remains mechanistically unclear. There has been rapid development in omics technologies, such as high-throughput sequencing and improvements in mass spectrometry, which have helped increase our ability to gather vast amounts of descriptive data, which are an important foundation for future hypothesis-driven investigation. In this review, we summarize some of these data from omics studies in BD from the last 18 months.

GENOMICS

Four genomic studies focused on genotype-phenotype associations, one on sex-specific genetic effects, and one on the functional relationship between *ERAP1-Hap10* and *HLA-B51* in Behçet's disease patients.

Casares-Marfil *et al.* [1▪] genotyped 436 (270 M/166F) Turkish Behçet's disease patients and calculated cumulative genetic risk scores (GRS) for each type of involvement. In order to identify novel risk loci for different types of organ involvement they applied a genome-wide association studies (GWAS) approach. They identified *HLA-B/MICA* (rs116799036) as an important susceptibility locus

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KEY POINTS

- Omics studies have created an unprecedented amount of data, and their wide application is changing biomedical science.
- Selection of adequate target and control populations remains critical to the generation of meaningful data.
- The gap between purely descriptive efforts and those aiming at understanding disease mechanisms is increasing.
- A critical appraisal of the data gathered may lead to the generation of new hypotheses and initiatives to create model systems that allow those to be tested.

in patients with Behçet's disease related uveitis (BDU) compared to non-BDU patients. The group also observed a significant association with BDU in *SLCO4A* (rs6062789) and with neurological involvement in *DDX60L* (rs62334264).

Jung *et al.* [2] conducted a GWAS including 1689 Korean Behçet's disease patients [379 with gastrointestinal Behçet's disease (GIBD)] and 2327 healthy controls (HCs) and analyzed it together with a previous Turkish GWAS (1215 BD patients, 1278 HCs) [3] in the discovery phase and a previous Japanese GWAS (101 GIBD patients, 492 non-GIBD patients, and 737 HCs) [4] in the replication phase. They identified three novel loci, including *NPHP4* (rs74566205), *TYW1–AUTS2* (rs60021986), and *SEMA6D* (rs4143322) as risk factors for Behçet's disease. They also identified that *HLA-B∗46:01* was significantly associated with GIBD but not with non-GIBD. Finally, they performed peptide binding prediction analysis and showed that mycobacterial peptides may bind more strongly to *HLA-B46:01*, than *HLA-B51:01*.

Su *et al.* [5] conducted a GWAS including 978 BDU patients and 4388 controls, and a replication study including 953 BDU patients and 2129 controls study to identify susceptibility loci for BDU in a Chinese population. They identified the strongest association within the *HLA* region (*HLA–B51, HLA–A26, and HLA–C0704*). The authors also confirmed seven previously reported loci (*IL23R-IL12RB2, IL10, STAT4, ERAP1, IFNGR1, LACC1, CEBPB-PTPN1*) and found 22 additional novel susceptibility variants in the outside of the *HLA* region. Then they performed a meta-analysis including their whole cohort and a previously reported Japanese GWAS (611 Behçet's disease patients and 737 controls) [4]. The group showed that *ZMIZ1, RPS6KA4, IL10RA, VAMP1,* and *SIPA1-FIBPFOSL1* reached genome-wide significance. Finally, they observed increased transcriptional activity and expression of *ZMIZ1* for the nonrisk allele of the susceptibility locus within ZMIZ1 in PBMCs.

Sornsamdang *et al.* [6] investigated *HLA* in a small sample of Thai Behçet's disease patients ($n = 56$) with different types of organ involvement and HCs ($n = 192$). They found associations between Behçet's disease and several *HLA* alleles, including *HLA-A∗26:01:01, HLA*-B∗39:01:01, *HLA-B∗51:01:01, HLA-B∗51:01:02, HLA-C∗14:02:01, HLA-DRB1∗14:54:01*, and *HLA-DQB1∗05:03:01*. Probably due to their small sample size, these alleles lost significance after Bonferroni correction. The authors demonstrated linkage disequilibrium in the haplotypes *HLA-A∗24:02:01*, *HLA-B∗51:01:01*, and *HLA-C∗14:02:02*. In terms of phenotype-genotype relationship, they found only one significant association, that is, between BDU patients and the *HLA DRB14:54:01* allele.

It is well established that male Behçet's disease patients have more severe disease, and Jo *et al.* [7▪▪] aimed to identify whether there are sex-specific genetic effects in Behçet's disease. They genotyped 2213 Behçet's disease patients (1762 M /1216 F) and 1533 HCs (897 M/636 F) in a Turkish population. They also performed a meta-analysis, including Spanish (106 M/118 F), Korean (98 M/96 F), Italian (67 M/ 59 F), Japanese (90 M/27 F), and Tunisian (71 M/33F) Behçet's disease patients to validate their results. Allele frequency of rs2848712 (*HLA-B/MICA)* was significantly higher in Turkish male Behçet's disease patients compared to Turkish female Behçet's disease patients. They observed the same trend, without statistical significance, in the other populations, except the Spanish cohort. Additionally, they calculated GRS in Turkish cohort and found that the loci in *HLA-B/MICA, HLA-C*, and *KLRC4* have significant impact on GRS in male Behçet's disease patients compared to female Behçet's disease patients. They also found that the *IFNGR1* variant was linked to a higher disease risk in female Behçet's disease patients.

The epistatic relationship between *HLA-B∗51* and *ERAP1-Hap10* was discovered in GWAS studies and has been shown to increase the risk of Behçet's disease to about 11-fold [8]. Cavers *et al.* [9▪▪] aimed to identify the biologic and mechanistic role of this epistatic relationship. They included 26 untreated active Turkish Behçet's disease patients with mainly ocular and vascular involvement and 22 age-sex matched HCs. The authors found that the frequency of homozygous *ERAP1-Hap10* in *HLA-B51+* Behçet's disease patients was 5% which is consistent with previous GWAS results. They also uncovered significant shifts in the frequency of naive versus antigen-experienced CD8 T cells in an HLA-B51 background, depending on *ERAP1-Hap10* carrier

status, suggesting HLA-B51-restricted antigen recognition in affected carriers. Modelling the low enzymatic activity feature of *ERAP1-Hap10,* which results in low peptide trimming, they generated ERAP1 knocked-outs in *HLA-B51+* LCLs and found significant differences in the immunogenicity and the composition of HLA class I-derived peptidomes of these cells when compared with wild type carrying an ERAP1 haplotype with higher trimming activity.

TRANSCRIPTOMICS

There are eight recent transcriptomic studies, which include different types of immune cells, circulating microRNA (ci-miRNA), and a multiomics approach.

Neutrophils are often observed in early target organ infiltrates in Behçet's disease, and their exact role is still being investigated. Yu *et al.* [10] studied the transcriptional profile of neutrophils from treatment-naive active Behçet's disease patients ($n = 10$) and age-sex-matched HCs ($n = 10$). Differentially expressed genes (DEGs) in Behçet's disease patients' neutrophils were primarily enriched in chemokine, MAPK, Wnt, and NF-κB signaling pathways. Studying additional cohorts of Behçet's disease ($n = 32$), HCs ($n = 32$), systemic lupus erythematosus (SLE) ($n = 28$), and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients ($n = 16$), they showed that chemotaxis-related genes (*CCL5, CCR6,* and *ETS1*) were similarly expressed in Behçet's disease, SLE, and AAV patients, but significantly overexpressed compared to HCs. CCL5 and ETS1 were significantly overexpressed in vascular Behçet's disease compared to other Behçet's disease subgroups.

Wang *et al.* [11▪] aimed to identify sex-specific differences in neutrophil composition and its association with BDU. Single-cell RNA sequencing (sc-RNAseq) from 18 active BDU (8 F/10 M) and 16 HCs (8 F/8 M) neutrophils. At first, the authors identified five neutrophil clusters including three conventional (with an inflammatory response gene signature) and two unconventional (with T cell regulation and interferon-α response gene signature) neutrophil ones in HCs. The group showed that gene expression for leukocyte chemotaxis, inflammatory responses, and responses to chemokines in conventional clusters was higher in men, with an increased abundance of conventional inflammatory neutrophils with high expression of S100A12. They showed the same pattern in male BDU patients suggesting a role for unconventional neutrophil subsets in BDU in men.

Le Joncour *et al.* [12▪▪] investigated the effect of phosphodiesterase 4 (PDE4) inhibition on neutrophil activation, reactive oxygen species (ROS) production, and neutrophil extracellular traps (NETs) formation in Behçet's disease patients ($n = 20$) and age-sex matched HCs ($n = 15$). They also performed transcriptomic analysis on neutrophils before and after the treatment. The group showed that Behçet's disease neutrophils are more activated, with increased ROS and NETs production compared to HCs. They also demonstrated that Behçet's disease neutrophils show increased expression of genes related to innate immunity, intracellular signaling, and chemotaxis compared to HCs. After treatment either *in vitro* with roflumilast or *in vivo* with apremilast, they showed reduced neutrophil activation, decreased ROS and NETs production, and downregulation of genes and pathways related to innate immunity, intracellular signaling, and chemotaxis in Behçet's disease patients.

Wu *et al.* [13] investigated the role of another immune cell type, macrophages, in Behçet's disease pathogenesis. They obtained monocytes from HCs, differentiated them into macrophages in culture, and then treated them with plasma samples from treatment-naive active Behçet's disease patients ($n = 45$) and age-sex matched HCs ($n = 25$). They demonstrated that exposure to Behçet's disease serum primes macrophages to polarize towards an M1-like phenotype, increases phagocytic activity, and promotes of Th1 cell differentiation. Transcriptome analysis revealed that this M1 polarization, after exposure to Behçet's disease serum, was driven by the activation of the NF-κB signaling pathway.

Two studies performed transcriptome analyses on all immune cells rather than focusing on a specific cell type. Okubo *et al.* [14] studied PBMCs from 23 Behçet's disease patients in remission or with low disease activity to detect inherent differences, rather than those related to excessive inflammation. They also included 28 age-sex matched HCs. The group used their own (ImmuNexUT) database consisting of RNAseq data and expression quantitative trait loci (eQTL) database of immune cell subsets from patients with immune-mediated diseases and HCs for analysis. They showed that the frequency of Th17 cells was increased in Behçet's disease patients, and transcriptome analysis of Th17 cells suggested activation of the NF-κB pathway. They also demonstrated upregulation of inflammatory pathways like MAPK and NF-κB in antigen-presenting cells form Behçet's disease patients. Additionally, they found an increased expression of *YBX3*, a gene in a plasmacytoid dendritic cell module associated with Behçet's disease, in individuals with the risk allele (rs2617170).

Zheng *et al.* [15▪▪] conducted sc-RNAseq (Behçet's disease $n = 4$, HC $n = 4$) and bulk RNAseq (Behçet's disease $n = 9$, HC $n = 10$) analyses on

PBMCs, and sc-RNAseq analysis on isolated monocytes (Behçet's disease $n = 4$, HC $n = 4$) in treatment-naive active Behçet's disease patients and age-sex matched HCs to investigate the immune landscape of Behçet's disease. They observed significant expansions and transcriptional changes in the monocytes of Behçet's disease patients and identified a distinct monocyte subtype, C1q-high ($C1q^{hi}$) monocytes, which accumulated in Behçet's disease patients and displayed proinflammatory characteristics. Their functional analyses showed that $C1q^{hi}$ monocytes were induced by activated interferon-γ (IFN-γ) signaling in Behçet's disease patients and were reduced by treatment with tofacitinib, suggesting this cell type could be a therapeutic target in BD.

Emmi *et al.* [16] conducted a study to determine ci-miRNA profiles associated with Behçet's disease that could be used as biomarkers. They enrolled 16 Behçet's disease patients and 18 age-sex matched HCs in the screening cohort, and 30 Behçet's disease patients and 30 age-sex matched HCs in the validation cohort. Additionally, they included 30 SLE and 30 giant cell arteritis (GCA) patients. The authors reported a unique ci-miRNA profile that can differentiate Behçet's disease patients from HCs and diseased controls (DCs). Pathway analysis revealed that the differentially expressed ci-miRNAs were mainly enriched in cell-matrix interactions, oxidative stress, and blood coagulation, which may have key roles in the thrombo-inflammatory processes observed in Behçet's disease.

Shi *et al.* conducted a multiomic single-cell study on 23 Behçet's disease patients and eight non-Behçet's disease individuals by integrating chromatin accessibility and gene expression analyses to identify cellular heterogeneity and disease-associated pro-inflammatory activity [17]. They found expansions in $CD8^+$ naive T cells, $CD8^+$ Tregs, double-negative T cells, cDC2, and activated monocytes, while NK cells showed no difference in frequency but were in a pro-inflammatory state in Behçet's disease. Their integrative analysis identified transcription factors, including AP-1, NF-kB, and ETS families, potentially regulating chromatin accessibility, gene expression, and inflammation in Behçet's disease. The non-Behçet's disease group was not defined as healthy controls and was not matched for age or sex with the Behçet's disease patients, making interpretation of these results somewhat difficult.

PROTEOMICS

Among five recent proteomics studies, four included Behçet's disease patients with uveitis, one with vascular involvement, and one with no specific type of organ involvement.

Wu *et al.* performed proteomic analysis on small and large plasma-derived extracellular vesicles obtained from HCs ($n = 32$), patients with ankylosing spondylitis (AS)-related uveitis ($n = 16$), BDU ($n = 16$), Vogt-Koyanagi-Harada (VKH) disease ($n = 16$), and posterior scleritis ($n = 16$) during acute ocular inflammation [18]. In BDU, the authors observed that proteins associated with the regulation of NF-κB signaling and the stress-activated MAPK cascade, complement activation and K63-linked protein ubiquitination, and immune effector processes and the regulation of autophagy were enriched in small extracellular vesicles, large extracellular vesicles, and plasma, respectively. They hypothesized that a possible microbial trigger could lead to the activation of NF-κB and NLRP3 inflammasome, initiating an inflammatory response in Behçet's disease. Additionally, they speculated that enzymes involved in protein ubiquitination may be present in plasma or extracellular vesicles, moving to target organ to regulate intracellular signaling pathways.

Zhang *et al.* [19] performed a proteomic analysis of aqueous humor-derived exosomes from uveitis patients with VKH ($n = 45$) and Behçet's disease ($n = 39$) who were in remission, as well as control patients with senile cataracts ($n = 45$), and integrated their findings with a publicly accessible scRNA-seq dataset of anterior segment tissue. They found differentially expressed proteins (DEPs) mainly enriched in the complement-related pathways and a significant increase in the expression of ceruloplasmin and C1QB in VKH and Behçet's disease compared to controls. They also showed that C1QB was primarily expressed by monocyte-macrophages in the anterior segment tissue. Results remain difficult to interpret, as the study did not demonstrate differences between VKH and Behçet's disease, two genetically, immunologically, and clinically distinct diseases, perhaps due to the inclusion of inactive individuals only.

Cai *et al.* [20] studied plasma-derived exosomes from active BDU patients ($n = 16$) and age and sex-matched HCs ($n = 16$) to evaluate their effect on neutrophil activation and DEPs. The group showed that exosomes from BDU patients induced neutrophil activation by increasing IL-17, IL-6, IL-8, MCP-1, and myeloperoxidase compared to HCs. Enrichment analyses showed some similarities with the previous two studies, primarily highlighting chemokine signaling, complement, and coagulation pathways. *PTPN11*, encoding SHP2, exhibited the most significant difference in expression between BDU and HCs. When the authors knocked down SHP2 in human neutrophil cell lines, levels of IL-17, IL-6, IL-8, MCP-1, and myeloperoxidase were increased, possibly suggesting an immune-protective effect of SHP2 in HD.

Liu *et al.* [21] analyzed the expression of immune response-related proteins in plasma from 55 active Behçet's disease patients and 53 age-sex matched HCs. They identified six upregulated (IL6, IL10, KLRD1, NCR1, AREG, CLEC6A) and 37 downregulated DEPs, including MGMT, IRAK1, FAM3B, IRAK4, SH2D1A, and DFFA, enriched in TLR-9 and NF-κB pathways. Six proteins (IL10, FCRL3, MASP1, NF2, FAM3B, and MGMT) were identified as potential biomarkers for Behçet's disease using machine learning algorithms. They used DEPs, clinical phenotypes, sex, and age to construct a disease classification model for Behçet's disease. The group found that patients with high disease activity had elevated expression of TRIM5, SH2D1A, PIK3AP1, HCLS1, and DFFA, while patients with low disease activity had higher expression of CCL11.

Cheng *et al.* [22▪] conducted a comprehensive proteomic analysis using plasma from 98 Behçet's disease patients and 31 age-sex matched HCs. They identified 220 DEPs between Behçet's disease patients and HCs and eight main biological processes, including leukocyte-mediated immunity, complement activation, angiogenesis, regulation of plasma lipoprotein particle levels, and wound healing. The authors performed unsupervised consensus clustering on DEPs and conducted a cluster analysis of the Behçet's disease cohort based on clinical data (sex, age, severity score, and clinical manifestations). By comparing clinical and proteomic classifications, they constructed a proteomic landscape to map organ-specific Behçet's disease profiles. The vascular phenotype had the highest number of DEPs, which were associated with platelet degranulation, blood coagulation, posttranslational protein phosphorylation, serine-type endopeptidase inhibitor activity, and the acute inflammatory response. Furthermore, they identified seven proteins that showed a linear correlation with disease severity and validated these findings in an independent cohort (Behçet's disease = 108, HCs = 29). Lastly, the group postulated that hyaluronic binding protein 2, tenascin, and serpin A3 could be potential biomarkers for vascular involvement in Behçet's disease.

METABOLOMICS

Metabolomics studies in Behçet's disease are relatively rare, with only two studies published in recent years. Park *et al.* [23] studied metabolomics using plasma samples and lipidomic using plasma and PBMC samples of Behçet's disease patients (metabolomic $n = 40$, lipidomic $n = 29$) and age-sex matched HCs (metabolomic $n = 18$, lipidomic $n = 18$) and DCs (metabolomic $n = 17$, lipidomic $n = 19$) [23]. They showed that carbohydrate, fatty acid, and phosphatidylethanolamine metabolisms were significantly altered in Behçet's disease patients' plasma and fatty acid dysregulation was present in Behçet's disease compared to HC and DC. The authors also found a positive correlation between disease activity and Sphingosine 1-phosphate, ether-linked lysophosphatidylethanolamine in plasma and PBMC in Behçet's disease patients. Depending on the disease phenotype, they only found significant differences in PBMC samples of active vs inactive uveitis patients. The authors included patients with two sets of mucocutaneous lesions (oral aphthae + genital ulcer or erythema nodosum) as DCs, making it difficult to definitively rule out incomplete expression of the Behçet's disease phenotype or mimics of the disease. The second study integrated microbiome and metabolome analyses in GIBD ($n = 35$), ulcerative colitis ($n = 35$), Crohn's disease ($n = 30$) patients, and HCs ($n = 41$) [24]. They performed microbiome analyses in colon tissue and stool samples, and metabolome analyses in plasma samples. Metabolomics profiles of GIBD and Crohn's disease were similar, whereas there were significant differences with HCs and UC. The authors linked these differences to the more common systemic features of GIBD and Crohn's disease compared to UC. Both fecal and tissue samples from GIBD patients had significantly different abundances of specific taxa compared to Crohn's disease, ulcerative colitis, and HCs. When combining microbiome and metabolome data, they observed that the mainly enriched metabolic pathways in GIBD were nucleotide metabolism and lipid and energy metabolism.

CONCLUSION

Omics technologies have had a tremendous impact on science over the past years. The vast amount of data generated by these approaches allows the deconstruction of complex biological systems in a completely unprecedented way. Omics studies have confirmed and uncovered susceptibility loci of the disease, identified disease-relevant pathways and provided first clues to molecules that may serve as candidates for biomarkers. Rapidly developing computational approaches propel these advances at a pace never observed in science before. Experimental work, however, has begun to lag behind these rapid advances. The highly heterogeneous clinical presentation of Behçet's disease, lack of consensus over possible differences in the expression of disease phenotypes within the context of specific genetic and environmental determinants, diagnostic issues related to mimics of the disease, varying patient characteristics in studies (e.g., active versus inactive,

treatment-naive versus treated), common difficulties with recruiting appropriate control groups, and the paucity of validation and replication studies, pose additional significant challenges. Continued efforts to address these issues and a conceptual re-orientation with the goal of pursuing insight in addition to oversight may enable the field to utilize the results of these studies as a steppingstone towards the design of experimental efforts that will advance our understanding of Behçet's disease pathogenesis.

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Conflicts of interest

Johannes Nowatzky is a member of the Medial Advisory Board at Soligenix.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
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VEXAS syndrome: an adult-onset autoinflammatory disorder with underlying somatic mutation

Ina Kötter[a,b] *and Martin Krusche*[a]

Purpose of review
VEXAS syndrome (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) was first described in 2020, where in a cohort of adults with unexplained fever or inflammation, systematic genetic testing was performed and 25 men with a median age of 64 years and somatic mutations in the *UBA1* gene were identified. In the current review, we aim to discuss the relevant literature from January 2023 until July 2024 to give new insights into the pathophysiology, epidemiology, diagnosis and treatment of VEXAS.

Recent findings
VEXAS affects 1 : 4269 in men over the age of 50. Janus-Kinase-inhibitors (JAKi) and IL-6-inhibitors are more effective immunosuppressants against hyperinflammation. Ruxolitinib is more effective than other JAKi. Azacitidine induces remission in many patients, but only few MDS-associated patients were treated. Allogeneic stem cell transplantation is feasible for selected cases. Infections are the major cause of death. Prognosis is still poor with a 5-year mortality rate of 18–40%.

Summary
In the current review, we discuss the novelties for VEXAS, including pathogenic pathways, epidemiological data, diagnostic criteria and algorithms, treatment options and complications. We hope that this review may improve rheumatologists understanding of VEXAS. We strongly recommend enrolling VEXAS patients in registries and clinical trials, to improve prognosis of VEXAS in the future.

Keywords
clinical spectrum, diagnosis, hyperinflammation, targeting the clone, treatment, UBA1 mutation, VEXAS

INTRODUCTION

VEXAS is an acronym for Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic and describes an adult-onset autoinflammatory disease. The syndrome was first described by Beck *et al.* in 2020 [1] who screened the exomes of 1477 people from a cohort at the NIH with undiagnosed recurrent fevers or systemic inflammation, focusing on genes related to ubiquitin. The authors identified 25 men with a median age of 64, each with somatic mutations affecting UBA1 on the X chromosome. In addition to fever, systemic inflammation and clinical manifestations such as neutrophilic dermatoses, ear and nose chondritis, vasculitis, pulmonary infiltrates, venous thromboembolism and cytopenias, the patients exhibited characteristic vacuoles in their myeloid and erythroid progenitor cells. The authors noted that VEXAS patients often additionally fulfilled diagnostic criteria for other inflammatory rheumatic and/or hematological disorders, namely relapsing polychondritis in 60%, Sweet's syndrome in 32%, polyarteritis nodosa (PAN) in 12%, giant cell arteritis (GCA) in 4%, myelodysplastic syndrome (MDS) in 24%, multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS) in 20%.

The aim of this review is to summarize the most relevant findings about VEXAS syndrome published from January 2023 to August 2024.

PATHOPHYSIOLOGY

UBA1 (ubiquitin-like modifier-activating enzyme 1) is a key enzyme of the ubiquitination process. As a

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KEY POINTS

- VEXAS is more common than previously thought (1 : 4269 in men over the age of 50); it rarely also occurs in women and young people. In case of typical symptoms, genetic testing is recommended.
- Characteristic "red flags" for diagnosis are systemic inflammation with macrocytic anemia, MDS and/or thrombocytopenia; neutrophilic dermatosis or leukocytoclastic vasculitis; nasal and/or auricular chondritis; pulmonary infiltrates and thrombosis.
- IL-6 antagonists and JAK inhibitors are most effective and ruxolitinib seems to be superior to other JAKi; azacitidine is effective and may significantly reduce the variant allele frequency, whereas allogeneic hematopoietic stem cell transplantation is effective in selected cases, eliminating the pathogenic clones and inducing complete remission, but is associated with a high transplantation- related mortality.
- Infections, often atypical, occur as a feature of VEXAS itself and the risk is increased by immunosuppressive treatment. Supportive treatment with antiinfective agents and prophylaxis against thrombosis is recommended.
- Mortality is high (18–46% in recently published cohorts).

prominent posttranslational modification, ubiquitination controls a multitude of signaling events inside cells including proteasomal degradation, DNA damage repair, cell cycle progression and more. A series of three enzymes (E1 activating enzyme, E2 conjugating enzyme and E3 ligase) sequentially bind and transfer the small modifying protein ubiquitin onto lysine residues within substrate proteins. Most mutations causing VEXAS occur in p.M41 the start codon for the cytoplasmatic isoform of UBA1 (UBA1b). The mutations lead to a loss of active UBA1b and emergence of a new catalytically impaired isoform [2▪].

The NIH group tempted to further elucidate VEXAS pathogenesis by transcriptome sequencing of single bone marrow mononuclear cells and hematopoietic stem and progenitor cells (HSPCs) [3▪]. They showed that myeloid progenitor and bone marrow mononuclear cells are the main producers of proinflammatory cytokines. Cells with the UBA1 mutation were only captured in a fraction of total cells, again with myeloid dominance. In the CD34+HSPC compartment in VEXAS, an unfolded protein response gene set was upregulated. A "VEXAS inflammatory signature" (TNFα, IFNγ, IL-6, IL-1ß) was described. A dysregulated protein ubiquitination/proteasome pathway due to UBA1 mutations with a lack of compensatory autophagy pathway for protein degradation leading to increased unfolded protein stress contributing to enhanced inflammation in VEXAS HSPCs was hypothesized. Apoptosis genes were upregulated and cell cycling genes downregulated, probably reflecting the ineffective hematopoiesis of MDS.

The hyperinflammatory environment positively selects other somatically mutated clones, DNMT3A and TET2 [4], which are frequent in VEXAS.

The auto-/hyperinflammatory cytokine profile (mainly TNFα and NFκB) was confirmed by Kosmider *et al.* [5▪▪] and hinted at inflammasome activation, as especially IL-1ß and IL-18 were upregulated at the transcriptional level. Single cell sequencing of monocytes revealed an increased unfolded protein and stress response. Enhanced neutrophil and monocyte cell death by apoptosis and pyroptosis and increased secretion of danger-associated molecular patterns (DAMP) may be an additional pathogenic mechanism [6].

To date, additional UBA1 mutations and splice variants have been described, and in one patient, even three UBA1 clones with different variant allele frequencies were detected [7▪▪,8–12].

Interestingly, there is considerable mosaicism for the UBA1 mutations: in hematopoietic tissues, the mutant allelic frequency (MAF) was 60.5%, and in nail samples (ectodermic tissue), it was 24.2% [13▪].

THE EVOLVING SPECTRUM OF CLINICAL MANIFESTATIONS

In 2022, the French VEXAS Group published the clinical and laboratory features of 116 patients [14] carrying UBA1 pathogenic variants. In 2023 thirty Spanish patients were described [13▪] and in 2024 an Italian VEXAS cohort of 41 patients was analyzed [15]. The clinical symptoms are summarized in Fig. 1.

Cutaneous involvement was in the focus of three recent publications. In a single-center cohort of 25 patients [16], 22 (88%) had skin lesions, which in 45% had occurred before other disease manifestations. The most common skin manifestation was neutrophilic urticarial dermatosis (25%) followed by leukocytoclastic/urticarial vasculitis (20%), urticarial tissue reaction (20%), neutrophilic dermatosis (15%), neutrophilic panniculitis (10%) and nonspecific chronic septal panniculitis (10%). Baur *et al.* [17] reviewed the cutaneous manifestations described in the literature in 2023: skin involvement occurred in 80–100%. In a US-American cohort of 112 patients, skin involvement was common with 83%. Predominantly leukocytoclastic vasculitis (36%), neutrophilic dermatosis (34%) and perivascular dermatitis

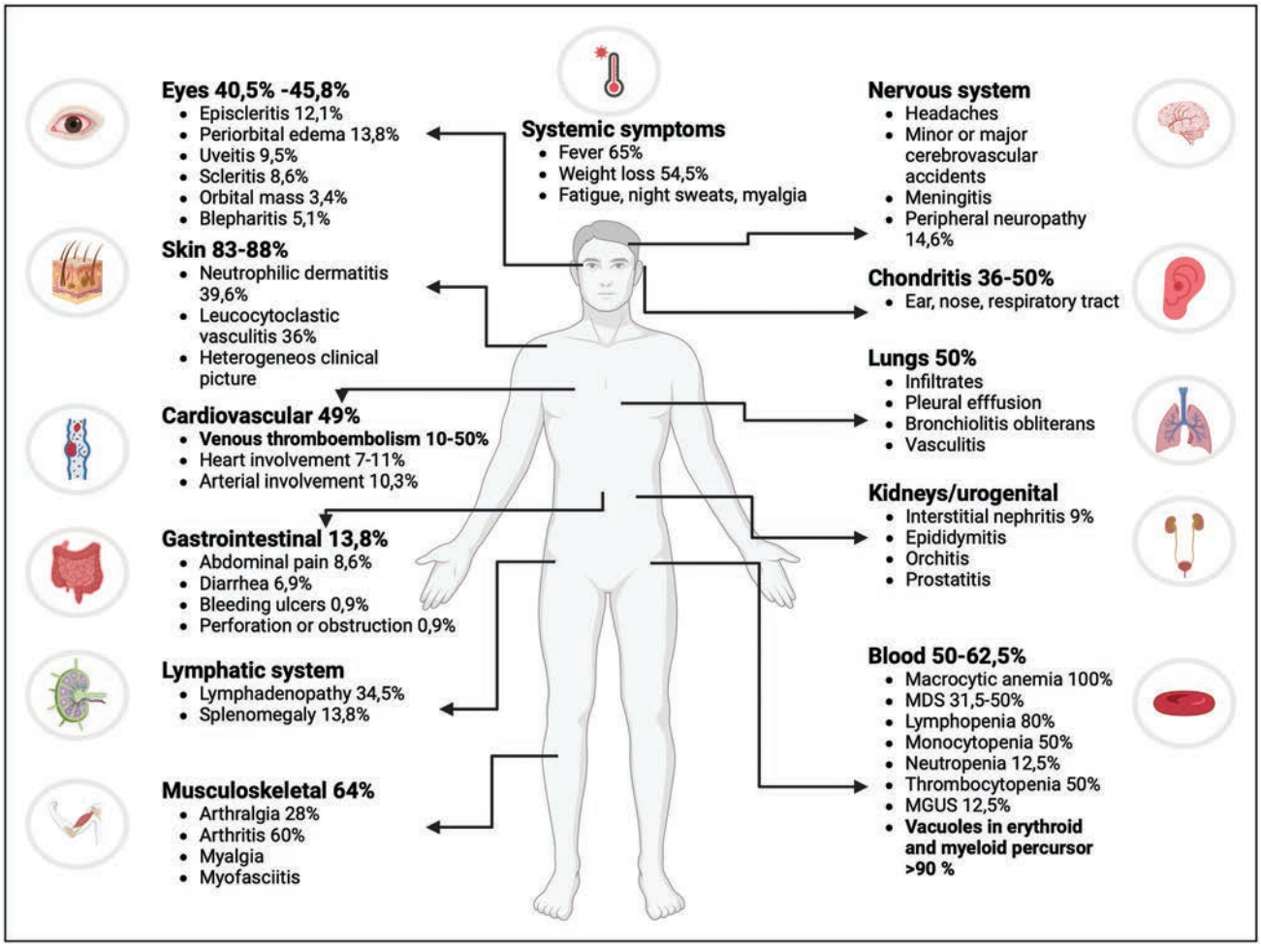

FIGURE 1. Organ manifestations of VEXAS. Data from [14,16,17,19,20,21▪▪,22▪▪,61], graphic created with BioRender.

(30%) were observed, Distinct pathogenic genetic variants were associated with specific cutaneous manifestations: p.Met41Leu variant was associated with neutrophilic dermatitis, often resembling Sweet's syndrome, whereas the p.Met41Val variant was associated with leukocytoclastic vasculitis [18▪▪].

Orbital and/or ocular involvement is also common in VEXAS; in the AIDA registry, 27 out of 59 (45.8%) patients had inflammatory orbital or ocular involvement [19]. Periorbital edema was most frequent (13.6%), followed by episcleritis (8.5%), scleritis (8.5%), uveitis (6.8%), conjunctivitis (6.8%), blepharitis (5.1%) and orbital myositis (3.4%). Twelve patients had relapsing polychondritis with a significant association to ocular/orbital manifestations. Similar results were found in a small single-center cohort from Israel [20].

Pulmonary manifestations were described in a cohort of 45 patients [21▪▪]. Chest CT revealed abnormalities in 91% of patients: parenchymal opacities (74%), most commonly ground-glass opacities (47%), along with mediastinal lymphadenopathy (29%), airway abnormalities (29%) and pleural effusion (24%).

Venous thrombosis is a prominent feature in VEXAS. An evaluation of 119 patients from the USA with respect to arterial and venous thromboembolism [22▪▪] revealed thrombosis in 49% of the patients, 41% of which were venous (VTE). Almost two-thirds of VTE were unprovoked, 41% were recurrent and 20% occurred despite anticoagulation. The cumulative incidence of VTE was 17% at 1 year from symptom onset and 40% by 5 years. Cardiac and pulmonary inflammatory manifestations were associated with time to VTE. p.Met41Leu was positively associated with pulmonary embolism. The cumulative incidence of arterial thrombosis was 6% at 1 year and 11% at 5 years. There was no difference according to survival between patients with or without thrombosis. Thromboembolism may be due to inflammation of the veins (venous vasculitis), as has been shown in two patients with VEXAS by Doppler ultrasound proving vein wall thickening which was even more pronounced than in Behçet's syndrome [23].

EPIDEMIOLOGY

Beck *et al*. [7▪▪] analyzed a large cohort from the Geisinger Health Initiative by microarray genotyping and whole exome sequencing. Among 163 096 participants, 11 (nine men, two women) harbored somatic variants at known pathogenic positions of UBA1, all of them had clinical manifestations consistent with a diagnosis of VEXAS. Together, disease-causing UBA1 variants were found in one of 13 591 unrelated individuals, one in 4269 men older than 50 years and one in 26 238 women older than 50 years.

Another cohort from the "The All of Us Program" reported whole genome sequencing in 245 368 individuals [24]. Seventy-four participants were identified who had at least one somatic UBA1 variant at the Met41 locus, 84% were women. Their ages ranged from 21 to 87 years. All 74 had a somatic DNA variant c.121A>C resulting in the protein variant p.Met41Leu, the variant which is least common and related with a milder VEXAS course. Their variant allele fraction (VAF) ranged from 4.5 to 33%. There was no difference in VEXAS-associated diagnosis codes when compared to people without UBA1 mutation.

Female VEXAS patients from existing literature were analyzed [25]. A total of 224 patient reports were collected (215 men and nine women). One of the women had a constitutional 45, X karyotype and four had an acquired X monosomy in the bone marrow karyotype. There were no differences in the clinical manifestations, nor were there differences in the type of UBA1 mutation, frequency or severity of MDS between men and women. The authors conclude that the UBA1 mutation should be sought for under the same conditions in both sexes.

Younger people may rarely be affected. The youngest patient described to date is a 23-year-old male Mexican who primarily presented with relapsing polychondritis [26]. Relapsing polychondritis patients carry UbA1-mutations in 10–50% [27,28]. Consequently, patients with relapsing polychondritis should carefully be evaluated for signs and symptoms of VEXAS, and if present, tested for UBA1 mutations.

In a prospective cohort study in patients with clonal cytopenias of undetermined significance, apart from typical somatic mutations found in MDS, UBA1 mutations were also found in a subgroup of patients (four of 90, 4%), who after re-evaluation fulfilled the criteria of VEXAS syndrome [29].

A cohort of 4168 Japanese patients with hematological malignancies was systematically tested for UBA1 mutations by whole genome and transcriptome sequencing [9]. The screening identified five patients with classical UBA1 variants, 16 with putative "nonclassical" UBA1 variants, 20 with putative germline UBA1-non classical UBA-1 variants and 16 where the origin of the UBA1 variant could not be defined. Most patients with UBA1 putative somatic variants had co-mutations in leukemic driver genes. Hematologists should therefore be aware of the possibility of hyperinflammation caused by UBA1 mutations in their patients, especially with MDS or plasma cell dyscrasias.

DIAGNOSIS

A VEXAS diagnostic scoring system was proposed by Japanese researchers [30▪] (Table 1). This score was not validated in other centers, and a study with 53 patients in France showed that the score has a low sensitivity of 33% in the first year of diagnosis, and a higher sensitivity of 83% after 18 months follow-up. Hence, it may not be suitable for the decision whether to do genetic testing in a patient with suspected VEXAS or not [31].

Of note, vacuoles in myeloid and erythroid precursor cells in bone marrow aspirations showed excellent performance for diagnosis [32,33]. Targeted testing of bone marrow specimens with cytoplasmic vacuolization helped identifying previously undiagnosed cases of VEXAS syndrome [34]. Bone marrow reports from 2014 to 2022 were reviewed for documentation of cytoplasmic vacuolization; available DNA was present in eight patients, seven of whom (87.5%) had a pathogenic somatic UBA1 variant.

Possibly, deep learning may help identifying vacuoles in peripheral blood smears, but to date, good performance was shown in a single center only and may be different when applied externally [35].

As proposed in a viewpoint article in RMD Open, we would recommend the criteria for genetic testing as depicted in Fig. 2 [36].

Table 1. Score for diagnosis of VEXAS

Clinical feature	Points
Age > 50 years	1
Cutaneous lesions	1
Pulmonary involvement	1
Chondritis of the auricular, nasal or respiratory tract	1
Macrocytic anemia	2
Total	0–6

Score 0–2, VEXAS unlikely; Score 3–4, VEXAS possible; Score 5, VEXAS likely; Score 6, VEXAS. Proposed by [30▪].

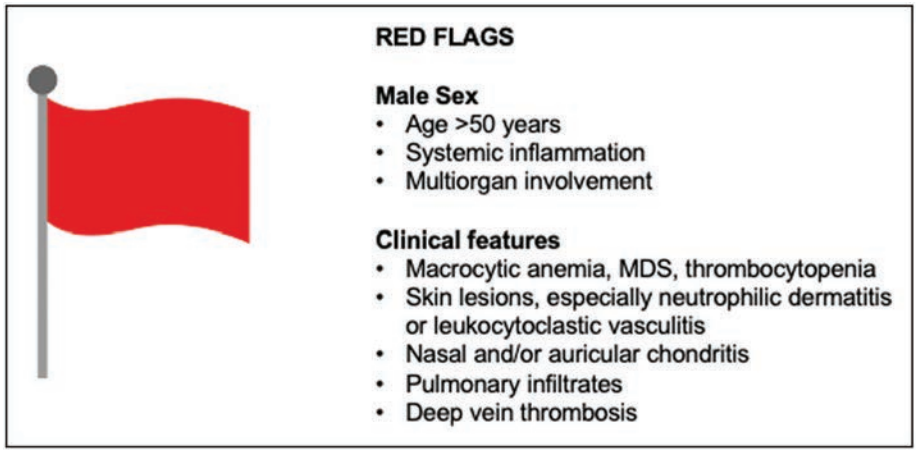

FIGURE 2. Red flags for genetic testing under suspicion of VEXAS syndrome. Proposed by [36].

A diagnostic algorithm was proposed for monogenic autoinflammatory diseases presenting with recurrent fevers in adults [37] (Fig. 3).

A novel XNA-based Luminex assay to detect UBA1 somatic mutations may facilitate genetic testing for the three most frequent mutations of the *UBA1* gene. It has a high sensitivity and allows high throughput testing [38].

TREATMENT OF VEXAS

There are two main principles for the treatment of VEXAS syndrome, addressing the symptoms of hyperinflammation by immunosuppressive drugs (conventional or targeted) or targeting the pathological clone bearing the somatic UBA1 mutation by hypomethylating agents or allogeneic hematopoietic stem cell transplantation (alloHSCT).

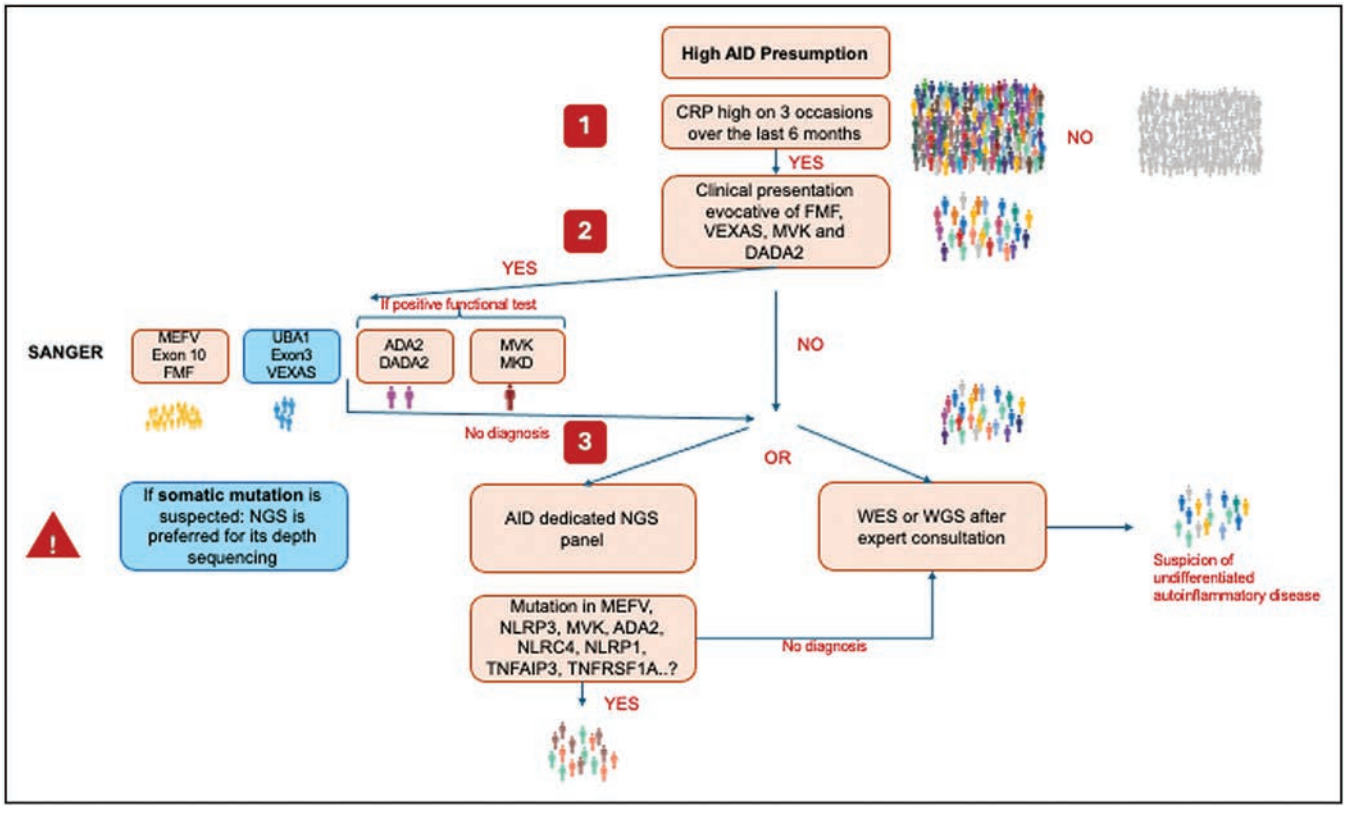

FIGURE 3. Diagnostic algorithm for autoinflammatory diseases in adults presenting with fever. As proposed by [37], slightly adapted (special colors for VEXAS). AID, autoinflammatory disease; NGS, next-generation sequencing; WES, whole exome sequencing; WGS, whole genome sequencing.

Targeting hyperinflammation

The French VEXAS Group recently evaluated the efficacy of targeted therapies in a cohort of 110 patients [39▪▪]. The patients received 194 targeted therapies, including 40% JAK inhibitors, 26% IL-6 inhibitors, 17% IL-1 inhibitors, 10% TNF inhibitors, 6% other targeted therapies. At 3 months, the overall response rate, comprising complete and partial remissions, was 24% with JAKi, 32% with IL-6i, 9% with IL-1i and 0% with TNFi or other targeted therapies. Survival without treatment discontinuation was significantly longer with JAKi than with the other targeted therapies. At the last follow-up, 22% of the patients had died, mainly from infections (13 of 24). Ruxolitinib was the JAKi most commonly prescribed (87% of cases treated with JAKi) and was found to be more effective than other JAKi used in rheumatology (complete remission at 3 months 83 vs. 18%; at 6 months 87 vs. 11%).

Treatment approaches published until April 2023 were summarized in a systematic review [40▪]. Thirty-six publications were identified with a total of 116 patients; 113 (98.3%) were men. Thirty-six patients had been treated with azacitidine, 25% achieved complete remission complete remission, 38.9% partial remission; 33 patients had received JAKi, with a complete remission in 33%, a partial remission in 27.3%; 15 patients were treated with tocilizumab, 20% achieved complete remission, 40% partial remission. alloHSCT was performed in seven patients, of whom 85% went into complete remission, one patient died; anakinra was applied in five patients, with 80% complete remission and 20% nonresponse. Canakinumab was given in two cases, with 50% complete remission and partial remission each. Six patients received glucocorticoid monotherapy, with 16.7% complete remission and 66.7% partial remission. Individual reports were available for TNFi, rituximab and methotrexate. Data on adverse events were available for 67 patients (57.8%) and included pneumonia in 17.9%, other infections in 13.4%, venous thromboembolism in 8.9%, cytopenias in 5.9% and acute and chronic graft versus host disease (GvHD) in 5.9 and 2.9%, respectively.

Hence, both publications hint at a superiority of JAKi and IL-6i, with ruxolitinib being more effective than other JAKi. Colleagues from France had already published a cohort of 30 patients treated with JAKi (12 with ruxolitinib, 11 with tofacitinib, four with baricitinib and three with upadacitinib) until February 2022 [41]. They described a superiority of ruxolitinib over other JAKi, without any differences between patients with or without associated MDS. However, under JAKi treatment, an expansion of the UBA1 mutated clones was observed under treatment ($n = 2$), which proves that treatment with JAKi does not cure VEXAS. Here, again, infections were the most frequent adverse event (36.7%), followed by thromboembolic complications (20%).

In the Italian cohort of 41 patients [15], variations of VAF were quantified during the treatment of four patients. In a patient under ruxolitinib, VAF increased, whereas after alloHSCT VAF was reduced to almost zero in three patients with VAT of up to 95% before the procedure. In a patient who was treated with erythropoietin for MDS, VAF remained stable at approx. 70% over 8 years.

Targeting the UBA1 mutated clone

Azacitidine as a hypomethylating agent, which is approved for the treatment of CMML, MDS and as a maintenance regimen for AML recently was shown to be effective after six treatment cycles in four patients with VEXAS and MDS [42▪]. Furthermore, the UBA1 mutated clones were reduced to very low or even undetectable levels, and a significant glucocorticoid dose reduction was made possible.

In another case report with two patients from Germany, complete clinical and molecular remission was described after azacitidine. Both patients had low or moderate-risk MDS with autoinflammatory features and a UBA1 mutation [43].

AlloHSCT may cure VEXAS and is one of the standard treatments for high-risk MDS in relatively young and fit patients. The EBMT (European Society for Blood and Marrow Transplantation) recently published 19 patients from their database who had undergone alloHCST [44▪▪]. Their median age was 59 years, the majority had concomitant MDS ($n = 13$), five had autoinflammatory manifestations only and one had myeloproliferative neoplasia. With a median follow-up of 14 months, the 2-year overall survival (OS) was 72% and transplant-related mortality (TRM) 25.8%. Of note, four patients died of bacterial infection or CNS toxicity ($n = 1$). Eleven survivors experienced clinical remission of their VEXAS phenotype and were able to discontinue immunosuppression. Molecular studies in six patients revealed disappearance (Sanger) or eradication (PCR) of UBA1 mutations by 3–6 months after alloHSCT. Acute graft GvHD occurred in 58%; in 26%, it was grade 2–4. Chronic GvHD presented in four patients at a median of 4.6 months from transplant.

A recent meta-analysis [45] revealed 39 patients in four publications, with a pooled OS rate of 86% and a pooled acute GvHD rate of 42%, chronic GvHD rate of 13% and a nonrelapse mortality rate of 14%. The pooled event-free survival rate was 42%.

Table 2. Response criteria for VEXAS syndrome

	Complete response (CR)	Partial response (PR)	Stable disease (SD)	Progressive disease (PD)
General and organ-specific clinical scales	Absence of clinical burden	<50% of baseline clinical burden	From <50% to >50% of baseline clinical burden	>50% of baseline clinical burden
CRP level	<5 mg/l for at least 1 month	<50% of baseline level	From <50% to >50% of baseline level	>50% of baseline level
Steroid dose	No steroid treatment for at least 1 month	<50% of baseline dose or < 7.5 mg/day	From <50% to > 50% of baseline level	>50% of baseline level
UBA1 VAF	Not detectable with sensitive technique	<50% of baseline VAF	From <50% to >50% of baseline VAF	>50% of baseline VAF

VAF, variant allelic frequency.
Proposed by [46▪].

Due to heterogeneity of the studies, evaluation of clinical response was difficult, and the follow-up after alloHSCT was short (8–18.5 months). One study with six cases reported complete remission in 100% (five of five evaluable cases).

As the definition of response differs between the publications, response criteria for VEXAS syndrome have been proposed by Sujobert *et al.* [46▪] (Table 2).

INFECTIONS

Infections are common in VEXAS patients, as demonstrated in an analysis of the French registry [47▪▪]. Here, 74 patients with 133 serious infections were reported. The most common sites of infection were lungs (59%), skin (10%) and urinary tract (9%). Fifty-two percent of infections were bacterial, 30% viral, 15% fungal and 3% mycobacterial. Among pulmonary infections, the main pathogens were SARS-CoV-2 (28.8%), legionella pneumophilia (21%) and pneumocystis jirovecii (PJP) (19%). Sixteen percent of infections occurred without any immunosuppressive treatment and with a daily glucocorticoid dose below 10 mg. In multivariate analysis, age at least 75 years, p.Met41Val mutation and arthralgia were associated with serious infections. JAKi were most associated with serious infections compared with biologics and azacitidine. After a median follow-up of 4.4 years, 27 (36%) patients died, including 15 (56%) due to serious infections.

In a cohort of 10 VEXAS patients from France [48], two experienced disseminated atypical mycobacterial infections (*M. avium*). At the time the infection was diagnosed, both were not intensively treated with immunosuppressive drugs and an intrinsic immunodeficiency due to VEXAS was hypothesized.

In another recent analysis from a US cohort, 94 VEXAS patients were screened for the prevalence of opportunistic infections and potential preventive measures were evaluated. Of these, 6% developed PJP, 15% had alpha-herpesvirus reactivation [with varicella zoster virus (VZV) being the most common] and 10% were reported to contract nontuberculous mycobacterial infection (NTM). A significant increase in risk of death was seen after the diagnosis of PJP (hazard ratio, 72.4 or NTM (hazard ratio, 29.1). The risk of death was also increased in patients with a history of herpes simplex virus (HSV) reactivation [odds ratio (OR), 12.10], but not in patients with VZV (OR, 0.89). Prophylaxis of PJP and VZV significantly reduced infection rates, underscoring the importance of prophylactic treatment [49]. Considering the existing data on treatments, prognostic factors and complications such as infections, management algorithm was proposed by Nakajima and Kunimoto [50] and Heiblig *et al.* [51▪▪], Fig. 4. Azacitidine should be considered for patients with 10–20% blasts. Nakajima and Kunimoto recommend considering HSCT also for patients without high-risk MDS but with risk factors negatively influencing survival.

INFLUENCE OF THE PRESENCE OF CLONAL HEMATOPOIESIS WITH SOMATIC MUTATIONS ON FREQUENCY AND COURSE OF ASSOCIATED AUTOIMMUNE DISEASE AND *VICE VERSA*

Clonal hematopoiesis with somatic mutations is a key feature in the pathogenesis of MDS; even in a cohort of low-risk MDS, 68% of the patients had at least one somatic mutation [52]. There is an association between specific mutations (especially DNMT3A and TET2) and prognosis of MDS as well as with concomitant autoimmune diseases [53].

The prevalence of autoimmune diseases in MDS in a recent meta-analysis of the literature was estimated 25% [54]. The frequency of UBA1 mutations in MDS cohorts was 1–12%, highest in an MDS cohort

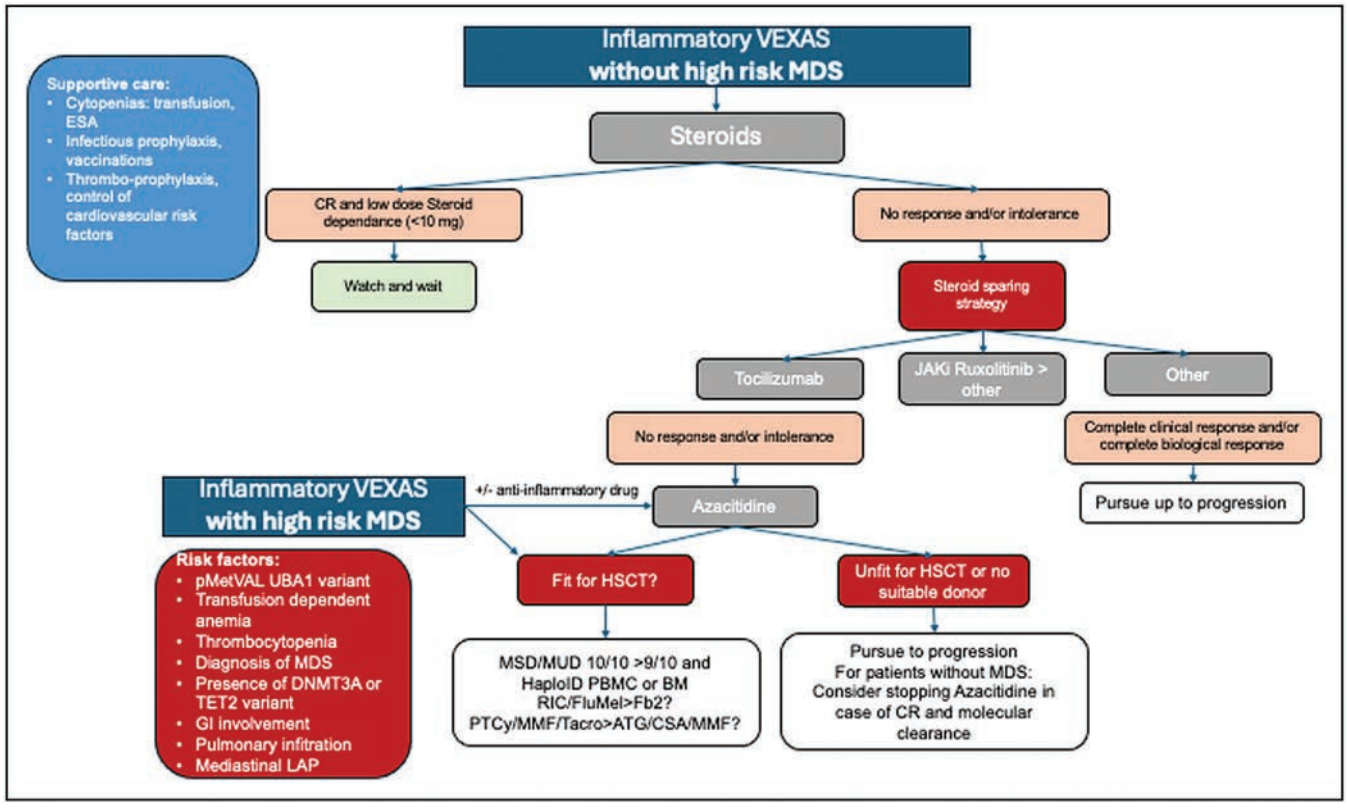

FIGURE 4. Therapeutic algorithm for VEXAS syndrome. As proposed by [50,51▪▪]. ATG, antitymocyte globulin; BM, bone marrow; CR, complete response; ESA, erythropoiesis-stimulating agent; FB", fludarabine+Busulfan conditioning regiman; FluMel, Fludarabine Melphalane conditioning regimen; HaploID, haploidentical; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MSD, matched sibling donor; PBMC, peripheral blood mononuclear cells; PTCT, post transplant cyclophosphamide; RIC, reduced intensity conditioning; Tacro, Tacrolimus.

with associated autoimmune diseases [55▪,56]. In an analysis of 92 relapsing polychondritis patients, 7.6% carried a UBA1 mutation and were older at disease onset, predominantly male, and they had ear and nose chondritis as well as hematological abnormalities [57].

MDS patients with autoimmune diseases had a significantly worse OS than those without [54]. This also holds true for VEXAS associated with MDS when compared to VEXAS without MDS [14]. To date, there are no data on OS of MDS patients with and without UBA1 mutations; however, a recent analysis showed that the more mutations are found in patients with clonal hematopoiesis of undetermined significance, the worse the OS [29].

On the other hand, the MDS-associated autoimmune diseases often respond less well to standard immunosuppressive treatments and were significantly more steroid dependent than the classical counterparts without underlying MDS [58,59].

Similarly, with respect to relapsing polychondritis, a comparative analysis of patients with and without UBA1 mutation apart from significant clinical differences showed that treatment response in patients with unmutated relapsing polychondritis is significantly better than in those with underlying UBA1 mutation [60]. Hence, there is an obvious correlation between clonal hematopoiesis with somatic mutations (including UBA1) and worse outcome of the myelodysplasia and treatment response of the associated autoimmune disease.

PROGNOSIS OF VEXAS

Data on prognosis rely on larger observational cohorts and registries and are sparse. In the French VEXAS Group [14], prognosis depended on the cluster the patients belonged to; Cluster 1 comprised patients with mild to moderate disease (47%), Cluster 2 patients with underlying MDS (16%), and Cluster 3 patients with constitutional manifestations, higher CRP levels and less frequent chondritis (37%). The 5-year probability of survival was 84.2% in Cluster 1, 50.5% in Cluster 2 and 89.6% in Cluster 3. The p.Met41Leu mutation was associated with a better prognosis.

In the Spanish VEXAS cohort, the mortality rate was 40%, and the observation period was not reported [13▪]. In the recent publication from the French VEXAS registry [47▪▪], mortality after 4.4 years was 36%; more than half of the deaths occurred due to serious infections.

A considerably better survival was reported in a cohort from the USA ($n = 119$), with 88% after 4.8 years [22▪▪]. In this cohort, age at diagnosis and pulmonary involvement were predictive for increased mortality risk without significant difference between UBA1 variants. Again, the most frequent cause of death was infections, followed by disease progression.

CONCLUSION

Almost 4 years after the first description, our knowledge of the clinical spectrum of VEXAS syndrome has expanded, mainly due to the formation of national and international VEXAS registries and cohorts. Additional UBA1 mutations were described, some associated with prognosis and/or clinical manifestations. Epidemiological cohort studies revealed that VEXAS is more common than previously thought; it also rarely occurs in women and young people. Diagnosis may still be difficult, and diagnostic scores have been proposed to select patients for genetic testing. Regarding pathophysiology, the consequences of the UBA1 mutations have been elucidated, hinting at hyperinflammation resulting from endoplasmic reciculum stress caused by an unfolded protein response due to a lack of UBA1 wild type. Although progress has been made concerning treatment, there is a considerable lack of prospective clinical trials. Treating hyperinflammation is clinically most effective with glucocorticoids, IL-6 inhibitors and JAK inhibitors, especially ruxolitinib. However, these do not cure VEXAS, as the frequency of mutant clones even increases. With the hypomethylating agent azacitidine and above all alloHSCT, the mutant clones can be significantly diminished or even disappear, as do the clinical symptoms of VEXAS. Prognosis is still poor with a 5-year mortality of 30–40%, mainly due to infections. Proposed treatment algorithms should include prophylaxis against opportunistic infections such as *Pneumocystis jirovecii*. Physicians should be aware of the "red flags" of VEXAS, and if finally diagnosed by genetic testing, patients should be included in registries and clinical trials to improve management and prognosis in the future.

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Polymyalgia rheumatica and giant cell arteritis: diagnosis and management

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Purpose of review
There have been advances in the diagnosis and treatment of giant cell arteritis (GCA) and polymyalgia rheumatica (PMR).

Recent findings
Themes in PMR and GCA include classification criteria, ultrasound imaging of temporal and axillary arteries replacing biopsies for diagnosis of GCA, faster diagnosis and treatment with rapid access clinics for suspected GCA, and expanding treatment options with the goal of rapid suppression of inflammation and sparing steroids.

Summary
Treatment is aimed at suppressing inflammation quickly in both GCA and PMR. Randomized trials have demonstrated success in reducing glucocorticoids when adding advanced therapies such as interleukin 6 (IL6) inhibitors. Other treatments including Janus kinase (JAK) inhibitors (especially a phase 3 trial of upadacitinib at 15 mg daily and secukinumab (an IL17 inhibitor) are being tested. Some uncontrolled GCA protocols are limiting glucocorticoids to initial IV pulse therapy only or rapid tapering of oral glucocorticoids with upfront treatment with tocilizumab. There is uncertainty of who should have an advanced therapy and how long to use it for and what order to consider advanced therapies when treatment fails. In PMR, studies are performed when patients cannot taper glucocorticoids effectively, whereas in GCA, advanced therapies are started with disease onset or with recurrent GCA.

Keywords
biologics, biopsy, giant cell arteritis, interleukin 17 inhibitors, interleukin 6 inhibitors, polymyalgia, rapid access clinics, sarilimumab, temporal arteritis, temporal artery, tocilizumab, treatment, ultrasound

INTRODUCTION

Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are inflammatory diseases affecting the aging population over 50 years of age [1]. GCA is a granulomatous vasculitis of the large and medium vessels, most commonly affecting the temporal arteries to cause cranial symptoms such as vision loss, headaches (especially scalp tenderness which is bilateral or unilateral), and jaw or tongue claudication, but can also involve extra-cranial vessels to cause systemic symptoms such as fever, and weight loss [2]. At onset, inflammatory markers are often very elevated. PMR is characterized by stiffness and pain in the shoulders and hip girdles associated with elevated inflammatory markers of C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR) [3]. GCA and PMR can manifest concomitantly, or serially in some patients and both depend on glucocorticoids as their mainstay of treatment.

Diagnosis of GCA and PMR both rely on recognition of clinical signs and symptoms. The diagnosis of PMR is clinical criteria with an elevated inflammatory marker whereas GCA also involves histopathologic findings on temporal artery biopsy [4], however this is an invasive procedure and false negatives can occur [5]. Although imaging modalities including ultrasound, PET/CT and MRI have been studied to identify GCA, more studies may be needed to determine the optimal standard of care for the diagnosis of GCA.

Glucocorticoids remain the mainstay treatment for GCA and PMR given its potent anti-inflammatory

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KEY POINTS

- Giant cell arteritis (GCA) rapid access clinics can improve time to diagnostic tests and treatment, and the standard of care for GCA diagnosis is changing from temporal artery biopsy which has many false negatives to ultrasound of the temporal and axillary arteries.
- Glucocorticoid sparing treatment in GCA is becoming the standard of care with upfront treatment with tocilizumab. Other randomized controlled trials (RCTs) are demonstrating the value of Upadacitinib and possibly secukinumab in GCA.
- Emerging data for very short-term prednisone/prednisolone with early GCA treated with tocilizumab are emerging and may change the standard of care.
- RCT data exist for sarilimumab in relapsing PMR or patients who cannot taper glucocorticoids.

and immunosuppressive properties, however its use is limited by various side effects including cardiovascular disease and dyslipidemia, hyperglycemia and insulin resistance, adrenal suppression, osteoporosis, and impact on mood and cognition [6]. Research efforts have therefore been primarily focused on identifying steroid-limiting and steroid-sparing therapies in treating GCA and PMR. The trend is to consider a steroid sparing drug and reduce the dose and duration of glucocorticoids in these diseases.

The scope of this review is to provide current knowledge and discuss recent developments in the diagnosis and treatment of GCA and PMR, with a focus on emerging steroid-sparing therapies and their efficacy and clinical utility.

Diagnosis of polymyalgia rheumatica and giant cell arteritis

PMR and GCA have long been thought of as entities that exist on the same disease spectrum. Although they can present in isolation, they can also manifest together but present sub-clinically, or co-occur simultaneously or disseminated in time. Indeed, PMR manifestations are seen in up to half of patients with biopsy-confirmed GCA [7,8], whereas as high as 20% of patients of PMR have been shown to have histologic features of GCA on temporal artery biopsy but not all of them present with GCA symptoms [9]. Even when GCA presents clinically, it may not be easy to recognize in the absence of cephalic signs as seen often in large-vessel GCA (LV-GCA), where symptoms can be constitutional and evolve slowly over months to years [10]. This can cause delays in diagnosis in addition to missing large vessel complications. Studies have shown patients with PMR and subclinical GCA tend to have higher rates of ischemic complications compared to clinical GCA [11–13]. Imaging modalities, such as [18F] fluorodeoxyglucose (FDG)-PET/CT, have utility in detecting subclinical GCA in patients with apparently isolated PMR [14–16], however systemic imaging modalities are still not part of routine care. Subclinical GCA may have important clinical implications. Recent studies have found increased relapse rates in patients with PMR and subclinical GCA compared to PMR alone [17▪,18▪▪,19▪]. Stratifying PMR into different clinical subsets using imaging modalities of ultrasonography, (FDG)-PET/CT, and MRI could provide insight into disease prognosis and steroid-responsiveness, however this remains an area of active research [20].

Diagnosis of PMR is primarily based of clinical manifestations, whereas diagnosis of GCA is based on both clinical and histopathologic findings from temporal artery biopsy or vessel involvement on imaging [21–23]. The 2012 EULAR/ACR classification criteria for PMR outlines a scoring algorithm whereby patients that are 50 years of age or older, have bilateral shoulder pain, and elevated inflammatory markers of CRP and/or ESR, may be classified as having PMR if they score minimum four points from having morning stiffness of more than 45 min, new hip involvement, absence of rheumatoid factor or anti-cyclic citrullinated peptide antibodies or other joint involvement; or minimum five points if including ultrasound findings of at least one shoulder or one hip with synovitis or bursitis, or both shoulders with ultrasound findings of synovitis or bursitis [22,23]. On the other hand, the 2022 ACR/EULAR classification criteria for GCA using a scoring algorithm whereby patients that are 50 years of age or older may be classified as having GCA if they score six points or above from a combination of clinical, laboratory, imaging, and biopsy criteria [21]. However, a positive biopsy occurs in 25% to 33% of patients with GCA, so a negative biopsy does not rule out GCA if clinical suspicion is high [24].

The 2024 Cochrane review looking at temporal artery ultrasound and temporal artery biopsy for giant cell arteritis used evidence (up to September 2022) to address two questions: whether ultrasound could be used to triage patients who need temporal artery biopsy, and whether ultrasound can replace temporal artery biopsy as a criterion of the ACR 1990 classification [25▪▪]. They found limited evidence on how accurate temporal artery ultrasound is for detecting GCA and whether ultrasound can replace biopsy for diagnosing GCA [25▪▪]. The TABUL study [26] performed ultrasound and temporal artery biopsies on 381 patients with suspected GCA and followed them for 6 months, and, found ultrasound

to be superior to biopsy in identifying patients with GCA (54% vs. 39% biopsy), but inferior in patients without GCA (none had positive biopsy; 19% had positive scan). More recently, a prospective study by Denis *et al.* enrolled 165 patients with high clinical suspicion of GCA and performed temporal artery ultrasound, then temporal artery biopsy on ultrasound-negative patients, with diagnosis confirmed in 44% and 17% of patients by ultrasound and biopsy, respectively [27▪]. This suggests temporal artery ultrasound may be a useful noninvasive way to diagnose GCA in patients with high clinical suspicion, in line with the EULAR recommendation of using imaging modalities such as ultrasound to confirm suspected diagnosis of large vessel vasculitis [28]. It may be optimal to ultrasound both temporal and axillary arteries to improve the diagnosis of GCA where a positive finding is a halo around the involved artery (halo sign) [29].

Management of polymyalgia rheumatica and giant cell arteritis

For both GCA and PMR, glucocorticoids remain the standard of care for treatment. The 2021 ACR/Vasculitis Foundation guidelines recommend high-dose oral glucocorticoids such as prednisone at 1 mg/kg/day up to 80 mg or equivalent for newly diagnosed GCA without cranial ischemia, and i.v. pulse glucocorticoids if there is threatened vision loss [4]. Additionally, it is now recommended to initiate oral glucocorticoids with tocilizumab over glucocorticoids alone for the treatment of newly diagnosed GCA as tocilizumab has demonstrated steroid sparing effects [4,30]. Other steroid sparing agents such as methotrexate can be considered if the patient is unable to tolerate or have limited access to tocilizumab [4]. Other glucocorticoid sparing drugs will be discussed below.

For PMR, the 2015 EULAR/ACR guidelines recommend initiating treatment with 12.5–25 mg of prednisone or equivalent daily, with adjustments of dose down to 7.5 mg and up to 30 mg depending on risk of relapse versus risk of glucocorticoid-related side effects [31,32]. This is followed by an individualized dose-tapering schedule, usually with initial taper down to 10 mg/day within 4–8 weeks, then tapering by 1 mg every 4 weeks once remission is achieved [31,32]. A large observational study led by Sattui *et al.* followed more than 26 000 patients with PMR seeing a rheumatologist and found two-thirds of patients remained on glucocorticoids between year 1 and 2 [33]. Recent work by Yates *et al.* in 39 000 patients with PMR, those who were still on glucocorticoids at two years were more likely to have started with lower initial glucocorticoid doses, and, have higher CRP levels [34▪▪]. These characteristics may help to inform individual steroid tapering strategies.

There are many trials that are trying to determine steroid sparing treatment for GCA and PMR, as outlined in Fig. 1. There is substantial evidence supporting interleukin 6 (IL6) inhibition in in the treatment of GCA (tocilizumab and other positive trials with other therapies) and sarilimumab in PMR (with other studies with positive results using other treatments). Patients with GCA treated with tocilizumab and steroids compared to steroids alone have higher remission rates, lower cumulative glucocorticoid use, and less serious adverse events [30,35]. Some study protocols used marked reductions in the duration and cumulative dose of glucocorticoid use in conjunction with tocilizumab. The GUSTO study was an open label single arm regimen with tocilizumab monotherapy following a short duration of glucocorticoids (e.g. 3-day pulse of methylprednisolone or 8 weeks of prednisone) in patients with new-onset GCA and this resulted in drug-free remission in a large proportion of patients [36,37,38▪▪]. Tocilizumab seems effective in patients with GCA presenting with or without concomitant PMR [39], resulting in significantly greater proportion of patients with PMR-activity score (PMR-AS) <10 and reduced prednisone requirements (SEMAPHORE trial) [40], as well as increasing the proportion of patients with glucocorticoid free remission at 16 weeks (PMR-SPARE trial) [41].

Another IL6-receptor inhibitor, sarilumab, has positive results from a randomized controlled trial (RCT) administered with a 14-week prednisone taper and resulted in an elevated sustained remission rate and lower cumulative glucocorticoid use at 52 weeks compared to placebo plus 52 weeks of prednisone taper (SAPHYR trial) [42▪▪]. Based on these results, the FDA approved sarilumab for treatment of PMR in patients with inadequate response to glucocorticoids or who cannot taper their steroids. Regarding GCA, the effect of sarilumab has been studied in a phase 3, double-blind study comparing sarilumab with glucocorticoid taper to placebo with glucocorticoid taper, however limited conclusions can be drawn given early termination of this study secondary to premature trial cessation during the COVID-19 pandemic [43▪▪].

A positive large RCT of upadacitinib (a Janus kinase (JAK) inhibitor) in GCA has been reported. The SELECT-GCA trial, found that patients with GCA treated with upadacitinib 15 mg daily together with a 26-week glucocorticoid taper result in greater percentage of patients achieving sustained remission and lower cumulative glucocorticoid use compared to placebo with a 52-week glucocorticoid

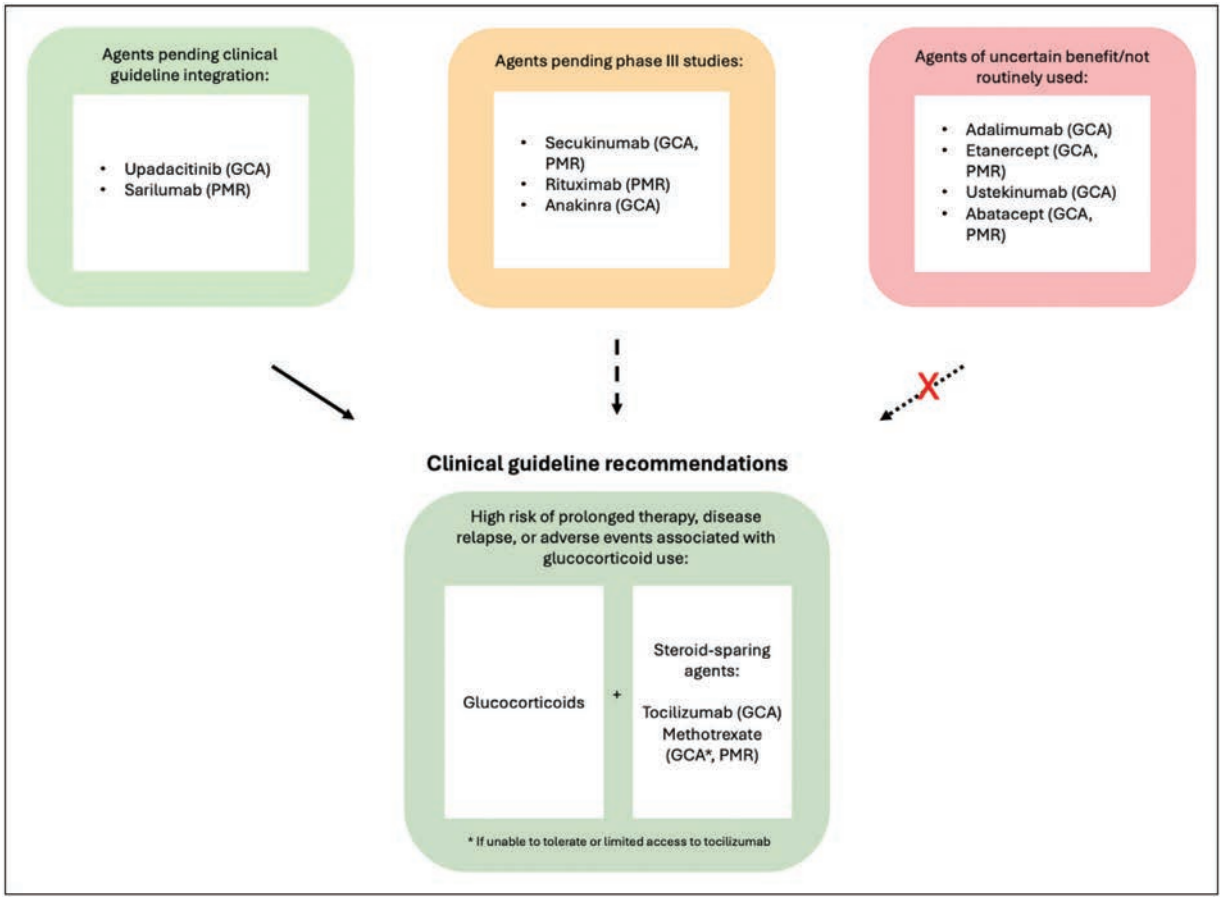

FIGURE 1. Current disease modifying antirheumatic drugs (DMARDs) for GCA and/or PMR based on their efficacy and development phase. GCA, giant cell arteritis; PMR, polymyalgia rheumatica.

taper [44▪▪]. Rates of MACE and serious infections were not numerically higher in patients treated with upadacitinib compared to placebo, but herpes zoster, lymphopenia, anemia, and nonmelanoma skin cancer were increased with upadacitnib [44▪▪]. The benefits of upadacitinib are likely similar (or slightly less) than the results in the tocilizumab trial but there have not been any direct comparisons. Baricitinib (a JAK2 inhibitor) was studied for relapsing GCA in a single site, open label study with 15 patients, where 13 patients achieved steroid discontinuation and remained in remission at 52 weeks [45]. In PMR, tofacitinib monotherapy appeared to have similar efficacy when compared to usual doses of glucocorticoids [46▪]. A phase 2 single arm study with 14 PMR patients with highly active disease treated with tofacitinib found 86% of patients reached remission at 24 weeks and a significant reduction in PMR-AS was seen as early as week 2 [47▪▪]. Sufficiently powered RCTs are needed to elucidate benefits of JAK inhibitors in PMR and see whether PMR can be treated without glucocorticoids especially in patients at high risk of steroid induced adverse effects.

Several agents appear to have benefit in treatment of GCA and/or PMR but are pending more validation through phase 3 studies. Secukinumab, an IL17 inhibitor, was shown in a phase 2 RCT (TitAIN) that patients with active GCA treated with a 26-week glucocorticoid taper experienced a higher sustained remission rate with secukinumab compared to placebo [48▪▪]. A 2-week randomized 3-arm study examining effects of single dose of secukinumab or canakinumab, or glucocorticoids on new onset PMR was terminated as it did not show secukinumab or canakinumab improved PMR disease activity as well as glucocorticoid treatment (https://clinicaltrials.gov/study/NCT01364389). A phase 3 study comparing secukinumab vs. placebo, combined with a glucocorticoid taper, in active PMR is currently recruiting (https://clinicaltrials.gov/study/NCT05767034).

Similarly, the BRIDGE-PMR trial observing the effects of rituximab vs, placebo in 49 patients with new-onset or relapsing PMR found a higher proportion of patients achieved glucocorticoid-free remission at 21 weeks with rituximab compared to placebo [49]. An extension study showed 47% of patients sustained glucocorticoid-free remission

compared to 23% in the placebo group [50▪]. Two phase 3 trials comparing rituximab on glucocorticoid free remission in new-onset and relapsing PMR are registered (REDUCE-PMR-1 and REDUCE-PMR-2, https://clinicaltrials.gov/study/NCT05533125, https://clinicaltrials.gov/study/NCT05533164).

Anakinra, an IL1 inhibitor, has previously shown steroid-sparing effects in a case series composed of six patients with refractory GCA [51]. A phase 3 trial comparing anakinra versus placebo in addition to glucocorticoids in treatment of GCA was registered, although its status is unknown (https://clinicaltrials.gov/study/NCT02902731).

There are several advanced therapies that do not appear to be beneficial in the treatment of PMR and/or GCA. TNF-alpha inhibitors collectively do not appear to be effective in treating GCA. Infliximab did not improve steroid sparing effects or reduce relapses compared to placebo [52]. In patients with new onset GCA, adalimumab did not yield superior remission rates compared to placebo [53]. Etanercept was shown to control GCA disease activity off steroids at 12 months in 50% of patients compared to 22% in placebo, however this difference was not significant, and the sample size was small [54]. In a RCT of 20 patients with new-onset PMR, etanercept monotherapy had a modest effect on disease activity with 24% reduction in PMR-AS [55]. Ustekinumab, an IL12/23 inhibitor, has shown mixed results; where, in one uncontrolled study, ustekinumab was associated with a significant reduction in glucocorticoids in refractory GCA [56], whereas in another prospective open-label trial, ustekinumab with prednisone had high rates of treatment failure [57]. Abatacept, a CTL4-Ig that modulates T cell activation, is associated with reduced risk of relapse in patients with GCA compared to placebo, however all patients in the study were exposed to abatacept for 12 weeks prior to randomization which confounds the conclusion [58]. The ALORS trial, a phase 3 RCT, showed half of 34 patients with PMR reached low disease activity with abatacept alone at week 12 compared to 22% in placebo; however, this was not significant suggesting the effect of abatacept alone may not be strong enough to warrant larger studies [59▪].

Future treatment options will likely be determined such as: which patients (vs. all patients) with PMR and GCA will require steroid sparing treatment, which treatment is optimal, how long to use glucocorticoids and at what dose (if using a steroid sparing treatment), how to diagnose and treat smouldering GCA with positive imaging (such as PET scanning) in large vessels or the arch of the aorta, and when to stop advanced therapies especially in GCA where there has been noted that after one year of tocilizumab, some patients flare when the treatment is stopped. These research questions to be answered for GCA/PMR are outlined in Table 1.

Table 1. Potential research questions for optimal treatment in GCA and PMR

Disease	GCA	PMR
Diagnosis		
	Optimal process with high sensitivity and specificity and if negative a high negative predictive value	Clinical diagnosis with elevated inflammatory markers
	Fast track GCA clinics allowing for ultrasound of carotid and axillary vessels and if negative and high index of suspicion consider temporal artery biopsy	Determination of asymptomatic GCA. Who to image and when and with what modality
Monitoring disease		
	When and how to investigate for subclinical disease activity	When and how to investigate for subclinical GCA
	Optimal biomarker to follow, esp if on IL6i that suppress CRP	Optimal biomarker to follow, esp if on IL6i that suppress CRP
Treatment		
	Optimal initial dose of prednisone/prednisolone	Optimal initial dose of prednisone/prednisolone
	Who should be offered a steroid sparing drug	Who should be offered a steroid sparing drug
	Duration and tapering of GCs	Duration and tapering of GCs
	Which steroid sparing drug to use and when to stop it	Which steroid sparing drug to use and when to stop it
	What order to use advanced therapies after an advanced treatment failure	What order to use advanced therapies after an advanced treatment failure
	Determine optimal cost-effective treatment options	Determine optimal cost-effective treatment options

CRP, C-reactive protein; GCA, giant cell arteritis; PMR, polymyalgia rheumatica.

CONCLUSION

While diagnosis of GCA and PMR remains anchored on clinical manifestations, imaging modalities like ultrasound, and PET-CT or MRI are emerging as useful noninvasive ways to assess patients with high clinical suspicion for GCA and to detect subclinical GCA. Management of GCA and PMR is undergoing rapid advancements focused on identifying new steroid-sparing or steroid-discontinuing therapies. IL-6 receptor inhibitors, namely tocilizumab and sarilumab, have been approved for treatment of GCA and PMR, respectively. JAK inhibitors, specifically upadacitinib, are showing good efficacy in treating GCA. Several agents such as secukinumab, rituximab, and possibly other therapies show promising results but phase3 RCTs are required to determine their overall benefit in these disease entities. The evolution of these therapeutic developments will hopefully contribute to refining clinical guidelines and elevating standard of care for patients with GCA and/or PMR.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
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Imaging in vasculitis

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Purpose of review
Systemic vasculitides are characterized by inflammation of blood vessels. Their classification is based on the size of the blood vessels involved – large, medium, or small. Vasculitis early diagnosis and reliable monitoring are crucial to establish a treatment plan and prevent serious complications. Based on these considerations and depending on the location of the affected vessels, the importance of imaging modalities including ultrasonography (US), magnetic resonance Imaging (MRI), magnetic resonance angiography (MRA), computed tomography (CT), computed tomography angiography (CTA), and [18F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (FDG-PET/CT) has progressively increased. In addition to physical exam and laboratory data, these imaging tools offer complementary information about vascular changes occurring in vasculitis.
This review summarizes the different imaging modalities being utilized to diagnose and monitor vasculitis.

Recent findings
The most recent update for the use of imaging in vasculitis is referenced in the 2023 European Alliance of Associations for Rheumatology (EULAR) recommendations and the American College of Rheumatology (ACR) guidelines in 2021. Recent advances in PET imaging in large vessel vasculitis include improved technological imaging acquisition and the use of novel radiotracers for cellular and immune targets. FDG-PET has now been demonstrated to have high sensitivity and specificity to detect temporal arteritis.

Summary
Imaging plays a significant role in the evaluation of vasculitis and continues to gain importance in the diagnosis and monitoring of disease activity. Differences exist between the ACR guidelines, which advocates for temporal artery biopsy, and the EULAR guidelines, which favors imaging modalities for the initial evaluation and diagnosis of large vessel vasculitis (LVV). Prerequisites for appropriate clinical management utilizing imaging in patients with vasculitis are the availability and access to skilled clinicians to interpret the images and the cost of these techniques not being prohibitive.

Keywords
imaging, magnetic resonance imaging, positron emission tomography, ultrasound, vasculitis

INTRODUCTION

Vasculitis is a group of complex and heterogeneous conditions characterized by inflammation of the blood vessels. The classification of vasculitis is based on the size of the blood vessels involved – large, medium, or small – and each type has distinct clinical features, etiologies, and treatment approaches. An image of a normal vessel with the various histologic structures as well as an overview of the various types of vasculitis is shown in Fig. 1. The diagnosis of vascular disease presents considerable diagnostic challenges due to its varied manifestations. Its accurate diagnosis is critical to guide treatment decisions. Fortunately, recent advances in magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), positron emission tomography (PET), PET-MRI, positron emission tomography-computed tomography (PET-CT), computed tomography angiography (CTA), and ultrasound (US) have enabled earlier and more accurate diagnoses. These cutting-edge imaging modalities offer unique advantages to visualize vascular inflammation and assess the extent and severity of the disease. They offer clinicians valuable insights

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KEY POINTS

- Advanced imaging modalities allow for the assessment of vessel involvement and treatment monitoring in vasculitis.
- There are pros and cons to the various modalities.
- Depending on the vessel size, degree and stage of inflammation, one modality may be preferred over the others.

into the disease's pathophysiology, improving detection, characterization, and monitoring of vascular inflammation and damage.

This overview aims to summarize the current state of imaging in vasculitis, highlighting the latest advances and their potential to transform patient outcomes (Table 1).

IMAGING IN VASCULITIS: DIAGNOSIS AND TREATMENT MONITORING

Recommendations in 2023 by the European Alliance of Associations for Rheumatology (EULAR) emphasize the importance of an early imaging test, depending on the availability of the imaging technique, to support the clinical diagnosis [1▪▪]. US is considered the first-line imaging diagnostic test for suspected giant cell arteritis (GCA), with axillary arteries being included in the examination. The latest American College of Rheumatology (ACR) management guidelines for GCA in 2021 gives preference for temporal artery biopsy (TAB) over US and MRI of cranial arteries for the diagnosis of GCA [2], which differs with EULAR and other recommendations [3–5]. As an alternative to US, cranial and extracranial arteries can be examined by FDG-PET or MRI [1▪▪]. Computed tomography angiography (CTA) only allows for assessment of GCA but no

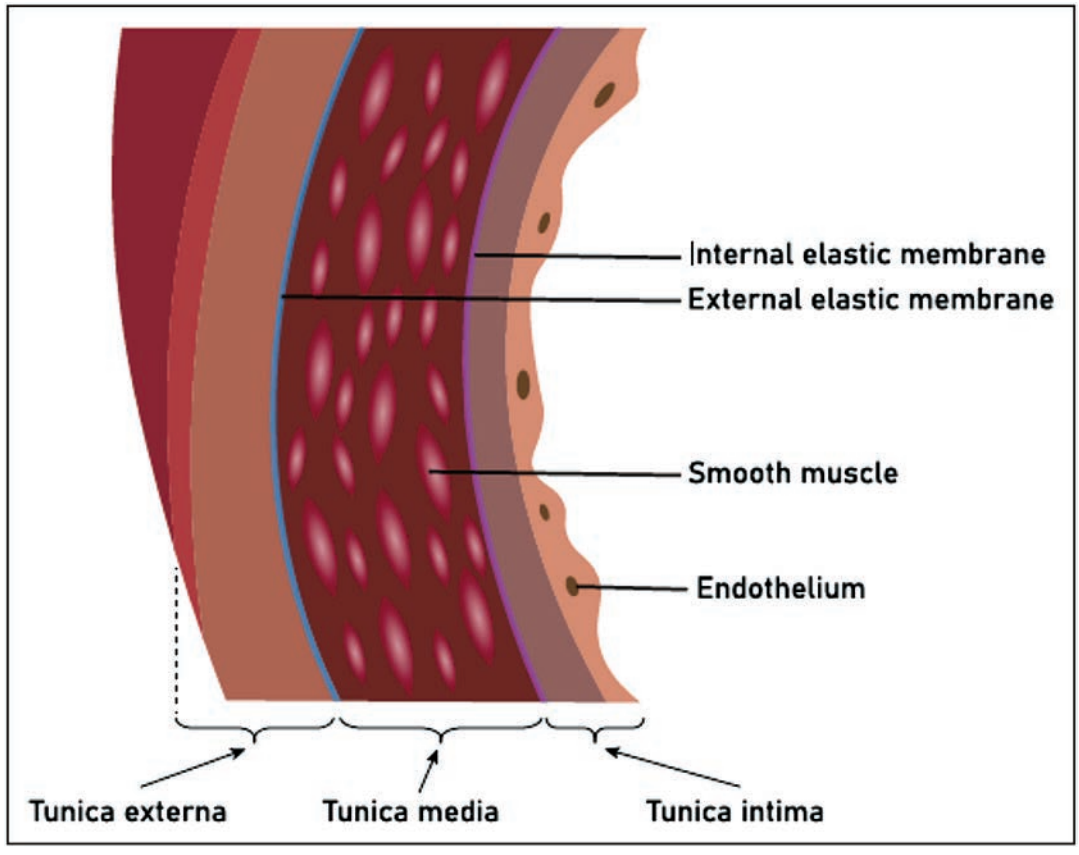

FIGURE 1. Histologic anatomy of a muscular blood vessel. This image shows the histologic anatomy of a muscular blood vessel. The three basic layers are the tunica externa, media, and intima. The layers are separated by thin connective tissue layers namely, the internal and external elastic membranes. In vasculitis, various areas are specially targeted by the immune system resulting in the clinical phenotype based on the size of the vessel involved. A general classification of the various vasculitides include: Large vessel: giant cell arteritis; Takayasu disease. Medium vessel: polyarteritis nodosa; Kawasaki disease. Small vessel: ANCA-associated: granulomatosis with polyangiitis; eosinophil granulomatosis with polyangiitis; microscopic polyangiitis. Immune complex associated: anti-GBM disease; IgA vasculitis; cryoglobulinemic vasculitis; anti-C1q vasculitis. Other: Behcet disease; Cogan's syndrome; cutaneous leukocytoclastic angiitis.

Table 1. Pros and cons of imaging modalities in vasculitis

Imaging modality	Tissue target	Pros	Cons
Ultrasound	Large vessels medium vessels	-Inexpensive -Short acquisition time -High spatial resolution -Can be performed during the clinical exam	-Requires a high frequency transducer -Limited access of the thoracic aorta
MRI/MRA	All vessels	-No radiation -No contrast needed -Noninvasive	-More expensive than US -Not for claustrophobic patients -Long imaging time
CT	All vessels	-Good for large vessels -Delineates inflammation	-Radiation exposure -Contrast agent exposure
PET/CT	All vessels	-Better than MRI -Identifies calcifications	-Can be expensive -Radiation exposure -Persistent tracer uptake - Difficulty differentiating active vasculitis from atherosclerosis

other forms of large vessel vasculitis. For Takayasu arteritis, MRI is the preferred imaging modality with FDG-PET, CT, or US being alternatives [1▪▪].

Imaging is not routinely recommended for follow-up, but it may provide important information regarding disease activity. US and MRI/MRA of cranial arteries may show improvement after treatment, while arterial wall thickening, stenosis, and aneurysms of the large vessels usually persist. Progression of the latter lesions may raise suspicion for inadequately controlled disease. Imaging follow up in Takayasu by MRA, potentially supplemented by US, is needed to monitor vascular damage progression. These imaging tools are preferred by not posing radiation risk to young patients with Takayasu vasculitis. The utility of US, CTA, MRI/MRA during treatment for GCA is less well established. CTA and MRA may demonstrate thoracic aorta aneurysm formation, but guidelines for monitoring aortic damage in GCA are lacking. After treatment, FDG PET/CT usually shows a decrease in arterial wall FDG uptake, but not always complete resolution [6]. Therefore, residual FDG uptake during treatment should not be considered as absolute evidence for active disease as it could represent vascular healing or remodeling. Overall, FDG PET/CT has a sensitivity of 77% and specificity of 71% for detecting relapsing or refractory disease in patients with GCA/Takayasu [7]. Thus, findings on imaging should be carefully interpreted.

Below each imaging modality will be discussed in detail regarding their utility in evaluating vasculitis.

Color-coded duplex ultrasound examination

US has been widely adopted and is readily available in European clinics to evaluate vasculitis. It assesses vascular changes of large vessels, but its utility also extends to medium vessel involvement. Duplex Doppler combines the color Doppler image with the 2D greyscale image. This fusion allows for a more comprehensive assessment of the vessel lumen and wall, including the surrounding perivascular tissues [8]. Compared with other noninvasive imaging modalities, color-coded duplex ultrasound examination has the highest resolution, permitting the detection of inflammatory changes in small arteries [9].

High-frequency linear transducers with a B-mode frequency of 15 MHz or greater allow for the visualization of small arteries, including the temporal arteries. A B-mode frequency of 7–15 MHz allows the assessment of extracranial supra-aortic and extremity arteries [10]. A curved array probe with a lower frequency (3.5–5.0 MHz) can be used for assessing large vessels like the aorta and its visceral branches [8]. US has been widely used in the assessment of vasculitis, especially in giant cell arteritis (GCA) [9,11].

For the greatest diagnostic value, US examination of both the central arteries like the axillary arteries and the peripheral arteries such as the temporal arteries should be performed. Using this combination, the diagnostic yield increases [12]. Although US and PET/CT showed a comparable diagnostic accuracy for GCA, there were discrepancies between the two within single vascular regions. Thus, they should be used as complementary methods, with a second imaging modality increasing the diagnostic yield by 16–20% [13].

Magnetic resonance imaging

MRI can help differentiate between active inflammation and chronic changes in vasculitis through several imaging techniques and sequences (Table 2).

Table 2. MRI technique/sequences used in assessing vasculitis

MRI technique/sequence	Pathogenesis of active inflammation	Findings
T2-weighted imaging	Vasculitis presents with increased water content and edema in affected blood vessel walls	Sensitive to water content and edema. T2-weighted sequences can reveal thickening and high signal intensity in the vessel walls, indicating acute inflammation.
Contrast-enhanced imaging	Increased blood flow and vascular permeability, leading to enhancement of affected vessel walls. This enhancement pattern is indicative of active inflammation	Provides insights into vascular perfusion and inflammation
Diffusion-weighted imaging (DWI)	Restricted diffusion due to cellular swelling and increased cellularity	Measures the random motion of water molecules in tissues. DWI detects restricted diffusion, showing high signal intensity in affected vessel walls, indicating active inflammation
Dynamic contrast-enhanced imaging (DCE)	Increased vascularity and vascular permeability, leading to rapid and intense enhancement of the vessel walls during the early phases of contrast injection. This enhancement pattern indicates active inflammation	Assesses the kinetics of contrast agent uptake and washout in tissues
Blood flow measurements	Increased blood flow or turbulent flow within the affected vessels, indicating ongoing inflammation	MRI can assess blood flow characteristics using techniques like arterial spin labeling or phase-contrast imaging

By analyzing the combination of these MRI techniques and sequences, differentiation between active inflammation and chronic changes in vasculitis can be determined. The hallmarks of active inflammation are increased vessel wall thickness, high signal intensity in T2-weighted images, enhancement on contrast images, restricted diffusion on diffusion-weighted imaging, rapid and intense enhancement on DCE-MRI, and abnormal blood flow characteristics. Alternatively, chronic changes in vasculitis show vessel wall thickening without active signs of inflammation or exhibit different enhancement patterns. However, MRI can overestimate the degree of severity of stenosis by up to 15% [14].

Magnetic resonance angiography

MRA is also a valuable imaging modality to diagnose and manage vasculitis by providing detailed images of blood vessels and detecting inflammation. MRA is used to detect and monitor pathological arterial abnormalities such as stenosis and aneurysm in large vessel vasculitis (LVV). It is preferred over CTA for follow-up monitoring as it avoids radiation and the need for iodinated contrast agents (Table 3). MRA imaging sequences identify edema, wall thickness, and contrast enhancement, suggesting ongoing vascular inflammation [15]. There has also been direct comparison between MRA and PET in LVV. As reported by Quinn *et al.* when assessing disease extent, there was fair agreement between MRA and PET, but MRA identified a greater extent of vascular involvement than PET by detecting both arterial wall (thickness, edema) and luminal (occlusion, aneurysm, and stenosis) abnormalities. When assessing disease activity, inter-rater agreement was greater for PET scan reads compared to MRA reads (kappa = 0.84 vs. 0.58), indicating that disease

Table 3. Application of MRA in assessing vasculitis

Visualization of blood vessels	MRA uses magnetic fields and radio waves to produce high-resolution images of blood vessels, allowing assessment of vessel walls, lumen, and surrounding tissues
Detection of inflammation	Identifies inflammation by detecting edema, vessel wall thickening, and contrast agent uptake
Identification of vessel stenosis/occlusion/aneurysm	Diagnoses narrowing, blockages, or aneurysms in blood vessels, which may occur in vasculitis
Monitoring of disease progression	Serial MRA scans track changes in blood vessel morphology and inflammation.
Treatment decisions	Guides treatment, such as identifying areas requiring angioplasty, stenting, or surgical intervention

activity assessed by PET is more reliable than MRA. Edema, especially in increasing number of arterial regions, was the strongest risk factor for an MRA to be interpreted as active. Compared to wall thickness and stenosis, edema also correlated with PET scan activity within the specific arterial regions. Wall thickness and edema on MRA were independently associated with global PET scan disease activity [16].

PET scan

PET imaging is a noninvasive tool that helps in diagnosing and monitoring vasculitis.

FDG-PET scan is a nuclear imaging technique that utilizes a glucose analogue labeled with a fluorine-18 molecule, which is a positron emitter. Once taken into the cells (similar to glucose), FDG undergoes phosphorylation to FDG-6 phosphate, which cannot undergo further glycolysis and thus accumulates in the cells. The emitted positron is detected by a scanner producing a bright signal in the FDG-PET scan reflecting increased glucose metabolism. Cells that have increased glucose metabolism include activated inflammatory cells and malignant cells. Additionally, there is an overexpression of glucose transporters that accumulate glucose and structurally related substances, such as FDG. In vasculitis, leukocyte infiltration of the vessel wall reflects reactive damage to regional tissues [17,18].

Although FDG-PET was previously considered an inadequate imaging modality for assessing temporal arteritis (TA) [10], more current studies have reported that TA can be detected with high sensitivity and specificity [19–21]. The combination of 18F-FDG-PET scan with CT or MRI can be useful in assessing active inflammation and structural damage in vasculitis. Even though FDG-PET has limited value in serial follow up and prediction of relapses, its sensitivity and specificity are acceptable in the early diagnosis of noncranial GCA, cranial GCA with negative biopsy, assessment of immediate response to treatment, and predicting prognosis [22]. In Takayasu arteritis, FDG-PET is useful for early diagnosis and likely to serially assess disease activity. However, the utility of FDG-PET in assessing medium and small vessel vasculitis is limited.

In the evaluation of LVV, FDG uptake is quantified in multiple arterial regions such as the abdominal aorta, aortic arch, carotid arteries, iliofemoral arteries, subclavian, supra-aortic trunk, and thoracic aorta [23]. The interpretation of PET images uses both qualitative and semi-quantitative methods but does not include a standardized definition of vascular inflammation based on the intensity of FDG uptake. The most used FDG intensity uptake classification is based on a 4-point scale suggested by Meller *et al.* and validated by Walter *et al.* [24,25]: none (grade 0), less than liver uptake (grade 1), similar to liver uptake (grade 2) and higher than liver uptake (grade 3). Grades 2 and 3 are relatively specific for vasculitis [26]. A second method is an aorta-to-liver maximal standardized uptake value which has proved highly sensitive (89%) and specific (95%) for GCA [27]. Another semi-quantitative classification is the aorta-to-lung and aorta-to-blood ratios. The third commonly used method is maximum standardized uptake value (SUV_{max}) showing very good sensitivity and specificity in assessing disease activity in Takayasu and GCA [28,29].

Recent advances in PET imaging in large vessel vasculitis include the introduction of digital long axial field-of-view PET/CT, dedicated acquisition, quantitative methodologies, highly sensitive PET camera systems (2.5 mm spatial resolution vs. 4 mm [30]), automated dynamic whole body (D-WB) PET imaging, and the availability of novel radiotracers [31▪▪]. Macrophages visualized by PET tracers target the somatostatin or folate receptor [32▪,33]. Other radiotracers targeting interleukin (IL)-2 receptor, $CD4^+$ and $CD8^+$ molecules on T-cells may offer another important avenue to assess vasculitis [34▪,35]. According to Parreau *et al.* stromal cells (fibroblasts) play an active role in the recruitment and activation of immune cells to the vessel wall [36]. Activated fibroblasts can be detected by using radioactively labeled fibroblast activation protein (FAP) inhibitor using 68Ga or 18F [37,38].

Vascular FDG uptake is not specific for vasculitis and is an important limitation as it may also be observed in atherosclerosis. The differentiation of atherosclerosis from LVV is considered less problematic with PET/CT compared to a stand-alone PET [39].

Another limitation is persistent uptake after treatment and difficulty in distinguishing between subclinical atherosclerosis, persistent disease activity and posttreatment vascular changes [40]. Limited availability, high cost and radiation exposure are other concerns.

CONCLUSION

Imaging plays a significant role in the evaluation of vasculitis and continues to gain importance in the diagnosis and monitoring of disease activity. It is imperative to accurately diagnose and initiate treatment as early as possible to control the disease and prevent long term sequelae resulting from unabated inflammation. In the United States, temporal artery biopsy remains the gold standard to diagnose GCA. However, the most current EULAR recommendations opt for imaging as the diagnostic modality of choice. FDG-PET imaging in vasculitis appears

to have the latest advancements with novel radiopharmaceuticals, a more highly sensitive camera system, and dynamic whole-body technique. Prerequisites for appropriate clinical management utilizing imaging in patients with vasculitis are the availability and access to skilled clinicians to interpret the images and the cost of these techniques not being prohibitive.

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Conflicts of interest

There are no conflicts of interest.

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Urticarial vasculitis

Tülin Ergun

Purpose of review
Urticarial vasculitis is a rare condition manifesting with a variety of clinical presentations ranging from skin limited lesions to life-threatening systemic illnesses. This review aims to highlight the recent findings on the etiology, diagnostic modalities, and therapeutic strategies and course of urticarial vasculitis.

Recent findings
In addition to well established triggers, urticarial vasculitis (UV) cases associated with severe acute respiratory syndrome coronavirus 2 (SARS-Cov2) disease and COVID-19 vaccines, vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome, and adenosine deaminase (ADA) deficiency have been reported. A clinical-dermoscopic model for differentiating urticarial vasculitis has been developed with purpuric patches and globules favoring UV diagnosis and thus diminishing the need for histopathology. The efficacy of treatment modalities has been reviewed, and antihistamines, systemic corticosteroids, omalizumab, cyclophosphamide, tocilizumab, anti-interleukin (IL)-1 agents, and rituximab were shown to have the highest success rates. Regarding the durability of remission, rituximab, dapsone, and MMF were related to long-lasting treatment free responses. The course of hypocomplementemic urticarial vasculitis was investigated in an epidemiological study, revealing 5- and 10-year survival rates of 92% and 83%, respectively. Chronic obstructive pulmonary disease, septicemia, and end-stage renal disease were identified as causes of mortality.

Summary
With the aid of dermoscopy, a noninvasive tool, differentiation from chronic spontaneous urticaria can be made, and the need for histopathological examination can be diminished. Although clear definitions and consensus criteria for performing disease severity are lacking, careful screening is needed to tailor the treatment on an individual basis. Emerging infections like SARS-CoV 2, vaccines, and autoinflammatory disorders like VEXAS syndrome and ADA deficiency are new associations. The optimal use of well established agents like systemic corticosteroids and immunomodulators are mainstay treatment modalities, whereas IL-1 inhibitors, omalizumab, rituximab and Janus Kinase inhibitors may represent viable alternatives in selected cases.

Keywords
complement, leukocytoclastic vasculitis, omalizumab, SLE, urticaria

INTRODUCTION

Urticarial vasculitis (UV) is a rare condition, characterized by urticaria-like lesions lasting longer than 24 h, and resolving with ecchymotic postinflammatory hyperpigmentation. Angioedema can also be seen. In contrast to urticaria which causes itching, UV lesions are painful. Histopathological findings include leukocytoclastic vasculitis and direct immunofluorescence reveals immunoglobulin, complement, or fibrin deposition around blood vessels and sometimes in the dermal-epidermal junction [1,2].

UV can be a skin-limited disease often not accompanied with systemic findings or may be associated with fever and arthralgias, and involvement of several organs including kidneys, lungs, gastrointestinal tract, eyes, heart and can be lethal in some instances [1–4]. UV is categorized into two subtypes based on serum complement levels: normocomplementemic UV (NUV) and hypocomplementemic UV (HUV). HUV is defined as a small-vessel vasculitis characterized by the presence of anti-C1q antibodies in more than half of the patients. A severe form of HUV, known as McDuffie syndrome, is characterized by six or more months of urticaria with hypocomplementemia, in the presence of systemic findings including arthritis/

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KEY POINTS

- Dermoscopic examination is a sensitive, cheap, and fast tool for differentiating UV from chronic urticaria.
- Hypocomplementemic urticarial vasculitis is a serious condition with a 10-year mortality rate of 17%.
- Antihistamines, dapsone, omalizumab, and systemic corticosteroids (CSs) are mainstay treatments in mild disease, whereas immunosuppressives interleukin-1 inhibitors, and rituximab, combined with CSs, are most effective options in severe disease.

arthralgias, glomerulonephritis, uveitis or episcleritis and recurrent abdominal pain [5,6].

UV is a rare condition and the worldwide prevalence is unknown. Its incidence in US population was reported as 0.5 per 100 000 person-years. Also, 9–21% of patients with leukocytoclastic vasculitis were found to have UV. Women are more frequently affected by UV. Pediatric UV is rare, and constitutes approximately 1% of children with vasculitis. Among patients with UV, 80% is normocomplementemic, whereas 9%-21% have hypocomplementemic UV (HUV) or hypocomplementemic UV syndrome (HUVS) [1]. In a recent comprehensive epidemiologic study from Sweden during the years 2000–2015, all HUV cases diagnosed by diagnostic HUV criteria and/or the 2012 Chapel Hill consensus definitions in combination with an anti-C1q antibody test were investigated. Accordingly, 16 patients (14 females, 88%) were identified, with an annual incidence rate per million inhabitants being estimated as 0.7. In line with the literature, urticaria-like lesions (100%), arthritis (88%), biopsy-proven glomerulonephritis (19%), episcleritis/scleritis (19%), and recurrent abdominal pain (13%) were the most common features [7]. The frequency of renal involvement was in line with previous studies from the US (18%) and France (50%) [1,2,8].

The authors estimated 1-, 5-, and 10-year survivals as 100%, 92%, and 83%, respectively. The causes of death were chronic obstructive pulmonary disease, septicemia, and end-stage renal disease [7].

The impact of UV on quality of life has been recently investigated by a web-based questionnaire. Consequently, a remarkable impairment in quality of life was found in patients with UV, mainly associated with long disease duration, high symptom burden, and a high need for therapy [9▪].

ETIOLOGY, TRIGGERS, ASSOCIATIONS

Although the specific etiology of UV remains unknown, it is considered to result from the formation of immune complexes and their deposition in vascular lumen followed by complement activation. The production of antibodies against a collagen-like region on C1q leads to the generation of antigen–antibody complexes and activation of complements, C3a and C5a, which cause mast cell degranulation, resulting in histamine release, vasodilation, enhanced vascular permeability, and the development of urticarial lesions. The complement fragment C5a is a potent chemoattractant for neutrophils, resulting in neutrophil infiltration, release of proteolytic enzymes and vessel damage [10–12].

Most UV cases are idiopathic. Nevertheless, UV can be associated with medications, infections (hepatitis B and C, Lyme disease, infectious mononucleosis), autoimmune disorders (systemic lupus erythematosus, primary Sjögren's syndrome, RA), malignancy, inherited or acquired deficiencies of C3 or C4, and autoinflammatory diseases [2,6,13–22]. Recently, several UV cases related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been reported. In these cases, skin lesions, with characteristic urticarial and annular morphology, were either the presenting sign or appeared a few weeks following recovery from COVID-19 [23–25]. Interestingly, an immunohistochemical study of two Brazilian cases demonstrated immunolabeled nucleocapsid for SARS-CoV-2 in eccrine glands, interstitial dermal cells and small blood vessels [26▪▪]. Thus, SARS-CoV-2 is claimed to serve as an antigen. COVID-19–associated pediatric UV is extremely rare and has not been observed in an international, multicenter study including 41 pediatric vasculitis cases [27]. Antihistamines, oral CS, antimalarials, colchicine, and omalizumab were administered with success. Also, few studies have linked UV with COVID-19 vaccines; mRNA, viral vector and inactivated whole virion. Individuals developed UV, 1–10 days following either dose of vaccination [28,29]. The most commonly used treatment modalities were antihistamines and systemic CSs.

The spectrum of autoinflammatory diseases, associated with UV also seems to be expanding. In addition to well established ones like Muckle-Wells and Schnitzler syndromes, few patients with co-existing vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome and ADA deficiency have been reported [30]. In a study of 25 genetically proven cases with VEXAS syndrome, 88% were found to have cutaneous manifestations. Among these, 4 patients revealed histopathological findings of LCV and urticarial vasculitis [31].

DIAGNOSIS AND WORK-UP

The diagnosis requires co-assessment of clinical and histopathological features. Wheals lasting longer

than 24 h, accompanied by pain/burning, resolving with postinflammatory hyperpigmentation should raise suspicion for UV. The most common challenge is to differentiate UV from chronic spontaneous urticaria (CSU) on clinical basis. Recently, 13 experts participated in a Delphi survey of the European Academy of Allergy and Clinical Immunology taskforce to define criteria for UV diagnosis and differential diagnosis with CSU. The panel agreed that essential criteria to guide a skin biopsy in patients with recurrent wheals should include at least one of the following features: wheal duration >24 h, bruising/postinflammatory hyperpigmentation, and systemic symptoms. Leukocytoclasis and fibrin deposits were identified as a minimum set of UV histological criteria [32▪].

Since clinical features are not specific, a clinical-dermoscopic model for discriminating urticarial vasculitis has been developed. Accordingly, the existence of purpuric patches and globules on dermoscopic examination was significantly more frequent in UV patients (OR: 20.94). In a clinical-dermoscopic analysis, purpuric patches/globules, red linear vessels, along with the persistence of wheals longer than 24 h, were associated with 63% sensitivity, 95% specificity. Positive, negative predictive values and correct diagnosis, confirmed by histopathological examination, were 77.3%, 91.2%, and 88.9% respectively. As a noninvasive, widely available, fast and cheap technique, dermoscopy can be utilized on daily clinical practice to improve the sensitivity of the standard visual inspection and also perhaps decrease the need for biopsy and histopathological examination, which is time-consuming and expensive [33].

Histopathological features, namely, perivascular neutrophilic infiltrate, fibrinoid necrosis, nuclear dust, endothelial cell swelling on upper dermal vessels, and erythrocyte extravasation, confirm the diagnosis. Eosinophils can be seen in the inflammatory infiltrate in patients with NUV [34]. Lesional direct immunofluorescence (DIF) examinations reveal the deposition of immunoglobulin (Ig)M, IgG, IgA, complement, and fibrinogen in /around blood vessels as well as dermoepidermal junction. DIF findings are present in 70–96% and 1–18% of HUV and NUV respectively. In affected cases, lung biopsies reveal leukocytoclastic vasculitis and renal biopsies have findings of proliferative glomerulonephritis, focal necrotizing vasculitis along with tubulointerstitial, crescentic and membranoproliferative glomerulonephritis with C1q deposits [8,35].

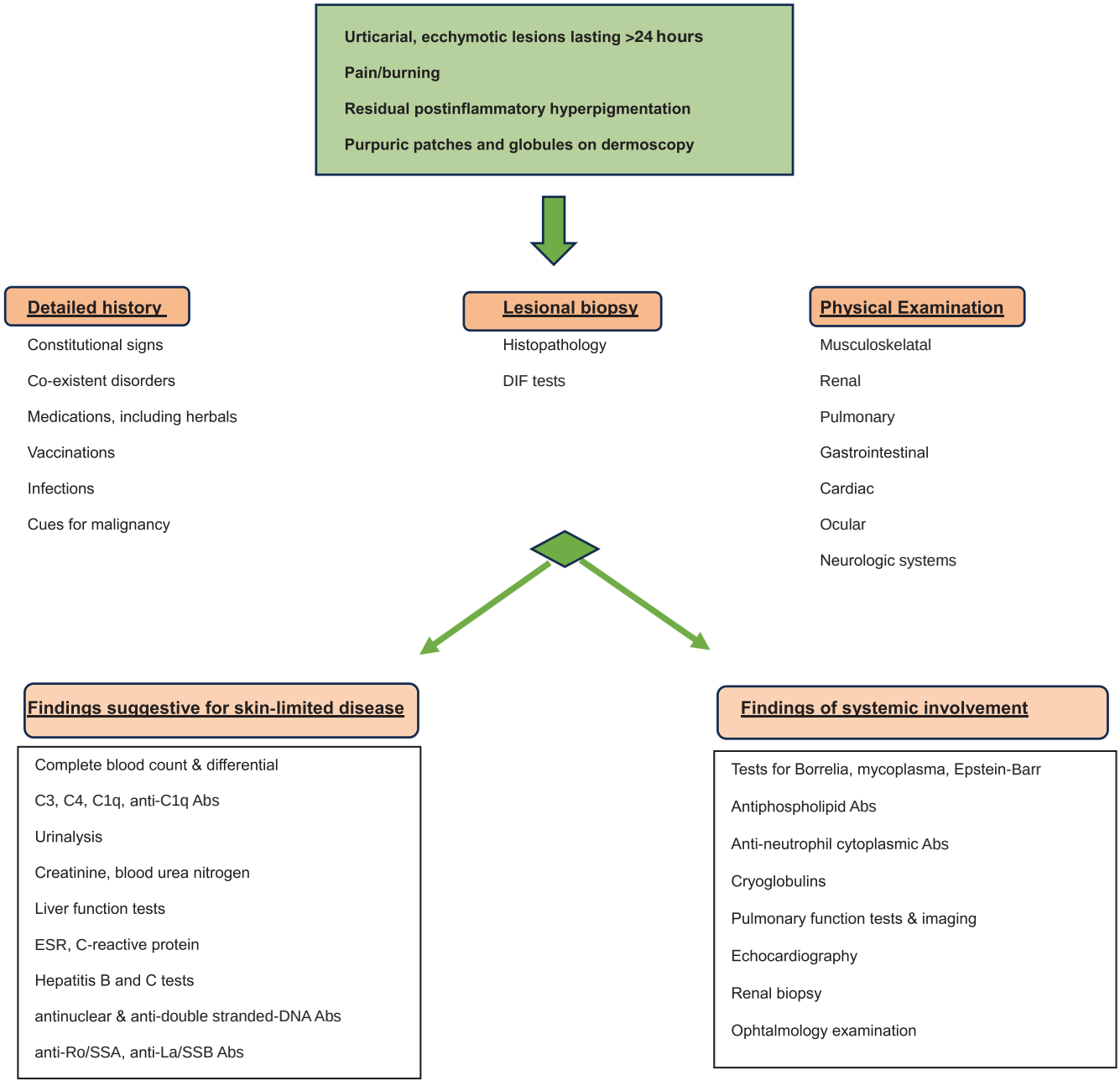
The evaluation of patients with UV poses challenges due to the wide spectrum of associated disease and lack of evidence-based protocols to guide initial evaluation. Nevertheless, a review of systems and examination, along with basic laboratory tests are mandatory to identify the causes and also systemic involvement. Among systemic features, arthralgia, and arthritis are the most common, seen in nearly half of patients, including normocomplementemic ones. Constitutional signs such as fever, malaise, and symptoms and signs of respiratory, renal, cardiac, gastrointestinal, ophthalmologic and central nervous system involvements should be asked carefully [36].

Since most cases with skin-limited disease have no long-term complications, basic laboratory tests, namely, lesional biopsy for histopathological and direct immunofluorescent examinations, C3, C4, C1q, complete blood count with differential, urinalysis, creatinine, blood urea nitrogen, liver function tests, ESR, hepatitis B and C tests, antinuclear and anti(double-stranded)-DNA antibodies should be ordered. Further tests, imaging studies and biopsies to evaluate extracutaneous involvement and triggers can be employed based upon the results of these tests and the presence of features suggestive of severe disease. In patients with no apparent cause, age- and sex-appropriate malignancy screening tests should be performed (Table 1) [34–39].

TREATMENT AND DISEASE COURSE

Treatment of UV is challenging due to the variable clinical spectrum, lack of approved medications, and evidence-based guidelines. Hence management is mainly determined by the severity of skin lesions, systemic involvement, and the co-existent systemic disease. When possible, offending medications should be withdrawn and underlying disorders should be treated. Most patients with skin limited disease are treated by H1-antihistamines (H1AHs), hydroxychloroquine, dapsone, omalizumab, and sCSs. Immunosuppressive medications including methotrexate, mycophenolate mofetil, cyclosporine, cyclophosphamide, azathioprine, tofacitinib, and biological agents such as tocilizumab, anti-TNFs, rituximab and anti-IL-1s are used in selected cases, mostly with severe disease, as monotherapy or as an adjunct to corticosteroids. Several case reports and series report the efficacy of colchicine, nonsteroidal antiinflammatory agents, doxepin, danazol, tranexamic acid, montelukast, thalidomide, pentoxifylline, dapsone, plasmapheresis and IVIG [36–40]. A recent systematic review aimed to address the efficacy of treatment modalities on skin symptoms, graded the response as complete, partial or none, and also evaluated the durability of response. Accordingly, systemic corticosteroids were found as the most effective agents, providing complete and

Table 1. Suggested diagnostic approach and work-up for patients with urticarial vasculitis (Ab: antibody)



partial response in 26% and 55% of patients respectively. Likewise, CS and H_1Ahs use is related to response in 81% of cases. Cyclophosphamide ± CS was effective in 87% and combination of CSs with other immunosuppressive agents, MTX, MMF, CYC, AZA provided 34–60 overall response [41]. Omalizumab (OMA), a humanized IgG1 monoclonal antibody directed against circulating IgE, is an effective agent in treating normocomplementemic urticarial vasculitis (NUV), with high success rates when used as monotherapy. In a 12-week study of 23 patients with NUV, 56.5% had extracutaneous symptoms including arthralgia, fatigue, fever and elevated baseline IgE levels (165.6 kU/l), 17.4% and 56.5% showed complete and partial responses, respectively. The time to significant improvement in wheals and general symptoms was 8 weeks. The lack of response, seen in 26.1% of patients, was related to low baseline total IgE levels (27.7 vs. 293.2 kU/l) and higher rates of extracutaneous symptoms (100.0% vs. 41.2%). No serious adverse events were observed [42]. In line with these results,

complete and partial response, which appeared at 4–16 weeks of treatment, was seen in 67% and 33% of the 6 NUV patients, all unresponsive to various agents including systemic CSs, in an Italian study [43▪▪]. Also, four children, aged 4–6 years, with CS unresponsive UV were successfully treated by OMA with good tolerability [44]. Considering the fact that OMA has an excellent safety profile, being approved for asthmatic patients aged ≥6 years and adults, it can be used as a first-line agent in pediatric and adult NUV cases with high baseline IgE levels. Other biologics, tocilizumab, anti-IL-1 agents, and rituximab, mostly used in HUV, are related to beneficial responses among 100%, 86% and 78% of patients, respectively. Of note, tranexamic acid, danazol, H1Ahs monotherapy, H2 antihistamines, montelukast, pentoxifylline, and doxepin were agents related to the highest chances of treatment failures, being inefficient in nearly 70–100% of cases [41].

A case with UV, unresponsive to various treatments, was reported to respond to tofacitinib [45]. Considering the beneficial responses reported with tofacitinib in case reports of cutaneous small-vessel vasculitis and polyarteritis nodosa, its use can be considered in selected cases, unresponsive to treatment [46–48]. Also, a case with treatment-resistant systemic lupus erythematosus-associated HUV successfully treated with belimumab was reported [49].

The course of UV is unpredictable. In patients with NUV, lesions may resolve within 3–4 weeks, may recur, or become persistent. However, mortality is not seen. In HUV patients, lung is the most difficult to treat site. Also renal involvement can be severe. COPD, end stage renal disease and sepsis are common causes of death [7,40,41].

CONCLUSION

UV is a rare and underestimated condition lacking evidence-based data on pathogenesis, management, and treatment. New studies are limited and mostly include case reports and small series. Although skin-limited or mild phenotypes have a good prognosis, serious forms are related to mortality. Thus, multicenter studies are needed for better understanding the disease pathogenesis and also to optimize treatment responses.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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REVIEW

OPEN

Autoantibodies as putative biomarkers and triggers of cell dysfunctions in systemic sclerosis

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Purpose of review
Antinuclear autoantibodies represent a serological hallmark of systemic sclerosis (SSc), with anticentromere, antitopoisomerase-I, and anti-RNA polymerase III antibodies routinely assessed for diagnosis, clinical subset classification, and prognosis. In addition, an increasing number of autoantibodies have been demonstrated to play a pathogenic role by mediating different SSc manifestations. This review aims to give an overview on autoantibodies as putative biomarkers in SSc and discuss their possible pathogenic role as triggers of cell dysfunctions.

Recent findings
Over the years, different autoantibodies have been proposed as biomarkers aiding in diagnosis, disease subtype classification, disease progression prediction, organ involvement, as well as in understanding treatment response. Increasing literature also indicates functional autoantibodies as direct contributors to SSc pathogenesis by exerting agonistic or antagonistic activities on their specific cognate targets.

Summary
In SSc, search and validation of novel autoantibodies with higher diagnostic specificity and more accurate predictive values are increasingly needed for early diagnosis and specific follow-up, and to define the best therapeutic option according to different disease subsets. Moreover, since autoantibodies are also emerging as functional pathogenic players, a better unraveling of their possible pathomechanisms becomes essential to identify new targets and develop promising therapeutic agents able to neutralize their effects.

Keywords
autoantibodies, biomarkers, pathogenic mechanisms, scleroderma, systemic sclerosis

INTRODUCTION

Systemic sclerosis (SSc; scleroderma) is an immune-mediated rheumatic disease characterized by a pathogenic triad comprising peripheral microvasculopathy, progressive tissue fibrosis, and autoimmunity. Autoantibodies represent a serological hallmark of SSc, as they are observed in the serum of more than 90% of patients [1–3]. The most relevant SSc-associated autoantibodies are antinuclear autoantibodies (ANA), particularly antitopoisomerase-I antibodies (ATA; anti-Scl70), anticentromere antibodies (ACA), and anti-RNA polymerase III antibodies (ARA), which are routinely assessed for diagnosis, clinical subset classification, and prognosis [1–3]. Other less frequently detected ANA in SSc react with different intracellular targets such as ribonuclear proteins (anti-U1 RNP, anti-U3 RNP/antifibrillarin, and anti-U11/U12 RNP autoantibodies) or nucleolar antigens [anti-Th/To, antinucleolar organizer region 90 (anti-NOR90), anti-Ku, and anti-polymyositis/Scl (PM/Scl) autoantibodies] [1–3]. SSc-associated ANA are usually disease specific and mutually exclusive, that is, patients do not switch ANA subset type throughout their disease duration [1–3]. Despite their diagnostic and prognostic value, the role of ANA in SSc pathophysiology has not been completely clarified. On the other hand, other autoantibodies detected in SSc, despite not being useful in clinical daily practice, have been demonstrated to play a pathogenic role by mediating several disease manifestations. The main targets of these so-called

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KEY POINTS

- Circulating antinuclear autoantibodies such as anticentromere, antitopoisomerase-I, and anti-RNA polymerase III antibodies are routinely assessed for SSc diagnosis, clinical subset classification, and prognosis.
- Other autoantibodies detected in SSc patients have been demonstrated to play a pathogenic role by mediating cell dysfunctions and a variety of disease manifestations.
- Search and validation of novel autoantibodies with higher diagnostic specificity and more accurate predictive values are increasingly needed for early diagnosis and follow-up of SSc patients, and to better shape therapeutic trajectories.
- A better understanding of the possible pathomechanisms of functional autoantibodies in SSc at the cell level is crucial to identify new targets and develop new therapeutic strategies able to block their effects.

functional autoantibodies are represented by cell types, such as endothelial cells (antiendothelial cell antibodies; AECA); cell surface receptors, such as angiotensin II type 1 receptor (anti-AT1R antibodies), endothelin (ET)-1 type A receptor (anti-ETAR antibodies), and platelet-derived growth factor receptor (anti-PDGFR antibodies); and extracellular matrix (ECM) components such as fibrillin or matrix metalloproteinases (MMP) [1,3,4]. The present review aims to give an overview on autoantibodies as putative biomarkers in SSc, and to discuss their possible pathogenic role as triggers of multiple cell dysfunctions.

AUTOANTIBODIES AS BYSTANDERS AND BIOMARKERS IN SYSTEMIC SCLEROSIS

ACA, which are mainly directed toward three centromere proteins, namely CENPA, CENPB, and CENPC, are the most frequently observed autoantibodies in SSc patients and are typically associated with the limited cutaneous SSc (lcSSc) subset, featuring long-lasting Raynaud's phenomenon followed by years of progressive thickening of the skin [2,5]. The small percentage of ACA+ patients displaying the diffuse cutaneous SSc (dcSSc) subset is characterized by a lower occurrence of visceral complications and improved survival [6]. ACA+ SSc patients have a higher risk of developing pulmonary arterial hypertension (PAH) [7–9], as well as calcinosis, esophageal dysmotility, and gastrointestinal manifestations [5,8,10]. ACA seropositivity also represents a positive prognostic marker, as patients manifest not only a higher postdiagnosis survival rate but also a lower incidence of scleroderma renal crisis (SRC), cardiac manifestations, and interstitial lung disease (ILD) [5,8]. In a recent study evaluating different ACA isotypes in very early SSc (i.e., patients with ACA-IgG, Raynaud's phenomenon, and/or puffy fingers and/or abnormal nailfold capillaroscopy, but not fulfilling the ACR/EULAR 2013 criteria for SSc) and definite SSc patients, ACA-IgG and ACA-IgM levels were found significantly higher in patients with definite SSc, with progression to established disease being associated with higher IgG isotype at baseline [11]. On these bases, the authors proposed ACA isotype levels as possible biomarkers to identify very early SSc patients at risk for disease progression [11].

Also known as anti-Scl70, ATA represent the second most common ANA in SSc patients and target topoisomerase-I, a nuclear protein that controls DNA tertiary structure during transcription. Usually associated with dcSSc, they have been found not only to correlate with the presence of digital ulcers [9], increased mortality rates in patients with SRC [12], and a higher incidence of ILD [1,5,8,9,13], but also to be a negative prognostic factor in dcSSc patients [8]. Nevertheless, not all ATA+ patients are characterized by a fast evolution of fibrosis, since some of them are only characterized by restrained cutaneous and pulmonary involvement [2,14]. This could be caused by the presence of different ATA isotypes, as SSc patients with both ATA-IgG and ATA-IgM more often experience progression of the disease with respect to those with only ATA-IgG [15]. In a recent study, Liem *et al.* [16] examined the sex-specific risk of ATA on mortality and found that although SSc male patients were more frequently positive for ATA and had an increased mortality rate compared with female patients, such higher mortality was only due to their sex and not to ATA frequency. Conversely, ILD development was found to strongly associate with ATA positivity but not with sex [16,17]. In a Thai SSc cohort of patients, high ATA levels were also recently found to correlate with a short ILD onset, cardiac involvement, and the presence of extensive skin stiffness [18]. Interestingly, when compared to ACA+ SSc patients, ATA+ individuals more frequently showed severe microangiopathy, as assessed by nailfold videocapillaroscopy [19]. In a recent literature study investigating if ATA prevalence in patients with very early SSc could be associated with disease progression, ATA positivity was found to be much lower in very early SSc than in individuals fulfilling the ACR/EULAR 2013 criteria, and ATA-IgG levels were demonstrated to have the tendency to be higher in patients with a more rapid disease

progression [20]. Of note, SSc patients with an inverted phenotype, that is, ATA+ but with a lcSSc subset, were reported to be characterized by an increased risk of developing ILD respect to those ACA+ [21]. The presence of ATA has also been found to correlate with a higher risk of cancer [22,23], particularly in the lung in patients with ILD [24]. Finally, circulating B cells from ATA+ SSc patients were seen to abundantly produce both ATA-IgG and ATA-IgA, demonstrating a recent and continuous immune activation that was shown to correlate with the presence and severity of ILD [25].

The presence of ARA is almost exclusive of dcSSc patients and associates with a rapid progression of skin involvement not further evolving after reaching the modified Rodnan skin score (mRSS) peak and, in some cases, also regressing [2,5,26,27]. ARA positivity can also be considered a strong predictor of SRC [1,2,8,12], gastrointestinal manifestations [10], gastric antral vascular ectasia (i.e., a SSc vascular manifestation possibly leading to iron deficiency anemia or acute gastrointestinal bleeding) [2,28], and cancer [1,2,5,29,30]. Interestingly, an association of ARA with silicone breast implant rupture was demonstrated in a multicenter Italian study performed on SSc patients [31]. As far as other clinical associations, ARA are generally not correlated with either SSc-ILD or PAH [2,8]. A recent blood/skin transcriptional and proteomic analysis revealed that different circulating protein markers of fibrosis and different gene expression profiles were displayed by SSc patients positive for ATA or ARA, suggesting that patient stratification according to ANA antibody subtypes may account for different outcomes in clinical trials targeting specific pathogenic mechanisms [27]. A different cell cluster gene expression profile between ATA+ and ARA+ SSc patients was subsequently confirmed by the same group through sc-RNAseq on SSc skin, with transforming growth factor β (TGFβ) ligand-receptor interactions occurring mainly in fibroblasts and smooth muscle cells in early ATA+ dcSSc patients, while mostly in endothelial cells in early ARA+ dcSSc individuals [32▪]. In addition, in a study performing gene expression profiling of SSc skin lesions followed by functional enrichment analysis, Inamo identified several pathways that were specifically upregulated according to the type of SSc autoantibody, namely "keratinocyte differentiation" pathways for ACA, "nuclear factor κB signaling" and "cellular response to TGFβ stimulus" pathways for ARA, "interferon α/β signaling" pathways for anti-U1 RNP, and "cellular response to stress" pathways for ATA [33].

Anti-U1 RNP antibodies target the U1 small nuclear ribonucleoprotein (snRNP) complex and are not SSc-specific, as they are usually employed for the diagnosis of mixed connective tissue disease [5]. In a recent monocentric retrospective study, SSc patients positive for anti-U1 RNP were found to be more often of Afro-Caribbean origin and to frequently manifest overlaps with Sjögren's syndrome and/or systemic lupus erythematosus when compared with anti-U1 RNP negative SSc individuals [34]. Moreover, these individuals not only commonly developed ILD, myopathy, and kidney involvement, but also had a worse overall survival [34]. In another study on SSc patients with more than one disease-associated autoantibody type, Clark *et al.* [35] demonstrated that double positivity significantly influences the clinical phenotype of patients. The authors found indeed that, when compared with single positive patients, those with anti-U1 RNP and ATA were younger, commonly showed the dcSSc subset, and had higher rates of overlap features, while those with anti-U1 RNP and ACA had a significantly higher prevalence of PAH and were more frequently affected by myositis [35]. In two other studies, anti-U1 RNP+ SSc patients were also found to be characterized by a higher incidence of SRC or an increased risk of cancer within 2 years from diagnosis [12,22].

Anti-U3 RNP (antifibrillarin) antibodies are directed against a protein of the U3 snRNP complexes called fibrillarin and are detected in 5–14% of SSc patients, where their presence has been associated with the highest incidence of PAH and cardiac involvement [5,8]. Anti-U3 RNP positivity also correlates with severe gastrointestinal involvement and poor prognosis [36]. Although in early SSc, anti-U3 RNP+ patients are characterized by a higher hazard of death, their long-term survival was found to be better compared to anti-U3 RNP negative SSc patients [8].

Anti-U11/U12 RNP antibodies, which target the U11/U12 RNP complex consisting of several proteins involved in alternative mRNA splicing, have been recently demonstrated to associate with the presence of both gastrointestinal manifestations and ILD [37–39]. Moreover, as these antibodies have been found to correlate with an augmented risk of cancer at SSc onset, the possibility of cancer-induced autoimmunity in this subset of patients has been suggested [40].

Anti-Th/To antibodies, directed against numerous proteins of the RNase mitochondrial RNA processing complex, are quite specific for SSc, where their prevalence varies between 1 and 13%, and where they have been associated with lcSSc, ILD, PAH, and SRC [2,5,9,41].

The anti-NOR90 antibodies, which target a 90 kDa nucleolar protein and can be detected in other

autoimmune diseases, are present in approximately 5% of SSc patients, where they mostly associate with the lcSSc subset and have a favorable prognosis [2]. In a very recent multicentric cohort study, Dima *et al.* [42] found that anti-NOR90+ SSc patients were more frequently female, had lower mRSS, and lower prevalence of gastrointestinal symptoms respect to anti-NOR90 negative individuals. Anti-NOR90 antibodies were also associated with the presence of SRC, proteinuria, and renal dysfunction [9].

Anti-Ku antibodies, that recognize the Ku complex, that is, a heterodimer involved in DNA repair, transcriptional regulation, and telomere activity, are present in about 2–6% of SSc patients and are commonly associated with the scleroderma-polymyositis overlap syndrome [2].

Anti-PM/Scl antibodies target the human exosome, a macromolecular complex involved in RNA degradation and processing, and are found not only in SSc, but also in other diseases such as polymyositis, dermatomyositis, and scleroderma-polymyositis overlap syndrome [2,43]. In a study performed on a multicenter international cohort of SSc patients, anti-PM/Scl+ subjects were clinically characterized by a higher incidence of myositis, ILD (with a good functional outcome in the first decade of the disease), calcinosis, cutaneous signs of dermatomyositis, and pulmonary fibrosis [2,43,44]. On the contrary, esophageal involvement, PAH, digital ulcers, and cardiac involvement were less frequent [2,43,44]. Anti-PM/Scl+ patients were also found to be at very low risk of death only in the first 10 years of the disease [8]. In another recent study, Richardson *et al.* [45] reported that anti-PM/Scl antibody positivity may confer an increased risk of a heavy burden of calcinosis in SSc patients.

Antibodies to Ro52, a ubiquitous protein overexpressed during inflammation and apoptosis, can be detected in different systemic autoimmune diseases and represent the second most common autoantibody in SSc, overlapping with disease-specific autoantibodies [2]. Several authors demonstrated a significant association between anti-Ro52 positivity and a higher frequency of ILD diagnosis in SSc [2,46,47]. In particular, the presence of anti-Ro52 strongly correlated with pulmonary function loss over time, with the loss rate linearly increasing with anti-Ro52 antibody levels [46]. According to a recent study performed on an Australian cohort of SSc patients, the presence of anti-Ro52 antibodies represents an independent prognostic factor for mortality, and a risk factor for the development of PAH, independently from ILD occurrence [48]. In a recent case series, Kruzer *et al.* [49] characterized ANA+ SSc patients lacking SSc-specific ACA, ATA, and ARA (triple negative), reporting that these individual manifested a high prevalence of anti-Ro52 antibodies, an enrichment for myositis specific antibodies, and an increased risk of ILD. Finally, anti-Ro52 antibodies were found to be more prevalent in cancer-associated SSc [24,29]. Nevertheless, the presence of anti-Ro52, together with anti-U1 RNP or anti-Th/To autoantibodies, was reported to be associated with a decreased risk of cancer when compared with the presence of anti-Ro52 autoantibodies alone [30].

Anticarbamylated protein antibodies (anti-CarPA), that is, antibodies targeting proteins subjected to the posttranslational modification termed carbamylation, which is almost irreversible and can be triggered by inflammation, have been detected in SSc patients, where they have been reported to correlate with the dcSSc subset, ILD, and the presence of digital ulcers [50,51]. No relevant clinical association was conversely found between anti-CarPA positivity and SSc skin involvement assessed with mRSS [52].

Antibodies against vinculin, a protein involved in cell adhesion, were reported not only to be augmented in SSc patients respect to healthy individuals but also to be significantly higher in patients with PAH when compared to those with ILD [53]. Moreover, increased levels of antivinculin autoantibodies were reported to associate with slower gastric transit in SSc patients, thus representing a potential useful marker of SSc-related gastrointestinal manifestations [54,55].

By means of a proteome-wide planar antigen array on a small cohort of SSc patients, antiphosphatidylinositol-5-phosphate 4-kinase type 2 beta (PIP4K2B) and anti-AKT serine/threonine kinase 3 (AKT3) antibodies were found to be the most promising candidates as potential skin and lung fibrosis-associated autoantibodies in SSc [56].

In recent years, numerous novel autoantibodies, including antieukaryotic initiation factor 2B (eIF2B), antiamino acyl-transfer ribonucleic acid synthetase (ARS), and anticyclic citrullinated peptide (CCP) antibodies have been found to be associated with SSc-ILD but, due to their low prevalence, such a clinical association remains to be further validated in larger cohorts of patients [57–59].

In a very recent study, antiinterferon gamma inducible protein 16 (IFI16) autoantibodies were reported to correlate with lcSSc, PAH, and reduced overall survival in patients negative for all SSc-specific autoantibodies [60].

Autoantibodies against annexin V, a calcium-regulated phospholipid-binding protein involved in cell life cycle, exocytosis, and apoptosis, were evaluated in a SSc population at baseline and after a 24-month follow-up and were found not only to be stable overtime but also to be associated with a

higher occurrence of PAH and digital microangiopathy [61].

As far as gastrointestinal involvement is concerned, Mc Mahan *et al.* [62] identified antibodies to gephyrin, a protein of the enteric nervous system expressed in human myenteric ganglia, as novel SSc-associated autoantibodies significantly higher among patients with severe constipation, distention, and bloating. In addition, Nakane *et al.* [63] reported a correlation between antiganglionic nicotinic acetylcholine receptor antibodies and various digestive-system problems in SSc patients, such as gastroesophageal reflux, constipation, appetite loss, vomiting, and diarrhea.

In a very recent study employing a global antibody profiling strategy to identify novel antibodies as possible biomarkers in SSc, Liang *et al.* [64] found a significant increase in antiprotein arginine methyltransferase 5 (PRMT5) antibody among SSc patients. These antibodies exhibited strong diagnostic accuracy in distinguishing patients with SSc from healthy individuals, and their titer was also found to correlate with disease progression [64]. Interestingly, immunization of mice with PRMT5 and consequent anti-PRMT5 autoantibody production was shown to induce skin and lung fibrosis [64].

In another study employing a protein array-based approach to identify and validate SSc-specific autoantibodies, antismall nuclear ribonucleoprotein polypeptide A antibodies were proposed as a novel serological biomarker for SSc diagnosis [65]. Moreover, SSc patients with antibodies against Sjögren's syndrome/scleroderma autoantigen 1 were found to manifest an increased risk of cancer compared with negative patients [66].

High levels of anti-AT1R and anti-ETAR antibodies have been reported to be associated with severe SSc vascular manifestations such as digital ulcers and PAH, and to be putative biomarkers to predict the development of such complications and overall mortality [67–69].

The detection of antiphospholipid autoantibodies frequently occurs in SSc, and a significant association of antiβ2 glycoprotein 1 positivity was found with active digital ulcers [70]. Moreover, a higher risk of cardiovascular events was reported in SSc patients positive for antiβ2 glycoprotein 1 and anticardiolipin antibodies [21,71]. A meta-analysis also revealed that antiphospholipid autoantibody positivity correlated with PAH, renal disease, digital ischemia, venous thrombosis, and miscarriage [72,73].

Finally, in an interesting study comparing the performance of three different SSc patient stratification models (i.e., according to LeRoy's cutaneous subtypes, autoantibody profile, or combination of both) in predicting survival, disease progression, and different organ involvement, Elhai *et al.* [74▪▪] demonstrated that the antibody-only model outperformed the cutaneous-only one in predicting overall survival, disease progression, and renal and lung fibrosis, while the combined model was more suitable for the prediction of digital ulcers and PAH.

A schematic representation of the main antigenic targets of SSc-associated autoantibodies is shown in Fig. 1, whereas a summary of the main associations between autoantibody profile and SSc clinical phenotypes is reported in Table 1.

AUTOANTIBODIES AS PATHOGENIC PLAYERS IN SYSTEMIC SCLEROSIS

Apart from representing relevant disease biomarkers, increasing evidence also suggests a direct pathogenic role of autoantibodies in SSc [3,4,75–77]. Autoantibodies can be defined as pathogenic when they contribute to the development of a disease by mediating its organ and/or systemic manifestations. In particular, functional autoantibodies exert their pathogenic function by directly activating (agonistic effect) or inhibiting (antagonistic effect) a specific molecular pathway through the binding to their cognate autoantigen. In particular, functional autoantibodies can be classified into six different categories based on their mechanism of action, namely activation of target receptors, blockade of target receptors, induction of receptor internalization, neutralization of target ligands, neutralization of other soluble extracellular antigens, and disruption of protein-protein interaction [78].

ATA have been suggested to be pathogenic mainly by promoting incessant inflammation and chronic fibrosis. Indeed, it has been reported that, after the release of topoisomerase-I from apoptotic endothelial cells and its subsequent binding to the fibroblast membrane, ATA can be recruited on the cellular surface of fibroblasts, possibly via CCR7 or proteoglycans, where they lead to increased monocyte adhesion and activation, ultimately resulting in the release of both proinflammatory and profibrotic cytokines [75–77,79] (Fig. 2). Interestingly, in a very recent work, May *et al.* [80▪▪] have demonstrated for the first time that ATA are also able to penetrate live cells and localize in the nucleus, where they exert direct effects on intranuclear processes, in particular causing a dose-dependent inhibition of topoisomerase-I activity.

As far as ACA are concerned, it has been proposed that these autoantibodies lead to vascular complications by inhibiting CCR3/CENPB-mediated vascular repair on vascular smooth muscle cells [75,77,79] (Fig. 2). Further evidence of the

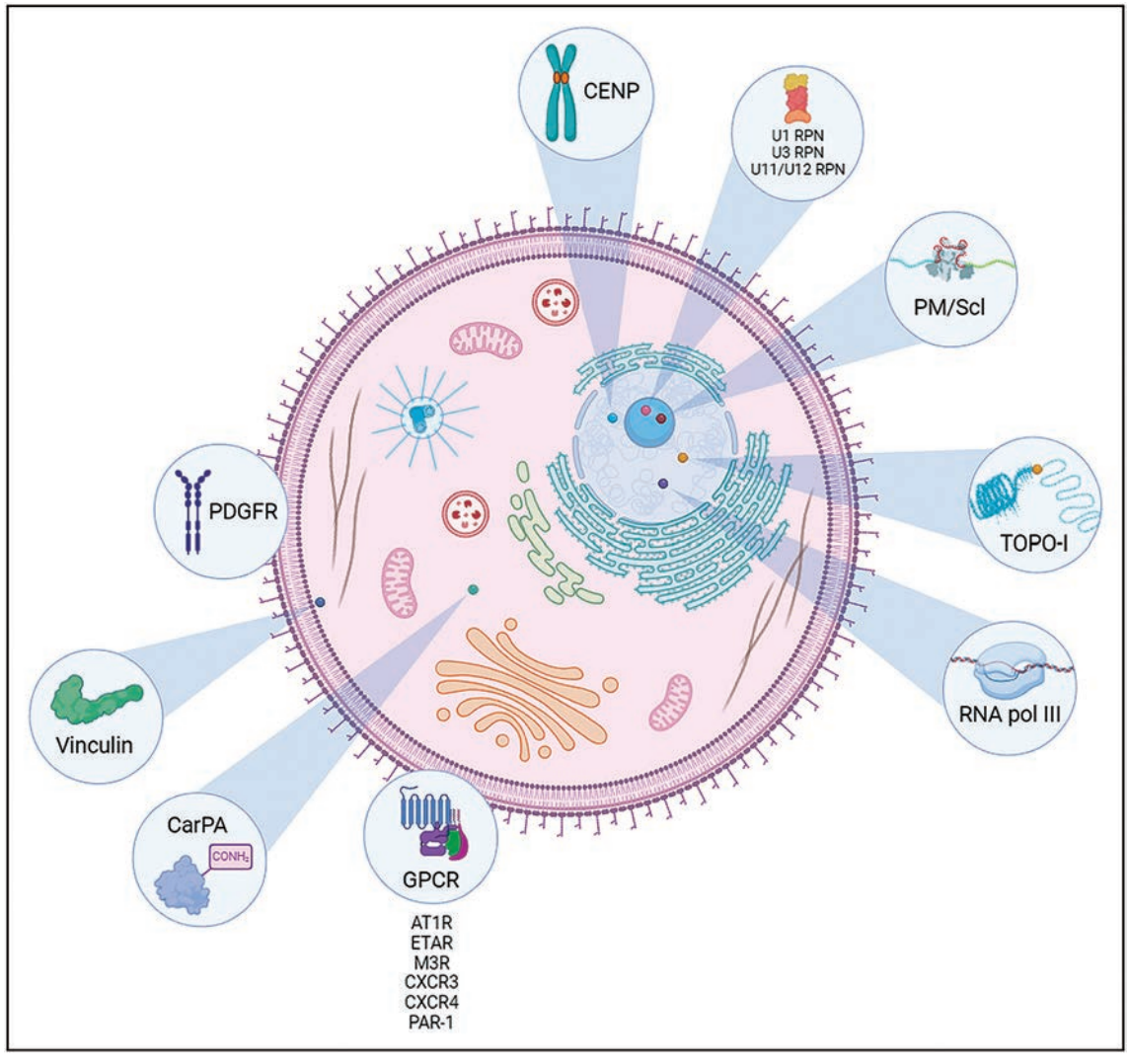

FIGURE 1. Antigenic targets of the most relevant systemic sclerosis-related autoantibodies. Among antinuclear autoantibodies, anticentromere antibodies are directed toward three centromere proteins, namely CENPA, CENPB, and CENPC; antitopoisomerase-I antibodies target topoisomerase-I (TOPO-I); and anti-RNA polymerase III antibodies bind to RNA polymerase III, the enzyme involved in DNA transcription. Within the nucleolus, anti-U1 RNP, anti-U3 RNP, anti-U11/U12 RNP autoantibodies recognize ribonuclear proteins, while anti-PM/Scl autoantibodies are directed toward a macromolecular complex involved in RNA degradation and processing. The most important plasma membrane and cytoplasmic targets are represented by platelet-derived growth factor receptor (PDGFR); vinculin; carbamylated proteins (CarPA); and several G protein-coupled receptors (GPCR) such as angiotensin II type 1 receptor (AT1R), endothelin-1 type A receptor (ETAR), muscarinic acetylcholine receptor M3 (M3R), C-X-C motif chemokine receptor 3 and 4 (CXCR3, CXCR4), and protease-activated receptor-1 (PAR-1).

pathogenicity of both ATA and ACA also derives from the fact that in-vitro stimulation of human dermal fibroblasts with such antibodies can induce a significant increase in profibrotic markers and apoptosis resistance [81], and that incubation of calf pulmonary arterial endothelial cells with SSc sera containing ACA and ATA is able to accelerate endothelial cell aging [82].

A possible pathogenic role of ARA has been proposed on the basis of a study reporting that ARA+ SSc patients with cancer were characterized by a missense mutation in the gene encoding the RPC1 subunit of RNA polymerase III, with the production of an altered protein that, once recognized by the patient's immune system, was able to induce a cross-reactive immune response against the

Table 1. Main associations between autoantibody profile and systemic sclerosis clinical phenotypes

Autoantibody	Antigenic target	Association with clinical phenotypes	References
ACA	Centromere proteins CENPA, CENPB, and CENPC	lcSSc; PAH; calcinosis; esophageal dysmotility; GI manifestations; higher postdiagnosis survival rate; lower incidence of SRC, cardiac manifestations, and ILD	[2,5–10]
ATA	Topoisomerase-I	dcSSc; DU; ILD; increased mortality rates in patients with SRC; severe microangiopathy; higher risk of cancer	[1,2,5,8,9,12,13,15–23]
ARA	RNA polymerase III	dcSSc; SRC; GI manifestations; gastric antral vascular ectasia; cancer	[1,2,5,8,10,12,26–30]
Anti-U1 RNP	U1 RNP	ILD; myopathy; kidney involvement; worse overall survival; SRC; increased risk of cancer	[12,22,34]
Anti-U3 RNP	U3 RNP/Fibrillarin	PAH; cardiac involvement; severe GI involvement; poor prognosis	[5,8,36]
Anti-U11/U12 RNP	U11/U12 RNP	GI manifestations; ILD; cancer	[37,38,39,40]
Anti-Th/To	Proteins of the RNase MRP complex	lcSSc; ILD; PAH; SRC	[2,5,9,41]
Anti-NOR90	90 kDa nucleolar protein	lcSSc; SRC; renal dysfunction; lower mRSS; lower prevalence of GI symptoms	[2,9,42]
Anti-Ku	Ku complex	Scleroderma-polymyositis overlap syndrome	[2]
Anti-PM/Scl	Human exosome	myositis; ILD; calcinosis; dermatomyositis; pulmonary fibrosis; calcinosis	[2,43,44,45]
Anti-Ro52	Ro52	ILD; PAH; cancer	[2,24,29,46,47,48,49]
Anti-CarPA	Carbamylated proteins	dcSSc; ILD; DU	[50,51]
Antivinculin	Vinculin	PAH; GI manifestations	[53–55]
Anti-PIP4K2B and anti-AKT3	PIP4K2B and AKT3	Skin and lung fibrosis	[56]
AntieIF2B, anti-ARS, and anti-CCP	eIF2B, ARS, and CCP	ILD	[57–59]
Anti-IFI16	IFI16	lcSSc; PAH	[60]
Antiannexin V	Annexin V	PAH; digital microangiopathy	[61]
Antigephyrin and antigAChR	Gephyrin and gAChR	GI symptoms	[62,63]
Anti-AT1R and anti-ETAR	AT1R and ETAR	DU; PAH	[67–69]
Antiphospholipids	β2 glycoprotein 1 and cardiolipin	DU; cardiovascular events; PAH; renal disease; digital ischemia; thrombosis	[21,70–72]

ACA, anticentromere antibodies; AKT3, AKT serine/threonine kinase 3; ARA, anti-RNA polymerase III antibodies; ARS, amino acyl-transfer ribonucleic acid synthetase; AT1R, angiotensin II type 1 receptor; ATA, antitopoisomerase-I antibodies; CarPA, carbamylated protein; CCP, cyclic citrullinated peptide; dcSSc, diffuse cutaneous SSc; DU, digital ulcers; eIF2B, eukaryotic initiation factor 2B; ETAR, endothelin-1 type A receptor; gAChR, ganglionic nicotinic acetylcholine receptor; GI, gastrointestinal; IFI16, interferon gamma inducible protein 16; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; MRP, mitochondrial RNA processing; NOR90, nucleolar organizer region 90; PAH, pulmonary arterial hypertension; PIP4K2B, phosphatidylinositol-5-phosphate 4-kinase type 2 beta; PM/Scl, polymyositis/Scl; RNP, nuclear ribonucleoprotein; SRC, scleroderma renal crisis; SSc, systemic sclerosis.

normal RPC1 protein, thereby contributing to the development of SSc [75].

Interestingly, immune complexes purified from the sera of SSc patients bearing different autoantibody specificities (ACA, ATA, ARA, or anti-Th/To) were found to induce proinflammatory and profibrotic pathways in healthy fibroblasts and human umbilical vein endothelial cells, presumably via toll-like receptors [83].

In the last decade, a growing body of evidence has highlighted that several G protein-coupled receptors (GPCR) are targeted in SSc by functional autoantibodies, whose altered levels, cross-reactivity, and synergistic effects may influence the disease pathogenesis [4,84]. Even if not specific to SSc, autoantibodies reactive to AT1R and ETAR, two vascular GPCR, are present in the majority of patients, where they exert agonistic effects [85]. Their capacity to determine SSc-like lesions was initially tested *in vitro* by using IgG fractions of SSc patients positive for anti-AT1R and anti-ETAR on different cell types [69,86,87]. In human

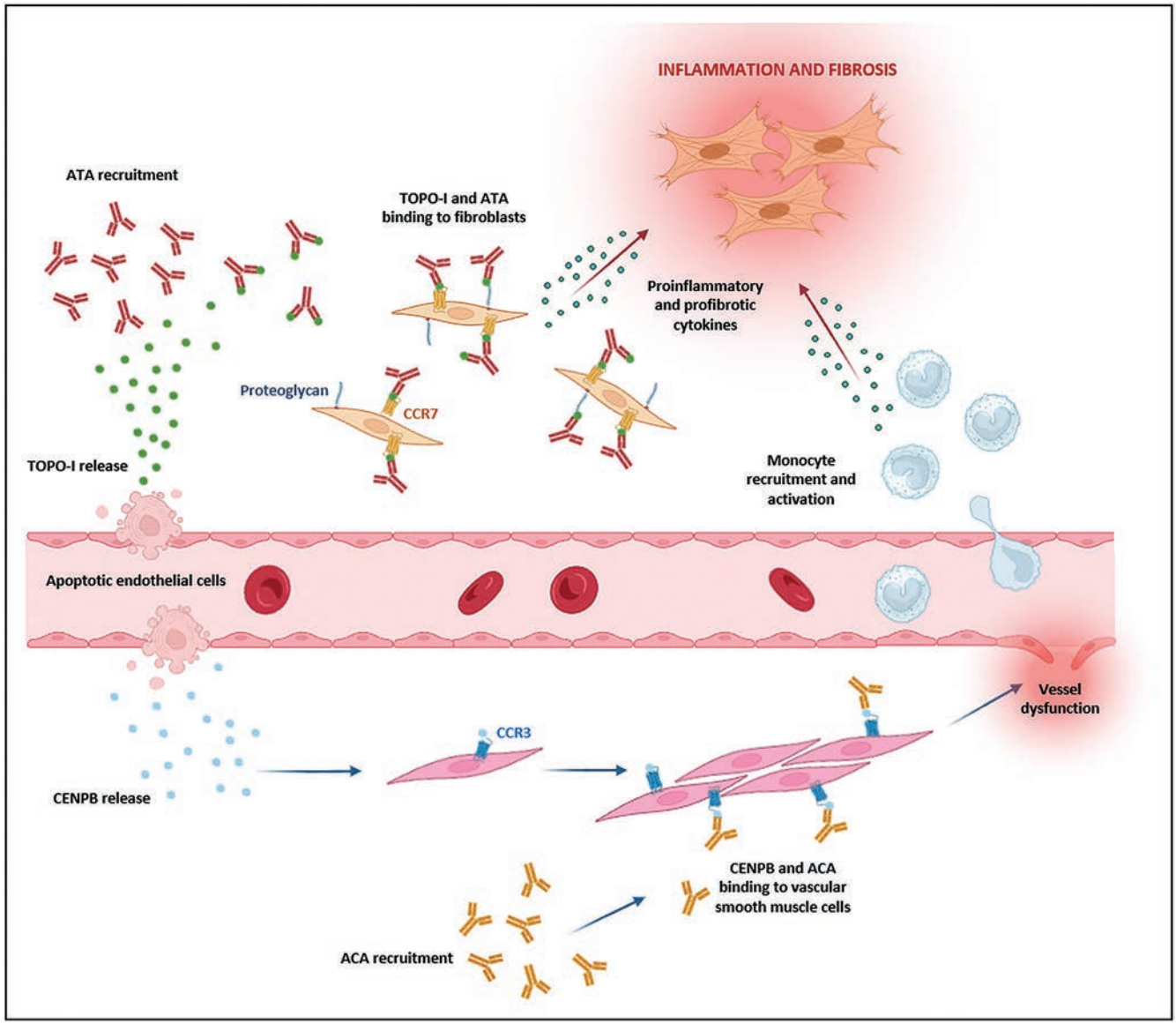

FIGURE 2. Pathogenic mechanisms of action of antitopoisomerase-I antibodies and anticentromere antibodies. After the release of topoisomerase-I (TOPO-I) from apoptotic endothelial cells and its subsequent binding to the fibroblast membrane, ATA can be recruited on the cellular surface of fibroblasts, possibly via CCR7 or proteoglycans, where they lead to increased monocyte adhesion and activation, ultimately resulting in the release of both proinflammatory and profibrotic cytokines. Similarly, after the release of CENPB by apoptotic endothelial cells, ACA leads to vascular complications by inhibiting CCR3/CENPB-mediated vascular repair on vascular smooth muscle cells.

microvascular endothelial cells, both antibodies were found not only to amplify profibrotic TGFβ gene expression [69] but also to cause a reduction in wound repair abilities and to induce the expression of interleukin (IL)-8 and vascular cell adhesion molecule 1, with consequent increased recruitment of inflammatory immune cells [86]. In addition, anti-AT1R and anti-ETAR IgG derived from SSc patients with SRC were found to trigger human microvascular endothelial cell proliferation through the mitogen-activated protein kinase pathway, with the subsequent activation of the E26 transformation-specific-1 transcription factor and the induction of tissue factor promoter [88]. Collectively, these data revealed an unknown connection among anti-AT1R and anti-ETAR IgG-induced intracellular signaling, endothelial cell proliferation, and coagulation in the context of SRC [88]. In healthy human dermal fibroblasts, these autoantibodies were reported to increase type-1 collagen expression [86], while in peripheral blood cells, they were found to induce the expression of different proinflammatory cytokines such as interferon-γ, tumor necrosis factor-α, IL-1, IL-6, IL-8, and IL-17, as well as of

chemokine (C-C motif) ligand-18, a marker for alternative monocytic activation [87,89,90]. Potential pathogenic effects of anti-AT1R and anti-ETAR antibodies were also tested *in vivo* by the passive transfer of anti-AT1R and anti-ETAR positive IgG fractions of SSc patients into healthy C57BL6/6J mice [68,86]. Interestingly, when histologically assessed, the animals displayed structural lung alterations such as increased cellular density and interstitial infiltrations [86], as well as inflammatory pulmonary vasculopathy [68]. Moreover, after a single IgG transfer, increased neutrophil count was found in bronchoalveolar lavage fluid of SSc IgG-treated mice when compared with healthy control IgG-treated mice, whereas no differences were observed in the counts for macrophages, lymphocytes, or eosinophils [86]. The ability of anti-AT1R to amplify angiotensin II-mediated effects was confirmed in AT1R-immunized mice that, indeed, developed skin and pulmonary inflammation, as well as dermal fibrosis [91].

Antibodies targeting the muscarinic acetylcholine receptor M3 (M3R), an organ-specific GPCR, have been found to contribute to gastrointestinal dysmotility in SSc by inducing neuropathy through the blockade of cholinergic neurotransmission in early disease stages, and by causing myopathy through the inhibition of acetylcholine action on smooth muscle cells with disease progression [3,4,76,92,93]. Interestingly, the administration of intravenous IgG was demonstrated to improve SSc-related gastrointestinal symptoms presumably by neutralizing anti-M3R antibodies [93–95].

Among the GPCR, C-X-C motif chemokine receptor (CXCR)3 and CXCR4 regulate several cell functions and have been involved in the pathogenesis of fibrosis, with anti-CXCR3 antibodies showing potential agonistic activity [4,77]. Although anti-CXCR3 and/or anti-CXCR4 antibodies have been found to be increased in SSc, with anti-CXCR3 autoantibodies from SSc patients preferentially binding to intracellular epitopes instead of extracellular epitopes as in healthy individuals, their pathological functional activity remains to be elucidated [4,76,77,96].

Antibodies against protease-activated receptor-1 (PAR-1), a GPCR with key roles in regulating the interplay between coagulation and inflammation, have been detected in SSc patients with SRC, where they agonistically activate PAR-1 and engage a signaling cascade that finally induces IL-6 production by endothelial cells [3,4,97].

Figure 3 shows a schematic representation of the main pathogenic effects of autoantibodies targeting GPCR.

Stimulatory autoantibodies directed against PDGFR, whose activation leads to the induction of proliferation, chemotaxis, and actin reorganization in fibroblasts and smooth muscle cells, have been detected in SSc sera and have been demonstrated to stimulate the conversion of normal fibroblasts into myofibroblasts [78,98,99]. Nevertheless, in the wake of subsequent studies reporting that these antibodies did not exert an agonistic activity and were not SSc-specific [100,101], the authors performed additional experiments and concluded that in the same SSc patient both agonistic and nonagonistic anti-PDGFR autoantibodies may coexist, and that stimulatory autoantibodies can be detected only in SSc but not in healthy individuals or patients with other connective tissue diseases [102,103]. The first indirect evidence of anti-PDGFR antibody pathogenicity was provided by a small study demonstrating that dermal fibroblasts isolated from SSc patients treated with the immunosuppressant rituximab displayed a downregulation of type-1 collagen expression and of the anti-PDGFR autoantibody-triggered intracellular signaling [99,104]. The first direct evidence of anti-PDGFR antibody pathogenicity was instead demonstrated in a skin-humanized severe combined immunodeficiency mouse model engrafted with skin biopsies of healthy donors injected with total IgG purified from the serum of SSc patients [105]. Indeed, mice acquired a cutaneous SSc-like phenotype when injected with pooled SSc IgG as well as with human agonistic anti-PDGFR mAbs obtained from SSc B cells [99,105]. Stimulatory anti-PDGFR autoantibodies from SSc patients have also been demonstrated to induce proliferation and migration of human pulmonary vascular smooth muscle cells *in vitro* [99,106].

In SSc patients, antibodies specific to estrogen receptor-α have been shown to correlate with clinical parameters of disease activity and severity, and with both a higher T cell apoptotic susceptibility and alterations in T regulatory cell homeostasis [107]. Furthermore, anti-CD22 antibodies have been found to reduce the phosphorylation of the inhibitory B cell response regulator CD22, thus stimulating B cell response [108].

Anti-MMP-1 and anti-MMP-3 antibodies were found to inhibit MMP-1 and MMP-3 activity, respectively, suggesting their potential role in impaired ECM turnover and consequent fibrosis development [109,110]. In addition, antifibrillin-1 antibodies purified from the sera of some ethnic groups of SSc patients were demonstrated to activate normal fibroblasts by inducing gene and protein expression of ECM components [111]. However, these latter were not detected in other SSc cohorts [3,112].

Although AECA are not specific to SSc, numerous studies reported their association with disease pulmonary and vascular involvement [3,75]. *In vitro*,

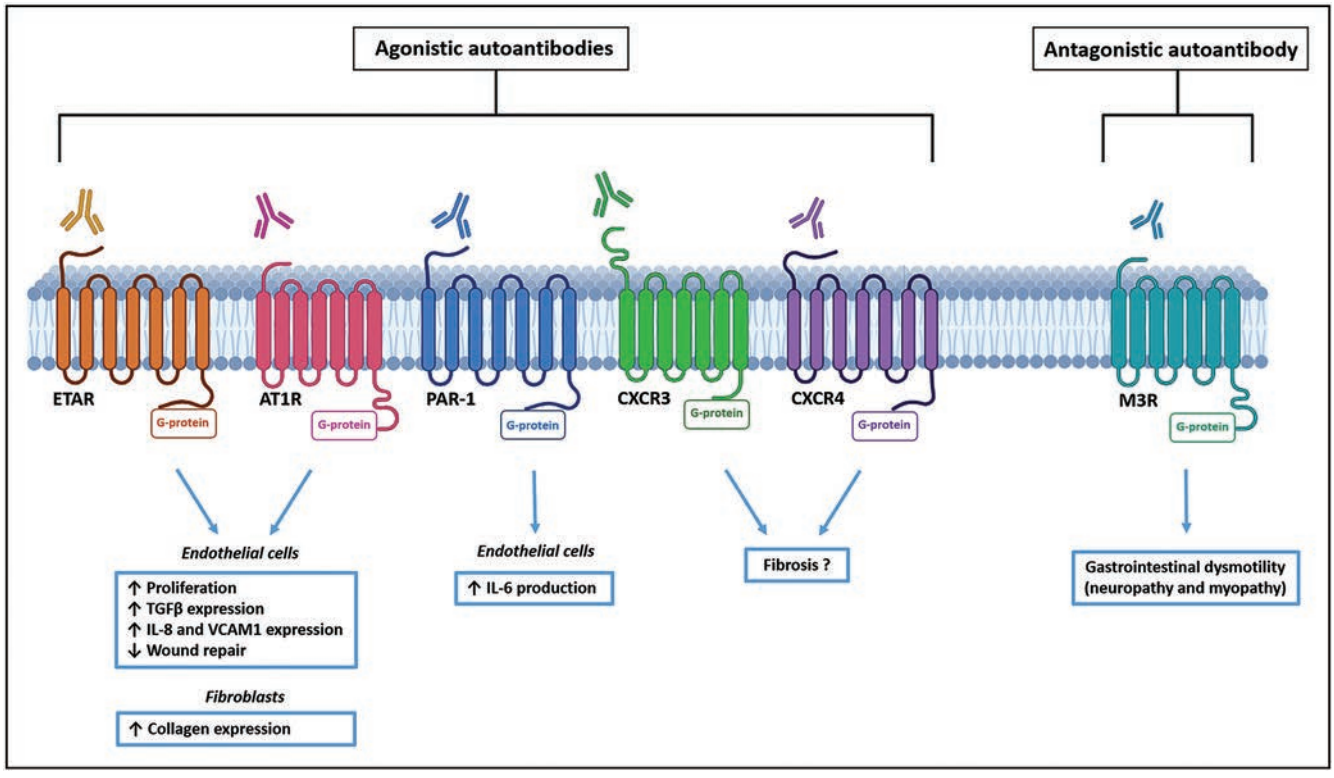

FIGURE 3. Main pathogenic effects of functional autoantibodies targeting G protein-coupled receptors in systemic sclerosis. Autoantibodies against endothelin-1 type A receptor (ETAR), angiotensin II type 1 receptor (AT1R), protease-activated receptor-1 (PAR-1), C-X-C motif chemokine receptor 3 (CXCR3) and CXCR4 exert agonistic effects, while autoantibodies targeting the muscarinic acetylcholine receptor M3 (M3R) carry out antagonistic activity. IL, interleukin; TGFβ, transforming growth factor β; VCAM1, vascular cell adhesion molecule 1.

AECA have been demonstrated to induce apoptosis in human dermal microvascular endothelial cells and to activate human umbilical vein endothelial cells by upregulating adhesion molecules and increasing interleukin secretion while, *in vivo*, the transfer of these antibodies in a chicken model of SSc resulted mainly in increased endothelial cell apoptosis [3,75].

In a recent study, antibodies targeting skin keratinocytes were detected in SSc circulation and were found to activate human keratinocytes *in vitro* by inducing toll-like receptor-2 and receptor-3 signaling finally leading to IL-1α release, likely contributing to downstream skin fibrosis [113▪].

Antibodies targeting telomerase and the shelterin protein telomeric repeat-binding factor 1 were found in an American cohort of SSc patients, where they associated with lung disease and short lymphocyte telomere length, suggesting that these autoantibodies may exert direct pathogenic effects on telomeres [114].

Finally, in a study by Murthy *et al.* [115], IgG serum fraction of SSc patients was found to modulate the secretome of monocyte-like cells toward a proinflammatory and profibrotic activity profile, suggesting their possible direct contribution in regulating immune cell activity.

CONCLUSION

In SSc, autoantibodies are currently valuable clinical tools routinely used for both diagnosis and disease subtype classification, and may even be prognostic for disease progression, organ involvement, and/or cancer development. Nevertheless, search and validation of novel autoantibodies with higher diagnostic specificity and more accurate predictive values are increasingly needed for early diagnosis, a specific follow-up of patients, and to better define therapeutic choices across the different SSc subsets. Moreover, since autoantibodies are also emerging as functional pathogenic players in SSc, a better unraveling of their possible pathomechanisms becomes essential to both identify new targets and develop promising therapeutic agents able to neutralize their downstream effects at the cell level.

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Conflicts of interest

There are no conflicts of interest.

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The role of neutrophil extracellular traps in inflammatory rheumatic diseases

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Purpose of review
Dysregulation in neutrophil extracellular trap (NET) formation and degradation has been reported in several inflammatory rheumatic diseases. This review summarizes the recent advances in the understanding the role of NETs in the context of inflammatory rheumatic diseases.

Recent findings
NET formation is enhanced in peripheral blood of patients with large vessel vasculitis and polymyalgia rheumatica. NETs are detected in affected organs in autoimmune conditions, and they might play pathological roles in tissues. Several understudied medications and supplements suppress NET formation and ameliorate animal models of inflammatory rheumatic diseases. NETs and anti-NET antibodies have potential utility as disease biomarkers.

Summary
Growing evidence has suggested the contribution of NET dysregulation to the pathogenesis of several inflammatory rheumatic diseases. Further research is warranted in regard to clinical impact of modulating aberrant NET formation and clearance in inflammatory rheumatic diseases.

Keywords
biomarker, disease pathogenesis, inflammatory rheumatic diseases, neutrophil extracellular traps, therapeutic target

INTRODUCTION

Neutrophil extracellular traps (NETs) are web-like structures composed of extracellular DNA fibers and neutrophil granule proteins. They were originally described as a defense mechanism against bacteria [1]. Since then, beyond important roles in infections, NET formation and its significance have been studied in the context of autoimmunity and various chronic inflammatory diseases. This is partly because NETs contain several key autoantigens such as double-stranded DNA, myeloperoxidase, and native and modified histones, known to be key targets of the immune system in autoimmune diseases [2]. NETs also induce endothelial cell apoptosis, activate inflammasome pathways, and enhance production of type I interferons (IFNs) via cytosolic DNA sensors and endosomal toll-like receptors (TLRs) [3]. Furthermore, recently, chronic stress has been reported to contribute to tumor metastasis-promoting NET formation through glucocorticoid receptor stimulation [4▪], suggesting that glucocorticoids, key in the treatment of inflammatory rheumatic diseases, directly modulate NET formation. Therefore, growing evidence has revealed the putative contribution of NETs to pathogenesis of inflammatory rheumatic diseases. This review aims to summarize the recent updates of the roles of NETs in inflammatory rheumatic diseases and potential therapeutic implications.

Systemic lupus erythematosus

The contribution of NETs to the pathogenesis of established systemic lupus erythematosus (SLE) has been extensively studied to date [5]. Incomplete SLE (iSLE) is known as an early stage of lupus, which does not meet classification criteria for the disease, but does manifest SLE-associated symptoms. High

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KEY POINTS

- Neutrophil extracellular trap (NET) formation in peripheral blood neutrophils is enhanced in several inflammatory rheumatic diseases.
- NETs can be identified in affected organs in patients with systemic rheumatic diseases and contribute to disease pathogenesis.
- Several medications may be effective in rheumatic diseases, at least in part, through their modulation of NETs.
- NETs and anti-NET antibodies may have a potential use as biomarkers of disease diagnosis and severity.

frequency of low-density granulocytes (LDGs), a distinct subset of proinflammatory neutrophils with enhanced NET-forming abilities [6], and increased circulating NET remnants have been described in iSLE as well as in established SLE patients [7▪▪]. This suggests that dysregulated neutrophil function and enhanced NET formation and/or impaired clearance also occur in early phases of SLE and may contribute to enhanced autoantigen externalization and modification.

Neutrophils and NETs are present in affected organs of murine lupus and SLE patients. For example, neutrophils infiltrate the kidney of lupus-prone MRL/*lpr* mice [8], and a deficiency in receptor for advanced glycation end-products (RAGE) leads to improved proteinuria accompanied by decreased glomerular neutrophil infiltration and NET formation [9]. Additionally, defensin-α3 transcript, which encodes for a neutrophil antimicrobial peptide bound to NETs, is upregulated in human lupus kidney in correlation with proteinuria and *IFNα2* mRNA levels [10▪]. These reports support that neutrophil infiltration in lupus kidney may play pathological roles in lupus nephritis. Nevertheless, the exact contribution of neutrophils and NETs to lupus kidney damage need to be further investigated.

Some previously understudied lupus medications and supplements may affect NET formation. Transcriptomic study of whole blood showed that anifrolumab, a type I interferon receptor 1 blocking antibody, inhibits cell death pathways, such as NETosis and apoptosis [11]. In addition, taxifolin, a flavonoid that exhibits antioxidative and anti-inflammatory effects, inhibits NET formation *in vitro* and *in vivo*, as well as inflammatory cytokine and autoantibody production, and venous thrombosis in murine models of SLE and antiphospholipid syndrome (APS) [12]. Furthermore, the steroid doses were recently found to negatively correlate with the degree of NET formation [13]. Whether this inhibition of NET formations is due to direct inhibitory effects by corticosteroids or is secondary to amelioration of disease need to be clarified in future studies.

NETs and antibodies targeting NETs may be useful as biomarkers for predicting treatment responses or stratifying SLE patients. Circulating NET remnant, defined by serum HMGB1-DNA and neutrophil elastase–DNA complexes, are higher in patients with active disease and proliferative lupus nephritis. Additionally, higher circulating NET levels at flare may predict future severe renal failure and nonachievement of complete remission [14]. Antibodies targeting NET components are elevated in patients with SLE, although their pathophysiological role and their utility in clinical practice remain to be elucidated in multicohort studies [15].

Rheumatoid arthritis

A hallmark of rheumatoid arthritis (RA) is the production of autoantibodies including rheumatoid factor, anticitrullinated, and anticarbamylated protein antibodies. A role of dysregulated NET formation in RA has been described for over a decade [16]. Peptidyl arginine deiminase 4 (PAD4) plays an important role in NET formation by citrullinating histones thereby promoting chromatin decondensation. In granulocyte-colony stimulating factor (G-CSF)-modified collagen-induced arthritis (CIA) model in the C57/B6 (B6) background, mice with global *Padi4* deficiency and mice with hematopoietic lineage–specific *Padi4* deficiency showed amelioration of arthritis and decreased NET remnants in plasma and synovial tissue [17]. Furthermore, the PAD4 inhibitor JBI-589 inhibits NET formation and shows efficacy in murine models of RA, such as the CIA model in DBA/1 mice and G-CSF-modified CIA model in B6 mice [18]. Hence, targeting PAD4 could represent a candidate therapeutic strategy in this disease.

Similar to citrullination, carbamylation is one of the posttranslational modifications which play putative pathogenic role in RA pathogenesis and its association with bone damage. Carbamylated NETs (and more specifically carbamylated histones present in these structures) can promote bone-erosive osteoclastogenesis in an accelerated manner, at least in part, through activation of TLR-4 [19▪]. This report suggests that carbamylation of NET proteins may be an alternative pathway to promote bone damage in this disease. Additionally, a recent study reported that low-dose ethanol inhibits NET formation and alleviates arthritis of CIA model in DBA/1 mice [20]. The implications of this finding remain to be determined.

Antineutrophil cytoplasmic antibody-associated vasculitis

Previous studies have implicated dysregulated NET formation and aberrant neutrophil function in the pathogenesis of antibody-associated vasculitis (AAV) [21]. In recent reports, serum from patients with AAV enhance NET formation when compared to healthy donors [22,23]. Furthermore, DNaseI activity (implicated in clearing NETs from circulation and tissues), was reported to be lower in patient with otitis media with AAV at onset, compared with patients in remission or healthy volunteers with otitis media with effusion [23].

Previously, NETs have been reported to be a source of the mitochondrial DNA that can activate cytoplasmic inflammatory pathways [24]. Plasma total cell-free DNA and mitochondrial DNA, but not genomic DNA, were reported to be increased in AAV compared with healthy donors. Plasma mitochondrial DNA associated with AAV diagnosis and disease activity [25] but the specificity remains to be determined given previous links with other systemic rheumatic diseases.

NETs may also play pathogenic roles in affected organs. Previous studies have demonstrated that NET components cause endothelial injury [26]. In kidneys, NETs were observed in kidney biopsy samples from active AAV glomerulonephritis patients. NETs, defined by the colocalization of citrullinated Histone H3 (CitH3) and MPO, were mainly found in glomerular lesions with active inflammation such as crescent formation, fibrinoid necrosis, and disruptions in glomerular basement membrane [22]. Of note, NETs have been found to evade phagocytosis by macrophages (efferocytosis) via cell surface CD47 expression on neutrophils, which may promote AAV initiation and perpetuation of disease features due to due to the decrease in the clearance of NETs and prolonged autoantigen exposure [27▪].

Several recent reports have proposed the therapies for AAV that inhibit NET formation. These have included Bruton's tyrosine kinase inhibitor tirabrutinib [28], the mitochondrial matrix protein cyclophilin D [29], astaxanthin, a carotenoid with anti-inflammatory and antioxidant effects [30], nuclear factor-erythroid 2-related factor 2, a transcription factor that works as an antioxidative stress controller [31], and a modulator of Sphingosine-1-phosphate (S1P) receptor FTY720 [32]. These compounds were reported to inhibit ANCA-induced NET formation, and/or ameliorate rat or mouse models of AAV.

Large vessel vasculitis

Giant cell arteritis (GCA) and Takayasu arteritis (TAK) are the most common forms of large vessel vasculitis. Among recently identified three novel loci (*MFGE8*, *VTN*, and *CCDC25*) that associate with risk for giant cell arteritis, *CCDC25* is associated to NETs [33]. Mechanistically, *CCDC25* encodes NETs transmembrane receptor to enhance motility of recipient cells [34], suggesting a putative association between NETs and the pathogenesis of large vessel vasculitis. Indeed, NETs are detected in the adventitia, adjacent to the *vasa vasorum*, in temporal artery biopsies from GCA patients [35], while levels of circulating NET remnants are increased in patients with large vessel vasculitis [36].

Recent reports have extensively investigated the levels of NET remnants in AAV and large vessel vasculitis. Circulating NET remnants, as defined by plasma neutrophil elastase-DNA complexes, are increased in GCA and TAK, independent of disease activity [37]. In addition, NET degradation is impaired in patients with large vessel vasculitis and AAV [37]. Antihistone antibodies, previously linked to anti-NET antibodies, have been identified in patients with TAK [37]. Of note, plasma NET remnants positively associate with thrombospondin 1 levels, a platelet activation marker [37]. These observations may suggest an interaction between platelets and NETs in the context of large vessel vasculitis, although implications remain to be better characterized.

Antiphospholipid syndrome

NETs have previously been linked to APS by promoting thrombosis through several mechanisms that include activation of coagulation factors, endothelium, platelets, and the complement system [38▪▪].

NETs have been proposed as biomarkers in APS. Levels of plasma calprotectin, a heterodimeric S100a8/a9 protein and a surrogate marker of NETs, are increased in pediatric APS compared with healthy donors [39]. Additionally, these levels negatively correlate with platelet counts, while plasma with high levels of calprotectin impairs platelet viability [39]. While these observations need to be validated in larger cohorts, plasma calprotectin may potentially serve as a candidate biomarker in pediatric APS [39]. Anti-NET antibodies are detected in 45% of patients found to be positive for antiphospholipid antibody without SLE [40▪▪]. Furthermore, anti-NET IgG antibody recognizes NET-associated proteins while anti-NET IgM antibody was reported to target DNA [40▪▪]. The detailed functions of these antibodies and their relation to clinical manifestations remain to be better characterized.

Several medications and other agents targeting NETs have been reported to be effective [38▪▪]. Solubilized ginger extract was reported to inhibit human

NET formation both *in vitro* and *in vivo* [41]. Additionally, oral intake of ginger attenuates autoantibody production and large-vein thrombosis in murine models of SLE and APS, respectively [41].

Idiopathic inflammatory myopathies

Idiopathic inflammatory myopathies (IIMs), characterized by chronic muscle inflammation and myositis-specific autoantibodies, have also been linked to neutrophil/NET dysregulation in previous studies [42]. In a study of transcriptomics and proteomics from whole blood, neutrophil granule-associated transcripts and protein levels were upregulated both in dermatomyositis and juvenile dermatomyositis, further supporting a neutrophil contribution to disease pathogenesis [43]. Furthermore, integrated transcriptomic analysis from several publicly available databases of muscle tissues from IIM patients showed an increased NET formation-associated signature [44], although further validation is warranted to assess if these signatures are bona-fide representation of NET formation in the context of IIM.

Systemic sclerosis

Compared to other autoimmune diseases, reports pertaining to the potential role of NETs in the pathogenesis of systemic sclerosis (SSc) and associated organ damage are limited. Previous reports have suggested that levels of circulating NETs are increased in SSc patients, and that SSc platelet-derived microparticles enhance NET formation [45]. Recently, NETs were found to promote differentiation of M2-like macrophages, leading to the promotion of skin fibrosis in the bleomycin-induced mouse model of SSc [46]. Nevertheless, the implications for human disease remain to be determined, although it should be emphasized that a link between NETs and fibrosis has been established for other conditions [47].

Behçet's disease

Behçet's disease (BD) is an inflammatory disorder characterized by recurrent oral aphthous ulcers and other systemic manifestations. Levels of circulating NET are increased in active BD [48], and BD neutrophils display enhanced NET formation compared to healthy donors [49]. In addition, NET formation in BD could be inhibited *in vitro* and *in vivo* by a phosphodiesterase 4 inhibitor, which is currently used in BD [49]. The number of LDGs is also increased in BD patients with high disease activity when compared patients with remission [50]. Additionally, the number of LDGs was higher in patients with vascular manifestations than in those without vascular manifestations [50]. These results suggest that LDG-derived NETs might contribute to the pathogenesis of vascular manifestations in BD, although this needs to be confirmed and further tested in other cohorts.

Adult-onset Still's disease

Adult-onset Still's disease (AOSD) is an inflammatory disorder characterized by fevers, arthritis, and rash. Increase in the levels of circulating NETs have been previously reported [51]. One of the hallmarks of AOSD is hyperferritinemia. Ferritin enhances NET formation via macrophage scavenger receptor 1 (Msr1), which leads to cytokine storm characteristics of AOSD [52]. Plasma levels of Pentraxin 3 (PTX3), an acute-phase inflammatory protein, are elevated in patients with active AOSD, and they positively correlate with circulating NET remnants, as assessed by MPO-DNA and CitH3-DNA [53]. Mechanistically, PTX3 has been proposed to induce NETs via mitogen-activated protein kinases pathway [53].

The interaction between AOSD NETs and monocytes or monocytic cells line was recently reported. The frequency of intermediate monocytes ($CD14^{bright}CD16^{+}$) is higher in active AOSD patients compared with that of healthy donors [54]. The stimulation of PBMCs with AOSD-derived NETs induced the expansion of intermediate monocytes and inflammasome activation [54]. In addition, serum levels of CCL2, also known as monocyte chemoattractant protein-1, is upregulated in patients with AOSD [55]. AOSD NETs upregulate CCR2 and pro-inflammatory cytokines such as interleukin (IL)-6, IL-1β, and IL-18 in THP-1 cells [55]. These results implicate NET/monocyte interactions in inflammatory responses in AOSD.

Polymyalgia rheumatica

Polymyalgia rheumatica (PMR) is an inflammatory condition characterized by aching and stiffness for the shoulders, pelvic girdles, and neck. NETs remnants, measured by plasma MPO-DNA complexes, were reported to be increased in PMR [56] and this was not altered by corticosteroids [56]. Whether NETs contribute the pathogenesis of PMR remains unclear.

Psoriasis

A putative role for NETs in the pathogenesis of psoriasis was previously described, including the presence of LDGs [57]. $CXCR4^{high}$ neutrophils,

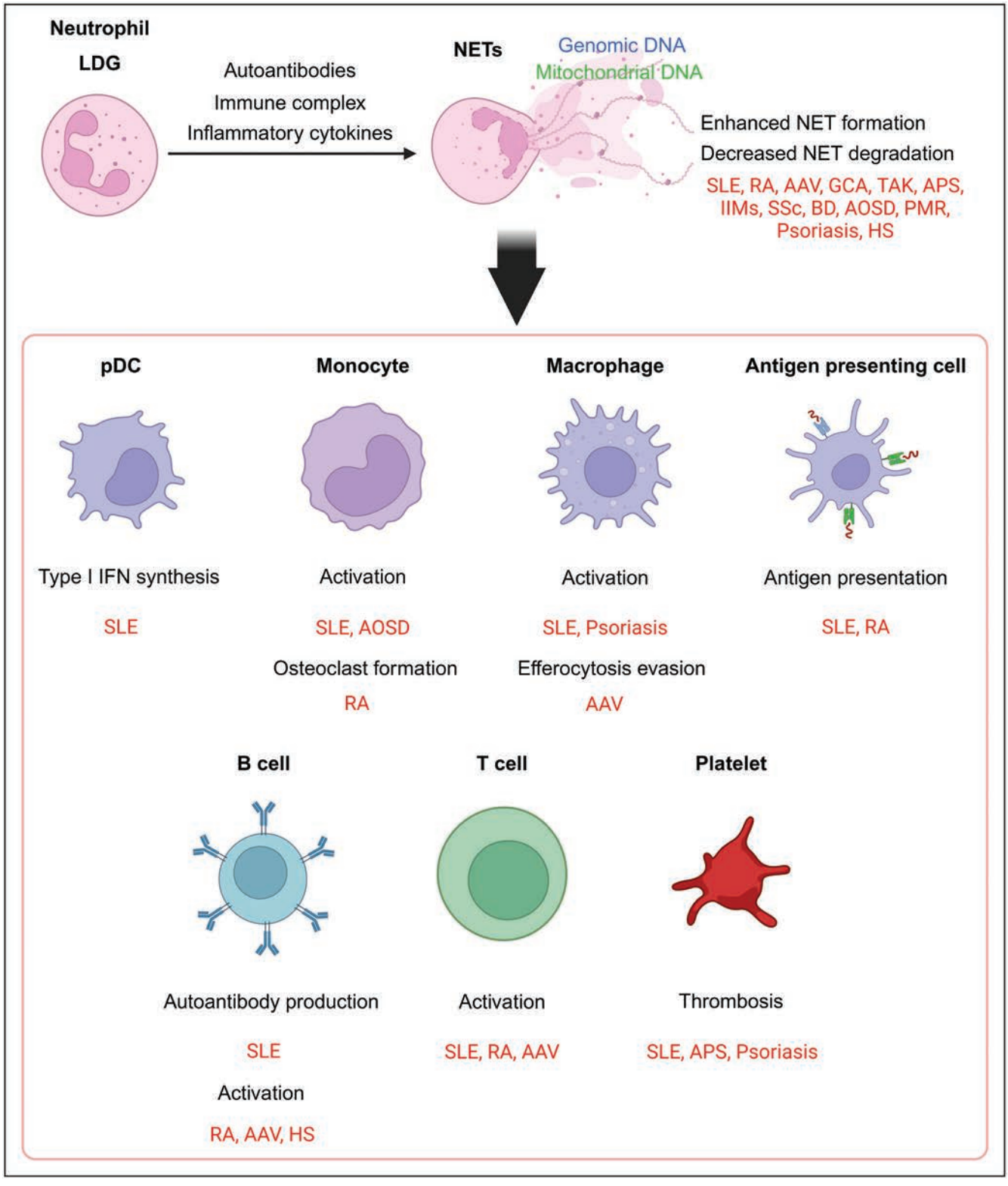

FIGURE 1. Dysregulation of NET formation and degradation in inflammatory rheumatic diseases and interaction with immune cells. Enhanced NET formation and decreased NET degradation are hallmarks of several inflammatory rheumatic diseases. NETs interact with several immune cells to contribute to disease pathogenesis. AAV, ANCA-associated vasculitis; AOSD, adult-onset Still's disease; APS, antiphospholipid syndrome; BD, Behçet's disease; DNA, deoxyribonucleic acid; GCA, giant cell arteritis; HS, hidradenitis suppurativa, IIMs, idiopathic inflammatory myopathies; LDG, low-density granulocytes; NETs, neutrophil extracellular traps; pDC, plasmacytoid dendritic cell; PMR, polymyalgia rheumatica; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TAK, Takayasu arteritis.

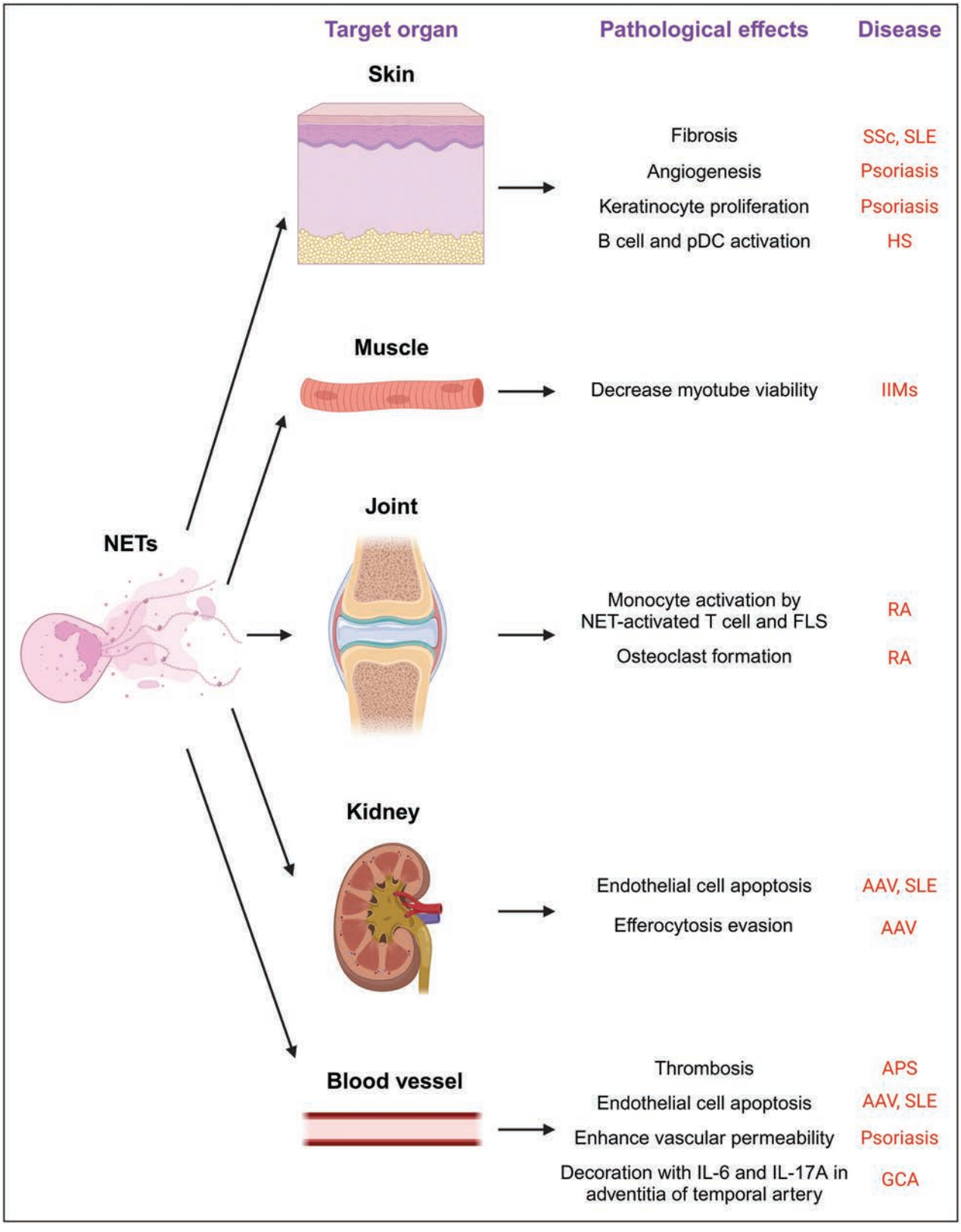

FIGURE 2. Contribution of NETs to organ damage in inflammatory rheumatic diseases. NETs contribute to disease pathogenesis in affected organs through the interplay with immune and stromal cells. AAV, ANCA-associated vasculitis; APS, antiphospholipid syndrome; FLS, fibroblast-like synoviocyte; GCA, giant cell arteritis; HS, hidradenitis suppurativa; IIMs, idiopathic inflammatory myopathies; IL, interleukin; NETs, neutrophil extracellular traps; pDC, plasmacytoid dendritic cell; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

which exhibit enhanced NET formation, are increased in psoriatic skin lesions and peripheral blood of patients with psoriasis [58]. This CXCR4^{high} neutrophils may contribute to the pathogenesis of psoriasis by inducing skin inflammatory response and enhancing vascular permeability [58]. Other mechanisms of action of NETs include activation of B and T cells by citrullinated LL37 contained in NETs [59], absent-in-melanoma-2 inflammasome activation in human keratinocytes [60], and macrophage activation through stimulator of IFN genes (STING)/NF-κB pathway [61].

Hidradenitis suppurativa

Hidradenitis suppurativa (HS) is a chronic inflammatory/autoimmune skin disorder that affects skin and hair follicles in intertriginous areas. Enhanced NET formation in HS neutrophils and detection of NETs in skin lesions was previously reported [62]. As a recent update, serum from patient with HS shows impaired NET degradation capabilities due to the decreased activity of DNase I secondary to the presence of anti-DNase 1 and -DNase 1L3 antibodies [63].

CONCLUSION

Since the discovery of NETs, their association with various inflammatory rheumatic diseases has been investigated, mostly on SLE, RA, AAV, and APS. Recent reports have implicated dysregulated NET formation and clearance, as well as their interaction with immune cells and tissue, in other inflammatory rheumatic diseases (Figs. 1 and 2). However, their exact contribution to disease pathogenesis remains to be systematically determined. Further investigations are necessary to elucidate the significance of enhanced NET formation/impaired NET degradation in several inflammatory rheumatic diseases. Exploring novel therapeutic targets aiming to control the NET dysregulation and validating the utility of NETs as biomarkers will be an important advancement in future investigations.

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Conflicts of interest

There are no conflicts of interest.

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Giant cell arteritis: update on pathogenesis and clinical implications

Hafeez E. Ibrahim and Cosimo De Bari

Purpose of review
Giant cell arteritis (GCA) is an age-related autoimmune disease with a complex pathogenesis that involves several pathogenic mechanisms. This review provides recent critical insights into novel aspects of GCA pathogenesis.

Recent findings
The use of novel approaches, including multiomic techniques, has uncovered notable findings that broaden the understanding of GCA pathogenesis. TCF1^{hi}CD4^{+} T cells have been identified as stem-like T cells residing in tertiary lymphoid structures in the adventitia of GCA aortic tissues, which likely supply the pathogenic effector T cells present in vasculitic lesions. Studies have demonstrated that fibroblasts present in GCA-inflamed arteries are not innocent bystanders, but they contribute to arterial inflammation via maintenance of Th1 and Th17 polarisation, cytokine secretion (IL-6, IL-1B, IL-12, and IL-23) and antigen presentation. Additionally, deregulated cellular senescence programs are present in GCA as an accumulation of IL-6 and matrix metalloproteinase 9-producing senescent cells have been identified in vasculitic lesions.

Summary
Recent studies have unravelled interesting findings with potentially significant clinical relevance. Stem-like T cells are likely key contributors to vascular disease persistence, and targeted depletion or modulation of these cells holds promise in GCA management. Fibroblast-targeting therapies and senotherapeutics are also exciting prospects in the treatment of GCA.

Keywords
cellular senescence, fibroblasts, giant cell arteritis, pathogenesis, stem-like T cells

INTRODUCTION

Giant cell arteritis (GCA) is a granulomatous vasculitis of large-sized and medium-sized arteries that affects people over 50 years of age [1]. It is the most common systemic vasculitis in adults, and is strongly associated with Caucasian background [2]. GCA predilects the aorta and its major branches as well as the temporal arteries and other branches of the external carotid artery [3]. GCA manifests as two distinct yet related components that include systemic inflammation and vascular disease. The vascular disease is characterized by uncontrolled vessel wall inflammation, tissue destruction, and maladaptive vascular remodelling culminating in either vascular occlusion in medium-sized arteries or aneurysmal formation in large arteries [4,5]. These manifest clinically as severe or life-threatening complications such as irreversible visual loss and stroke [6,7]. Glucocorticoid therapy, the mainstay of GCA treatment, induces clinical remission and reduces the incidence of disease complications [8]. However, glucocorticoid therapy is associated with high incidence (34–75%) of disease relapse as well as adverse effects such as osteoporosis, diabetes and severe infections [9–11]. Tocilizumab (an antibody against IL-6 receptor), approved for GCA management as a steroid-sparing agent, is also associated with disease relapse (>30%) upon discontinuation [12]. These observations emphasize the need to advance our knowledge of GCA pathogenesis to identify new therapeutic targets. In this article, we review key findings from recent studies that

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KEY POINTS

- TCF1^{hi}CD4^{+} T cells are stem-like T cells that reside in tertiary lymphoid structures in the adventitia of GCA aortic tissues and they likely contribute to vascular disease persistence by repopulating lesional effector T-cell subsets.
- Fibroblasts in GCA-inflamed arteries contribute to arterial inflammation and vascular remodelling.
- Accumulation of senescent cells with pro-inflammatory and tissue destructive phenotypes in GCA vasculitic lesions supports a crucial role of cellular senescence in GCA pathogenesis.

investigated novel aspects of GCA pathogenesis and discuss their potential clinical significance.

OVERVIEW OF GIANT CELL ARTERITIS PATHOGENESIS

The factors that initiate GCA remain unclear. Studies suggest that GCA pathogenesis commences in the adventitia and then progresses inwards towards the vascular lumen [5]. Early events of GCA pathogenesis have been attributed to the dysregulation of the mechanisms that facilitate vessel wall breach, aberrant CD4^{+} T-cell activation and immune cell infiltration. Dysfunctional circulatory regulatory T cells and active CD28-mediated T-cell costimulatory signals in GCA arteries result in uncontrolled activation and proliferation of CD4^{+} T cells [13,14]. These aberrantly activated CD4^{+} T cells gain vascular entry via the vasa vasorum owing to defective PD1-PDL1 and CD155-CD96 immunoinhibitory checkpoints in GCA arteries, in addition to microvascular disruptive activities of matrix metalloproteinase 9 (MMP9)$^{+}$ monocytes and reactive oxygen species (ROS)-producing immature neutrophils [15–18]. Deregulation of the vascular endothelial growth factor (VEGF)-Jagged1-Notch signalling pathway also contributes to CD4^{+} T-cell activation, infiltration, and polarization [19].

Activated vascular dendritic cells secrete chemokines (CCL19, CCL20 and CCL21) and cytokines, which promote immune cell recruitment and activation [20,21]. On vascular entry, CD4^{+} T cells differentiate into IFN-γ-producing T-helper 1 (Th1) cells in response to Th1-promoting cytokines (IL-12 and IL-18), whereas Th17 polarization is promoted by IL-6, IL-1β and IL-23 [21]. Other vasculitogenic T-cell subsets (Th9, Th21, Th22, and GM-CSF producing T cells) are present in GCA and they elicit varied effector functions that contribute to arterial inflammation [16,22]. Cytokines secreted by CD4^{+} T cells, particularly IFN-γ, activate macrophages which consequently produce cytokines, matrix metalloproteinases and growth factors such as VEGF and platelet-derived growth factor [23–25]. These molecules further enhance arterial inflammation and promote maladaptive vascular remodelling by acting on endothelial cells and vascular smooth muscle cells (VSMCs) [23–25]. Endothelial cells and VSMCs then contribute to the propagation of inflammation via the expression of adhesion molecules (ICAM-1 and VCAM-1) and chemokines (CXCL9, CXCL10), respectively [26,27]. Additionally, neutrophils in GCA arteries promote inflammation via the formation of neutrophil extracellular traps containing IL-6 and IL-17A [28]. The pro-inflammatory activities of these infiltrating and tissue-resident immune cells create a feedback loop with intensification of vascular inflammation.

RECENT ADVANCES IN GIANT CELL ARTERITIS PATHOGENESIS

By employing newer techniques and using insights from cancers and other autoimmune diseases, recent studies have broadened the scope of GCA pathogenesis. Here, we discuss findings from these studies and their potential clinical implications.

CD4^{+} T cells in giant cell arteritis: from circulation to tissue stem-like cells

Over the years, studies on GCA have unravelled multiple subsets of vasculitogenic T cells, their multieffector functions and their contribution to disease pathogenesis. The use of multiomic technologies has brought new insights into GCA pathogenesis including the roles and subsets of vasculitogenic T cells in GCA vessels. In a study that explored the gene expression profiles of circulating CD4^{+} T cells in GCA patients, no difference was observed between healthy controls and steroid-free GCA remission patients, which indicated a possible restoration of CD4^{+} T cells transcriptome following glucocorticoid treatment. However, 14 deregulated genes were identified as being less steroid-responsive, some of which are involved in the Wnt canonical signalling pathway (RARG and WNT10A). Compared with healthy controls and steroid-free GCA remission patients, CD4^{+} T cells from the active disease group exhibited gene enrichment related to interleukin and chemokine signalling, angiogenesis, apoptosis and Notch signalling [29▪]. Similarly, when compared with the GCA remission patients on glucocorticoids, an enrichment in immune-related pathways such as the CD40/CD40L, I-kappaB kinase/NF-kappaB and

interleukin pathways (IL-2, IL-6, IL-9, IL-11, IL-12, IL-22 and IL-23) was observed [29▪]. Whilst some of these findings were known to be linked to GCA, deregulation of pathways such as IL-2 signalling and Wnt signalling are notable previously unappreciated findings in GCA. Interestingly, IL-2 signalling deregulation is associated with dysfunctional regulatory T cells [30], whereas dysregulated Wnt signalling contributes to the pathogenesis of autoimmune diseases such as rheumatoid arthritis [31]. These observations highlight the need to understand how they mechanistically relate to GCA pathogenesis.

Using computational methods, aberrant intercellular communications between circulatory $CD4^+$ T cells and $CD14^+$ monocytes in GCA were postulated [29▪]. Specifically, Notch ligands (DLL1, JAG1 and JAG2) expressed by $CD14^+$ monocytes interact with Notch1 and Notch2 expressed by $CD4^+$ T cells in active GCA [29▪]. This suggests that aberrant Jagged-Notch crosstalk exists between circulatory immune cells, in addition to the previously known interaction between circulating $CD4^+Notch^+$ T cells and vasa vasorum $Jagged1^+$ endothelial cells that contributes to GCA initiation. However, functional studies are required to validate these computational findings.

Histological analysis of repeat temporal artery biopsies obtained at up to 12 months intervals from steroid-treated GCA patients showed persistence of lesional T cells [32]. In line with this, active aortitis was detected histologically in the majority (82%) of GCA patients in clinical remission that underwent aortic surgery about 6 years (median) after initial diagnosis [33▪]. Of note, Th17 axis in GCA has been observed to undergo steroid-mediated suppression, whereas the Th1 arm displays steroid resistance in the circulation and vascular lesions [21,34]. Collectively, these findings emphasize the steroid-refractory nature of GCA and the need for targeted therapies.

In a separate study, GCA aortic tissues were reported to contain dense mononuclear aggregates, which formed tertiary lymphoid structures (TLS) located primarily around adventitial vasa vasorum networks [35▪▪]. These TLS comprised of varied proportions of proliferating T cells and B cells while harbouring $CD11c^+$ dendritic cells, $podoplanin^+$ lymphatic vessels and high endothelial venules (Fig. 1). In line with this, bulk RNA-sequencing analyses revealed significant enrichment of TLS-related genes (*CXCL13*, *CCL19*, *IL-7*, *IL15* and *TNFSF13B*) and gene signatures for activated memory B cells and $CD4^+$ memory T cells in GCA aortitis compared with disease controls [35▪▪].

Subcutaneous engraftment of human arteries into nonobese diabetic/severe combined immunodeficient IL-$2R\gamma^{null}$ (NSG) mice followed by adoptive transfer of active GCA peripheral blood mononuclear cells (PBMCs) was used to induce vasculitis [35▪▪]. These induced vasculitic arteries contained adventitial lymphoid aggregates with similar gene signatures to the GCA aortic TLS [35▪▪]. Single-cell RNA-sequencing of T cells isolated from the adventitia of these induced vasculitic arteries revealed a $CD4^+$ T-cell cluster, which expressed TCF7 (encoding TCF1) as well as genes associated with T-cell survival (IL-7R), TGF-β signalling, TLS development (LTB) and lymphoid tissue homing (CCR7, CXCR3 and CXCR5) [35▪▪]. Using pseudotime trajectory analysis, the $TCF1^{hi}CD4^+$ T cells were predicted to differentiate into other T-cell clusters, which include $IL\text{-}21^+BCL6^+$ T-follicular helper (TFH)-like cells, cytotoxic $EOMES^+$ $CD4^+$ T cells, and cycling T cells (Fig. 1) [35▪▪]. Positive correlation between TCF1 and IL-7R led to the use of IL-7R as a surrogate marker for $TCF1^{hi}CD4^+$ T cells in assessing stemness and disease-maintaining ability of $TCF1^{hi}CD4^+$ T cells [35▪▪]. Following serial transengraftment into empty NSG mice, $IL7R^+$ PBMC-induced vasculitic arteries contained significant T-cell proliferation, arterial inflammation, tissue damage and neoangiogenesis compared with vasculitis induced by IL-7R-depleted PBMCs [35▪▪]. Furthermore, immunohistochemical staining of GCA aortic tissues demonstrated the presence of $TCF1^{hi}CD4^+$ T cells in adventitial TLS as well as TFH-like cells and $EOMES^+$ $CD4^+$ T cells [35▪▪]. The presence of TFH-like cells and $EOMES^+$ $CD4^+$ T cells in GCA aortic tissues is likely the result of differentiation from $TCF1^{hi}CD4^+$ T cells, as inferred by the trajectory analysis [35▪▪]. Taken together, these findings indicate that the $TCF1^{hi}CD4^+$ T cells possess stem-like T-cell features as they exhibit self-renewal, cell survival and stemness capabilities, and are likely to replenish lesional effector T-cell populations, maintain vascular inflammation and promote vascular disease persistence in GCA. As such, therapeutic targeting of these stem cells may prove pivotal in GCA management.

Fibroblasts in giant cell arteritis: not innocent bystanders

Fibroblasts constitute a major cellular component of connective tissues where they regulate extracellular matrix homeostasis and maintain tissue architecture [36,37]. In disease states, fibroblasts were previously seen as innocent bystanders or mere targets of immune cell activities. However, findings from studies in chronic autoimmune and inflammatory diseases have unravelled the active roles fibroblasts play in disease pathogenesis [38–40].

Little was known about the contribution of fibroblasts to GCA, despite the adventitia being considered as the site of GCA initiation and early disease phase. A study demonstrated a significantly

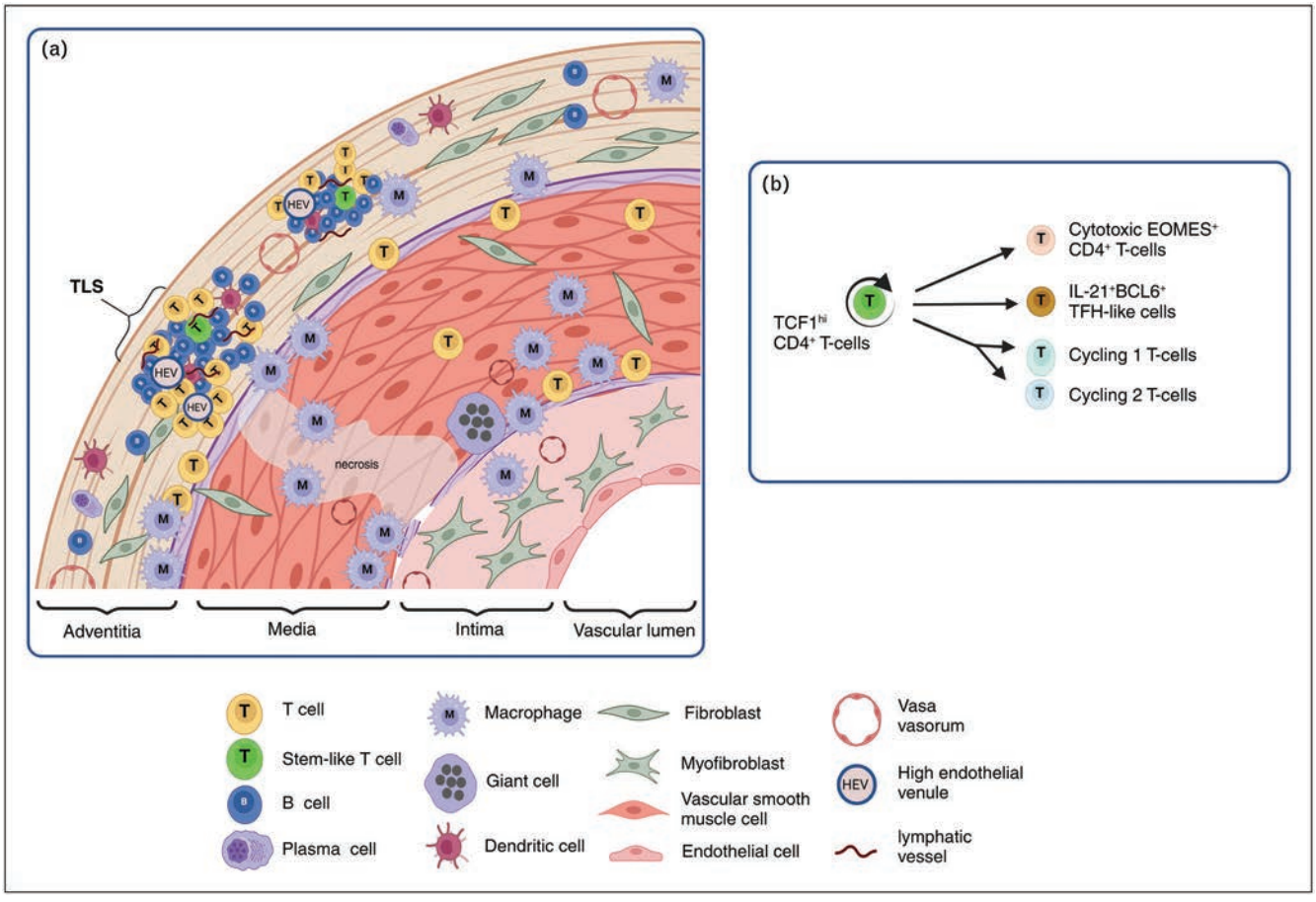

FIGURE 1. Stem-like T cells in GCA-affected aorta contribute to vascular disease persistence. (a) Stem-like TCF1$^+$CD4$^+$ T cells reside within tertiary lymphoid structures in the adventitia of GCA-affected aorta. (b) The stem-like T cells possess self-renewal capabilities and differentiate into cytotoxic EOMES$^+$ T cells, IL-21-producing T-follicular helper-like cells and cycling (1 and 2) T cells. The stem-like T cells repopulate effector T cells in vasculitic lesions, which suggests their contribution to vascular disease persistence. Figures were created using BioRender.com. GCA, giant cell arteritis.

increased proportion of CD90$^+$vimentin$^+$ASMA$^+$ fibroblasts in the adventitia and intima of GCA temporal arteries as opposed to control arteries and reported the presence of fibroblasts in destroyed areas of the tunica media forming 'invading wells' (Fig. 2a) [41]. This suggests an inward transmural migration of these cells and their possible contribution to the intimal myofibroblast population. Although VSMCs, presumably via phenotypic switching and migration, have been noted to contribute to neointimal hyperplasia, the cellular origins of the intimal myofibroblast population in GCA arteries are yet to be fully elucidated.

Using immunohistochemical staining, fibroblast activation protein (FAP), a serine protease, was found to be significantly expressed in all layers of GCA temporal arteries and in the medial layer of GCA aortas, with little or no expression in control arteries [42▪]. FAP was predominantly expressed by adventitial fibroblasts and intimal macrophages in GCA temporal arteries [42▪]. Interestingly, a proportion of the CD90$^+$FAP$^+$ fibroblasts co-localized with IL-6 and MMP9 protein expression, suggesting likely proinflammatory and tissue destructive capabilities of these cells (Fig. 2b) [42▪]. The role of FAP and the regulation of its expression in GCA remain unclear.

A study using spatial transcriptomics for analysis of GCA and control temporal artery biopsies reported that the number of differentially expressed genes (DEGs) increased inwards across the vascular layers with the intima having the most dysregulated genes as well as a more homogenous expression profile among donors [43▪]. Furthermore, the three vascular layers shared 32 common dysregulated DEGs, which are associated with major histocompatibility complex (CD74, B2M, HLA-B, HLA-DRA and HLA-DRAB1), macrophages (CD68, cathepsin B), immunoglobulins (IGHGs) and tissue remodelling (ADAM15, COL3A1) [43▪]. Of particular interest was CD74, a major histocompatibility complex class-II antigen, identified as the most upregulated

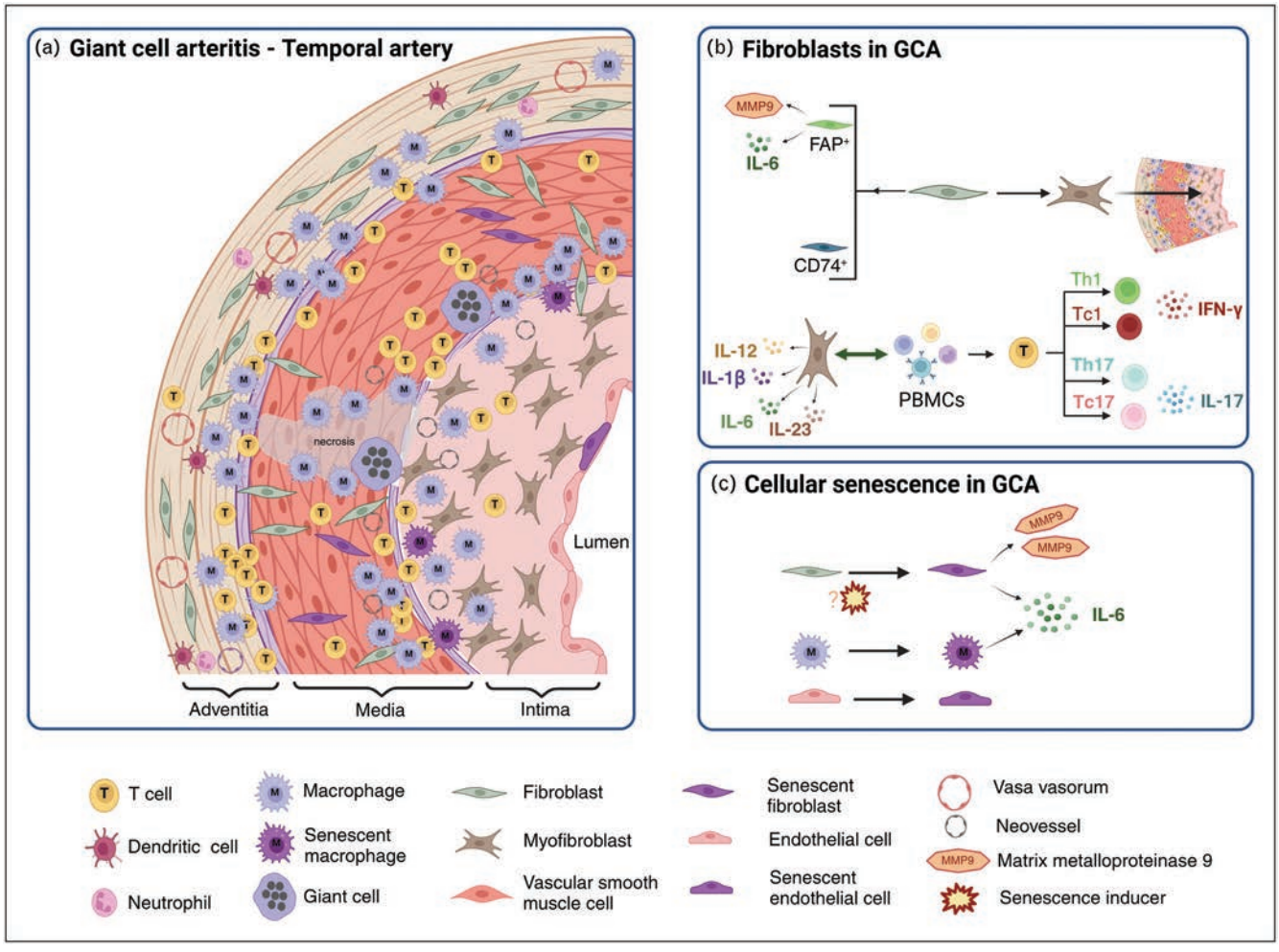

FIGURE 2. Fibroblasts and senescent cells are involved in promoting arterial inflammation and vascular remodelling in giant cell arteritis. (A) Schematic of GCA-affected temporal arteries showing immune cell infiltration, necrosis and tissue damage in media, neoangiogenesis, and intimal hyperplasia. (b) Fibroblasts become activated and migrate inwards to contribute to the intimal myofibroblast population, which consequently contributes to vascular occlusion. Multiple fibroblast phenotypes are present in GCA-affected arteries, and they include pro-inflammatory FAP^+ fibroblasts and $CD74^+$ fibroblasts. Additionally, fibroblasts promote arterial inflammation by maintaining Th1/Tc1 and Th17/Tc17 polarisation. Fibroblasts also secrete cytokines (IL-6, IL-1β, IL-12, and IL-23) following interaction with peripheral blood mononuclear cells (PBMCs). (c) Senescent cells present in GCA-affected arteries include fibroblasts, macrophages, and endothelial cells. Senescent fibroblasts express IL-6 and MMP9, which is suggestive of their pro-inflammatory and tissue destructive capabilities. Figures were created using BioRender.com. GCA, giant cell arteritis.

gene in the intima and media of GCA arteries in comparison to controls [43▪]. Notably, CD74 was expressed by fibroblasts ($CD90^+CD3^-$) in the intima–media border, the media and adventitia of GCA arteries with no expression detected in control arteries by immunohistochemical staining [43▪]. These data suggest that $CD74^+$ fibroblasts likely contribute to antigen presentation and immune response regulation in GCA.

In a separate study using myofibroblast-PBMC co-culture models, it was demonstrated that temporal artery-derived myofibroblasts significantly increased the percentage of Th1, Tc1, Th17 and Tc17 cells with notable predominance of Th1 and Tc1 polarization [44▪▪]. Interestingly, these temporal artery-derived myofibroblasts expressed significant amounts of IL-6, IL-1β, IL-12p19 and IL-12p40 transcripts following interaction with PBMCs in the co-culture model (Fig. 2b) [44▪▪]. Furthermore, myofibroblasts pretreated with IFN-γ and TNFα significantly upregulated IL-12p35 and IL-12p40 mRNA expression following co-culture with PBMCs [44▪▪]. These findings demonstrate that temporal artery-derived myofibroblasts, when stimulated, sustain T-cell polarization and secrete key cytokines involved in GCA pathogenesis. Therefore,

myofibroblasts are not only relevant to vascular remodelling but to arterial inflammation as well.

Cellular senescence in giant cell arteritis

Cellular senescence is a state of stable and generally irreversible cell-cycle arrest induced by adverse stimuli and damaging stressors [45,46]. Senescent cells feature macromolecular damage, deregulated metabolism and a senescent associated secretory phenotype (SASP) involving the release of pro-inflammatory cytokines, chemokines, growth factors and proteases [46]. Physiologically, cellular senescence contributes beneficially to tissue repair processes and tumour suppression [47]. With ageing, however, the presence of senescent cells is increased in several tissues including arteries [47]. In age-related diseases, there is aberrant accumulation of senescent cells, at least partly because of deficient immune clearance following cellular senescence [48–50].

Given the older age exclusivity in GCA, the presence of preexisting senescent cells as a component of vascular ageing is likely and it raises questions on the possible role of cellular senescence in GCA pathogenesis. A significant accumulation of $GL13^+$ senescent cells was indeed reported in GCA temporal arteries as opposed to non-GCA and polymyalgia rheumatica temporal arteries [51▪]. Using phenotypic markers, the senescent cells in the GCA temporal arteries were demonstrated to be $Vimentin^+$ fibroblasts, $CD68^+$ macrophages and $CD34^+$ ECs (Fig. 2c) [51▪]. These senescent cells were shown to express some SASP components with IL-6 and MMP-9 being expressed predominantly by senescent fibroblasts (Fig. 2c). Senescent macrophages also expressed IL-6, but to a lesser extent [51▪]. Another study demonstrated that $p16^{16INK4A}$ and $p21^{WAF/Cip1}$ senescence markers were expressed by cells across the three vascular layers in GCA temporal arteries with little or no expression in control arteries from age-matched individuals [52▪]. Macrophages and giant cells, in the intima–media border, were the predominant cell types expressing p16 and p21, and some of these cells co-localized with IL-6 and GM-CSF expression [52▪]. Some stromal cells, as identified by cellular morphology, were also noted to express p16 and p21 in GCA temporal arteries [52▪]. Taken together, the increased accumulation of senescent cells and their expression of GCA-associated SASP molecules suggest that cellular senescence has biological significance in GCA pathogenesis. Senescent cells in GCA arteries likely contribute to intensification of arterial inflammation as well as vascular remodelling based on the SASP components.

CLINICAL PROSPECTS AND FUTURE DIRECTIONS

The ongoing challenges in GCA management highlights the need for different and novel conceptual approaches to unravel the complexity of GCA pathogenesis and identify new therapeutic targets. Stem-like T cells in GCA aortic tissues likely contribute to vascular disease persistence and therefore represent a promising therapeutic target. Future studies aimed at defining the development and function of these stem-like T cells would be of additional biological and clinical relevance. Furthermore, cellular senescence in GCA introduces the potential use of senotherapeutics in GCA via either targeted elimination of senescent cells (senolytics) or modulation of senescent cells' phenotypes (senomorphics). However, more mechanistic studies are required to provide a more in-depth understanding of the cellular senescence programs in GCA to ensure precise and selective targeting of detrimental senescent components.

The presence of pathogenic fibroblasts in GCA-inflamed arteries is also of potential clinical importance with regards to imaging and targeted therapies. For instance, FAP^+ fibroblasts in GCA-inflamed arteries can be used for FAP inhibitor tracer-guided imaging as currently being evaluated in oncology and other autoimmune conditions such as rheumatoid arthritis [53–55]. Additionally, targeted deletion of $FAP\alpha^+$ fibroblasts has been demonstrated to suppress both inflammation and tissue damage in mouse models of autoimmune inflammatory arthritis [38]. This highlights the potential applications of fibroblast-targeting therapies in GCA. As such, depletion or modulation of pathological fibroblast subsets provides the possibility of curtailing vascular remodelling as well as arterial inflammation. With respect to fibroblasts, we propose that future studies should be geared towards deciphering fibroblast pathotypes, their activation pathways, spatial distribution and cellular interactions in GCA-inflamed arteries.

CONCLUSION

GCA is a difficult-to-manage autoimmune disease because of its complex pathogenesis which involves multiple dysregulated signalling pathways, several key cellular players, and a myriad of effector cytokines. The recent strides in GCA pathogenesis have resulted in very interesting findings that have the potential to influence the biological and clinical landscape of GCA. Single-cell technologies and spatial transcriptomics open new exciting avenues towards molecular targeting and precision interventions.

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Conflicts of interest

There are no conflicts of interest.

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Inclusion body myositis: an update

Nicolaas C. Anderson and Thomas E. Lloyd

Purpose of review
To review recent advances in our understanding of the epidemiology, pathophysiology, and management of inclusion body myositis (IBM).

Recent findings
Recent epidemiologic studies have highlighted the morbidity and mortality associated with IBM, including the impact of dysphagia. Multiomic analyses of IBM tissues have identified new pathogenic pathways and biomarkers for use in clinical trials. New diagnostic criteria and outcome measures have been proposed to improve clinical trial design. Ongoing clinical trials are targeting T cells and autophagy.

Summary
Improvements in our understanding of IBM pathogenesis are identifying new pathways and biomarkers that need validation in larger cohorts. Exercise remains the primary therapeutic modality available, and new treatment targets are needed.

Keywords
cytotoxic T cells, inclusion bodies, myositis, TDP-43, transcriptomics

INTRODUCTION

Inclusion body myositis (IBM) is the most common idiopathic inflammatory myopathy (IIM) in older adults, and yet the underlying pathophysiology of the disease remains unclear, and there are no disease-modifying therapies. The disease is distinguished from other IIMs by the insidious course, the distinctive pattern of muscle involvement, preferentially affecting finger flexors and knee extensors, and muscle pathology demonstrating both intense endomysial infiltration of CD8+ T cells and sarcoplasmic protein aggregation. New technologies, models, and therapeutic strategies are shedding light into the pathogenesis of this disease, offering patients new hope for a meaningful treatment. Here, we will focus on how findings in the last several years have impacted our understanding of this disease.

EPIDEMIOLOGY

Population-based studies continue to shed light on the prevalence, survival, symptoms, and associated conditions in IBM. A Swedish study found a prevalence of 32 per million people, in line with prior findings, while a smaller US study (Olmsted County) showed a prevalence of 182 per million in people over the age of 50 [1,2]. Another Swedish study found an incidence of young-onset IBM (<50 years) of 0.12 per million per year [3]. Dysphagia is increasingly recognized as an impactful symptom in IBM, eventually affecting over two-thirds of patients, and was the initial symptom in 23% of women and 10% of men [1,2]. Half of patients with IBM have another autoimmune disease, with rheumatoid arthritis and Sjogren's syndrome the most common reported [1,2,4]. Patients with IBM have also been found to be more likely to have polyneuropathy and hematologic malignancies [4]. Reduced life expectancy has been reported in IBM, with a mean survival from symptom onset of 14 years in one study [1,2,4].

Limited investigation into the economic impacts on patients with IBM has been completed. In a survey through a German IBM patient registry, the annual per capita cost of illness was found to be over $100 000, worse in those not working due to IBM when compared to those who are retired or still working [5]. More than 35% of patients with IBM reported symptoms of depression or anxiety [6]. A study on health-related quality of life in IBM

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KEY POINTS

- Single nuclei transcriptomics of muscle tissues have identified new hypotheses about inclusion body myositis (IBM) pathogenesis.
- Multiomic analyses of biofluids are identifying new biomarkers for future clinical trials.
- New European Neuromuscular Center diagnostic criteria and outcome measures for IBM have recently been proposed.
- A large, international clinical trial to deplete cytotoxic T cells in IBM may finally answer the question as to whether the immune system drives disease progression.

identified four sequential phases of the patient's experience of disease: uncertainty about physical vulnerability until diagnosis, pinpointing various treatment approaches, self-management and spousal support, and weak body, busy mind, and caregiver burden [7]. Understanding these phases may be important in providing supportive care to patients with IBM.

DEFINING THE INCLUSION BODY MYOSITIS CLINICAL PHENOTYPE

Recent work has highlighted new phenotypic features of IBM with distinct demographic differences. A single center, retrospective review of 54 patients found that the majority of patient with IBM (59%) had restrictive forced vital capacity (FVC) deficits at initial visit [8▪]. Patients with ventilatory pump impairment were more likely to be nonambulatory, and longitudinal analysis showed a mean decline in forced vital capacity (FVC) of 0.1 l/year, though the magnitude of FVC decline did not correlate with the decline in muscle strength.

There may be phenotypic differences in IBM depending on race, sex, and age-of-onset. Analysis of a large cohort of IBM patients showed that black patients had weaker arm abductors, hip flexors, and knee flexors when compared to nonblack patients and that female patients had stronger finger flexors and knee extensors when compared with males [9]. A population-based study showed that five of six patients with onset of IBM before age 50 had swallowing difficulty [3]. These patients had a median age of symptom onset of 36 years, a median survival of 14 years, and a high mutation load of large scale mitochondrial DNA rearrangements. Dysphagia has been found to be more common in anti-cN-1A positive patients in some but not other studies [10,11]. Of note, a meta-analysis on the diagnostic accuracy of anti-cN-1A antibody testing concluded that this test is not useful for IBM diagnosis [12]; this may be due to lack of standardized autoantibody testing methodology. Considerable effort has gone into developing consensus diagnostic criteria for IBM, and revised European Neuromuscular Center (ENMC) diagnostic criteria have recently been published [13▪▪].

Patients with IBM had significantly more impaired facial tasks when compared to healthy controls, especially noted in orbicularis oculi muscles, and patients with facial weakness reported more trouble with swallowing [14]. A study characterizing features in IBM patients with cricopharyngeal bar (CPB) on swallow study found that CPB(+) patients more frequently experienced obstruction-related dysphagia, had stronger knee extension, and less fatty replacement in muscle MRI when compared to patients without CPB [15]. In hospitalized patients, the risk for dysphagia, infection, and falls was increased in IBM when compared with other forms of IIM [16]. A study investigating the correlations between laboratory markers and clinical features found that the severity of endomysial inflammation correlated with severity of dysphagia in patients with IBM [17].

Altogether, these studies highlight the clinical heterogeneity of IBM and suggest that respiratory dysfunction and dysphagia may progress independently of limb weakness.

PATHOPHYSIOLOGY

Autoimmune or myodegenerative?

The million dollar question in IBM continues to be what drives disease progression. Is the intense endomysial collection of inflammatory cells, including cytotoxic T cells invading myofibers, truly causing muscle atrophy and weakness, or is the inflammation secondary to a myofiber-autonomous degenerative process? Perhaps the strongest evidence that IBM is triggered by autoimmunity comes from genome-wide association studies demonstrating a strong association between human leukocyte antigen (HLA) haplotypes and IBM. A recent Australian study showed that individuals carrying the DRB1∗03:01:01 but lacking the DRB4∗01:01:01 and DQA1∗01:02:01 alleles had a 14-fold higher risk of developing IBM than the general Caucasian population with an average of 5 years earlier onset [18▪▪].

Despite the strong evidence for an autoimmune trigger, it's unclear if disease progression is driven by inflammation. For one, numerous clinical trials targeting the immune system have failed to slow

progression. One theory, currently being tested in a clinical trial (see below), is that the pathogenic cytotoxic T cells attacking muscle in IBM are refractory to conventional immunosuppressive therapy. A recent immunophenotyping study of IBM muscle biopsies, following up on the pioneering work of Greenberg *et al.* [19], confirmed that infiltrating $CD8^+$ T cells express cell-surface markers [e.g. KLRG1 (79%), CD57 (48%), and PD-1] and lack CD28 expression [20], consistent with the hypothesis that these T cells are highly differentiated cytotoxic T cells with a senescent phenotype.

Recent work has also investigated T cell immunophenotypes in the peripheral blood of patients with IBM. In contrast to a prior study that suggested that 22/38 (58%) of IBM patients met diagnostic criteria for T cell large granular lymphocytic leukemia (T-LGLL) [21], a recent study suggests that only 2 of 85 IBM patients had counts above the threshold used for diagnosing T-LGLL [22]. Rather, this study showed that 34/85 (40%) of IBM patients have a mild T-LGL expansion, and suggest that this is likely to be a 'reactive' population rather than a driver of IBM. Immunophenotyping of peripheral blood followed by machine learning readily distinguishes patients with IBM from age-matched controls [23]. In particular, IBM patients have a higher proportion of TEMRA cells, highly differentiated and highly cytotoxic T cells [23,24]. Together, these new studies confirm that senescent cytotoxic $CD8^+$ T cells are expanded in the blood of patients with IBM, though the pathogenic significance remains unclear.

An alternative hypothesis of IBM pathogenesis is that myofiber degeneration progresses independent of inflammation. Numerous proteins form ubiquitinated sarcoplasmic aggregates and accumulate within rimmed vacuoles in myofibers in IBM muscle biopsies, a pathologic feature characteristic of neurons in neurodegenerative diseases. One such protein is the RNA binding protein TDP-43 which is lost from the nucleus and forms cytoplasmic aggregates in IBM, amyotrophic lateral sclerosis (ALS), and other neurodegenerative diseases. TDP-43 normally prevents incorporation of 'cryptic exons' into mature mRNA, and thus loss of nuclear TDP-43 can be detected with RT-PCR analysis of cryptic exons. 36 out of 44 (82%) muscle biopsies from IBM patients show expression of cryptic exons, whereas they are not detected in controls [25]. In a xenograft mouse model of IBM, depletion of 96% of T cells with intraperitoneal administration of an anti-CD3 antibody had no effect on p62-positive aggregates, cryptic exons, or rimmed vacuoles [25]. Furthermore, TDP-43 aggregates from IBM biopsies can form self-templating seeds, potentially allowing prion-like spread of pathology in IBM [26].

Omics approaches

Single nuclei RNA sequencing (snRNAseq) technologies are shedding novel insights into IBM pathogenesis by providing an unbiased and comprehensive analysis of transcriptional changes in myonuclei and adjacent cells. Spatial transcriptomics paired with snRNA sequencing of quadriceps muscles found selective loss of type 2 myonuclei in conjunction with T cell-predominant inflammation in IBM when compared with controls [27▪▪]. Consistent with this observation, two pathological analyses of IBM muscle biopsies showed atrophy and loss of type 2 muscle fibers [28,29▪▪].

SnRNAseq analysis also showed that IBM myonuclei upregulated either cell stress markers (GADD45A and NORAD) or protein degradation markers (RNF7 associated with p62-positive aggregates) [27▪▪]. In addition, ACHE (encoding acetylcholinesterase) is upregulated in IBM myonuclei, leading to the intriguing possibility that this may result in functional myofiber denervation [27▪▪]. Other RNAseq studies of IBM muscle tissue found dysregulation of genes involved in calcium homeostasis [30] and a specific upregulation of the cell adhesion molecule CDH1 [31]. Furthermore, another snRNAseq study identified a population of senescent fibro-adipogenic progenitors (FAPs) upregulated in IBM [29▪▪]. Together, these unbiased transcriptomic approaches are leading to novel hypotheses regarding disease pathogenesis.

These 'omics' approaches can also be performed on blood and peripheral tissues to identify biomarkers. Evaluation of circulating microRNA in patients with IBM found a significant alteration of two microRNAs that regulate muscle regeneration during aging [32]. An analysis of the transcriptome of fibroblasts obtained from skin biopsies of IBM patients showed altered inflammatory gene expression, suggesting fibroblasts may be a valid disease model [33▪]. Additionally, a combined metabolomic and transcriptomic study found upregulation in histamine and glycosaminoglycan biosynthesis and downregulation of carnitine and creatine metabolism [34]. Finally, an analysis of multiple samples from IBM patients (saliva, fibroblasts, urine, plasma, and muscle) identified intriguing metabolic abnormalities from IBM samples, including a reported 100% sensitivity and specificity of IBM diagnosis with upregulation of two urine metabolites [35]. Thus, these multiomics approaches on multiple tissues have the potential to identify novel diagnostic, prognostic, and therapeutic biomarkers in addition to shedding light on IBM pathogenesis.

OUTCOME MEASURES

A potential reason that clinical trials in IBM have failed to show efficacy in slowing disease progression

is the insensitivity of primary outcome measures. Thus, a major need in the field is the identification and validation of biomarkers and outcome measures that indicate disease activity or progression that can be used as surrogate endpoints. The IBM Functional Rating Scale (IBMFRS) has been used as the primary endpoint in recent treatment trials, however, data supporting validity, reliability, and sensitivity is limited. A recent study showed that the scale is a valid assessment of functional deficits, has a high degree of intra- and inter-rater reliability, and has equivalence when administered via phone instead of face-to-face [36,37]. Significant correlation was found between Functional Dexterity Test, Performance of the Upper Limb, Sollerman Hand Function Test and IBMFRS [38]. There was also strong correlation of IBMFRS to manual muscle testing, timed get up, and modified Rankin score [39].

Magnetic resonance imaging (MRI) has gained attention as a potentially sensitive biomarker of disease progression. A 1-year longitudinal study using quantitative MRI showed a statistically significant decline of thigh muscle volume and increase in intramuscular adipose tissue, and also suggested that inflammation may trigger fat infiltration [40]. In a placebo-controlled trial of sirolimus for IBM, quadriceps and medial gastrocnemius muscle fat fraction decreased and quadriceps contractile cross-sectional area increased more in the sirolimus group than placebo at one year [41]. Given its sensitivity to detecting small changes, there continues to be interest in incorporating MRI in future clinical trials.

Other imaging modalities also hold potential for noninvasively assessing IBM pathology. A PET tracer for $CD8^+$ T cells has potential for determining whole-body skeletal muscle endomysial inflammation [42▪]. Ultrasound (US) is another useful marker of disease progression in muscle diseases. Muscle echogenicity correlated with patient reported outcomes grip/pinch strength in patients with IBM in one study [43], however did not appear to change over 26 months in another study [44]. Thus, while US may be more accessible in the clinic, it has not yet shown to be sensitive to change over time.

DISEASE MANAGEMENT AND CLINICAL TRIALS

Unfortunately, there is little evidence to inform clinical management of IBM patients. An analysis of a large Japanese nationwide database showed that longer duration (>1 h) of daily rehabilitation was associated with improved activities of daily living when compared to shorter duration [45]. Patients with IBM who completed 8 weeks of inspiratory muscle training demonstrated improvement in respiratory function, though this did not lead to significant changes in quality of life, sleep quality, or two-minute walk test [46]. These recent findings are in line with prior studies showing that exercise can improve muscle strength and aerobic activity [47]. Analysis of a large US database shows that palliative care is likely underutilized in hospitalized patients with IBM, with only 7.1% of patients receiving palliative care services, despite a mortality rate of 2.8% [48].

Several drug treatment trials have proved disappointing. A multicenter, randomized, double-blind, placebo-controlled study to investigate the efficacy of arimoclomol showed no difference in change on IBMFRS score at 20 months [49]. An extension of the 1 year RESILIENT trial for the efficacy and safety of bimagrumab over a 2 year period showed no benefit in 6 min walk distance [50]. A randomized, double-blind, placebo-controlled, single center trial studying the efficacy of sirolimus did not show a significant benefit of maximal isometric knee strength, however, secondary outcome measures were encouraging [51]. Sirolimus targets both the autophagy pathway (potentially accelerating clearance of protein aggregates) and T cells, and a large, international phase III trial of sirolimus with IBMFRS as primary endpoint is underway.

A single-site randomized, double-blind, placebo-controlled, crossover study investigated the effect of testosterone on IBM [52,53]. Patients on testosterone cream showed an improvement in emotional wellbeing despite lack of significant improvement in strength over a 12 week period when compared to exercise alone [52]. Blood immunophenotyping during this study showed that testosterone resulted in a modest yet significant reduction in monocytes and eosinophils when compared to exercise alone [53].

Since KLRG1 was shown to be specifically expressed on highly cytotoxic T cells in IBM [19,24], an anti-KLRG1 antibody (Ulviprubart) is now in clinical trials for IBM. A phase 2/3 international, randomized, double-blind placebo-controlled trial evaluating safety and efficacy of two Ulviprubart doses at 76 weeks is expected to be completed at the end of 2025. If effective in IBM, this may finally settle the debate as to the pathogenicity of cytotoxic T cells in this disease.

CONCLUSION

New multiomic and imaging technologies hold promise for an improved understanding of IBM pathogenesis and an identification of disease biomarkers that may detect changes in disease activity

prior to changes in muscle function. Recent studies have highlighted the clinical heterogeneity of IBM and the need for effective treatments. While clinical trials of sirolimus and Ulviprubart are ongoing, an identification of additional drug targets is sorely needed.

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Conflicts of interest

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Molecular underpinnings of aging contributing to systemic sclerosis pathogenesis

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Purpose of review
Systemic sclerosis (SSc) is a systemic autoimmune disease characterized by diffuse organ fibrosis and vasculopathy. Aberrant aging has been increasingly implicated in fibrotic diseases of the lung and other organs. The aim of this review is to summarize the established mechanisms of aging and how they may contribute to the pathogenesis of SSc.

Recent findings
Shortened telomeres are present in SSc patients with interstitial lung disease (SSc-ILD) and associate with disease severity and mortality. Although the cause of telomere length shortening is unknown, immune mechanisms may be at play. Senescent cells accumulate in affected organs of SSc patients and contribute to a pathologic cellular phenotype that can be profibrotic and inflammatory. In addition to identifying patients with a more severe phenotype, biomarkers of aging may help identify patients who have worse outcomes with immunosuppression.

Summary
Aging mechanisms, including telomere dysfunction and cellular senescence, likely contribute to the progressive fibrosis, vasculopathy, and immune dysfunction of SSc. Further work is needed to understand whether aberrant aging is an initiator or perpetuator of disease, and whether this is cell or organ specific. A better understanding of the role aging mechanisms play in SSc will contribute to our understanding of the underlying pathobiology and may also influence management of patients exhibiting the aging phenotype.

Keywords
aging, interstitial lung disease, scleroderma

INTRODUCTION

Systemic sclerosis (SSc) is a complex systemic autoimmune disease characterized by diffuse organ fibrosis and vasculopathy. SSc has the potential to affect nearly every organ system and continues to carry a high mortality rate among the rheumatic diseases [1,2]. Although survival has improved over time, nearly 45% of patients with SSc will die within 10 years of diagnosis [3,4]. This high mortality rate is in part due to our limited understanding of the underlying disease pathogenesis, which has made the development of effective biomarkers and therapies challenging. An improved understanding of the primary drivers of disease is crucial for advancing care in the field.

Aging is associated with increased risk and severity of various diseases [5,6]. Aberrant aging has been independently shown to associate with fibrotic disease, including pulmonary fibrosis [7,8], as well as autoimmune conditions, including rheumatoid arthritis (RA) [9▪▪,10]. SSc represents a complex interplay of autoimmunity and progressive fibrosis and there is emerging evidence that a maladaptive aging process may play a role in SSc pathogenesis. Here we review how expected and aberrant aging mechanisms may be implicated in SSc and explore how this knowledge can be leveraged to advance care of SSc patients.

MOLECULAR MECHANISMS OF AGING

Aging is a complex and multifaceted process associated with cumulative molecular and cellular damage that leads to gradual physiologic decline over time. The incidence of most diseases increases with age, along with disease-specific morbidity and

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KEY POINTS

- Aging is a complex and multifaceted process associated with cumulative molecular and cellular damage that leads to gradual physiologic decline over time.
- Molecular mechanisms of aging, including telomere dysfunction and cellular senescence, are increasing implicated in systemic sclerosis (SSc), particularly SSc-interstitial lung disease.
- While unlikely the primary initiator of disease, maladaptive aging may become a more critical driver on in the disease course through sustained aging-related feed-forward loops that do not require high levels of inflammation or immune stimuli.
- A deeper understanding of the aging and senescence process can be leveraged to advance management of SSc patients, potentially identifying patients who have a more severe phenotype or may be less responsive to immunosuppression.

mortality [5,6,9▪▪,11]. In comparison to other rheumatic diseases, SSc has a later disease onset, with a mean age of about 45–60 years [11,12]. Patients with later onset disease have worse prognosis and often a more rapidly progressive disease course [13]. Although this may be compounded by other factors associated with age including frailty and comorbidities, aberrant aging mechanisms may be involved. Understanding of the biology of aging continues to expand and several key mechanisms are thought to play a role including telomere dysfunction, DNA damage and instability, and cellular senescence among others. Here we summarize some of the molecular drivers of aging, focusing on those that may be involved in SSc pathogenesis.

Telomere shortening and dysfunction

Telomeres are DNA structures composed of DNA repeats and supporting proteins, known as the shelterin complex, that play a crucial role in maintaining genomic stability. Telomeres shorten with each cell division and when their length becomes critically short, the shelterin complex can no longer bind and destabilizes the telomere [14,15]. As a consequence, the uncapped telomere leads to DNA instability and activation of the DNA damage response (DDR) and ultimately cellular senescence or apoptosis. Importantly, it only takes one to few critically short telomeres to activate the DDR [16]. The gradual attrition of telomeres is a key contributor to cellular aging and telomere length is inversely proportional with age [17–19]. Shortened telomeres are associated with all-cause mortality but more significantly with disease-specific morbidity and mortality [9▪▪,20].

DNA damage and instability

DNA damage, most often in the form of DNA double-stranded breaks, is another characteristic of aging. As cells age, there is accumulation of DNA damage due to factors such as replication error, oxidative stress, and environmental triggers [21]. These breaks activate the DDR pathway, which can lead to molecular and cellular consequences including a potent inflammatory response and cycle arrest. DNA repair mechanisms are in place to address these changes however their efficiency decline with age allowing for accelerated genomic damage over time [22,23]. Consequently, the rate of accumulation of somatic mutations is shown to scale with age and has an inverse correlation to lifespan [24].

Cellular senescence

Cellular senescence is characterized by a state of permanent cell cycle arrest, marked by expression of cell cycle inhibitors, p16 and p21 [25]. Senescence can be categorized as replicative or stress-induced, and can be brought about by telomere dysfunction, DNA damage, oncogene activation or organelle stress [26]. It is a mechanism to maintain tissue homeostasis by preventing proliferation of damaged cells. Importantly, senescent cells undergo a significant change of their cellular structure and function with alterations in their gene expression, metabolism, and morphology, which in aggregate drive the activation of a senescence-associated secretory phenotype (SASP) [26]. SASP is associated with secretion of proinflammatory mediators including cytokines, chemokines, and proteases, which actively influence the behavior of adjacent cells and alter the local microenvironment. Circulating inflammatory cytokines such as interleukin (IL)-6, a key SASP factor, increases with age and associates with all-cause mortality in aged populations [27]. In several aging associated diseases including lung fibrosis, atherosclerosis, and neurodegenerative diseases, senescent cells accumulate in affected tissues over time and likely contribute to promote local and systemic aging associated pathology [28].

DNA methylation, mitochondrial dysfunction, loss of proteostasis, and other mechanisms of aging

Several other mechanisms contributing to aging are also well described. Epigenetic modifications including DNA methylation and histone

modification, often change with age. A duality of global hypomethylation with hypermethylation of gene promoters and CpG islands is linked with aging mechanisms including activation of proinflammatory and interferon pathways and decrease in DDR [29,30]. Mitochondrial dysfunction also increases with age due to accumulation of mtDNA mutations, destabilization of respiratory chain complexes, and decreased clearance of damaged mitochondria [31]. This serves as a source of reactive oxygen species (ROS) that contributes to DNA damage, inflammation and cell death in a positive feed forward loop. Over time there is also disruption of protein homeostasis. With aging, there is an increase in errors of protein production due to inaccurate translation and ribosomal pausing, with a reciprocal decline in proteasome and autophagy pathways, leading to accumulation of misfolded and damaged proteins [32]. These mechanisms and others including stem cell exhaustion, deregulated nutrient sensing, and altered cellular communication have all been implicated in aging and aging-related diseases [33]. The following sections will explore how these processes may be implicated in SSc pathogenesis.

INTERSTITIAL LUNG DISEASE AS A MODEL OF AGING AND SENESCENCE

Interstitial lung disease (ILD) is the leading cause of morbidity and mortality in SSc [34]. Increased age is a well established risk factor for the development of ILD and associated with poor SSc-ILD outcomes [35]. Lung fibrosis is strongly associated with aging. Aging lungs exhibit reduced elasticity, decreased lung volumes and impaired gas exchange. These changes are in part due to stiffening of lung tissue resulting from accumulation of ECM [36]. On CT scans, lungs of healthy aged individuals demonstrate evidence of faint shadows suggesting scarring and altered architecture [37]. On a molecular level, gene expression profiles of aging human lung demonstrate an increase in senescence and pro-fibrotic pathways as well as expression of p16 and p21 [38]. Additionally, there is epithelial stem cell exhaustion of the lung with decreased number and dysfunction of alveolar type 2 cells (AT2), which have impaired differentiation to alveolar type 1 cells (AT1) [39]. These mechanisms appear to not only be present but accelerated in SSc-ILD pathogenesis (Fig. 1).

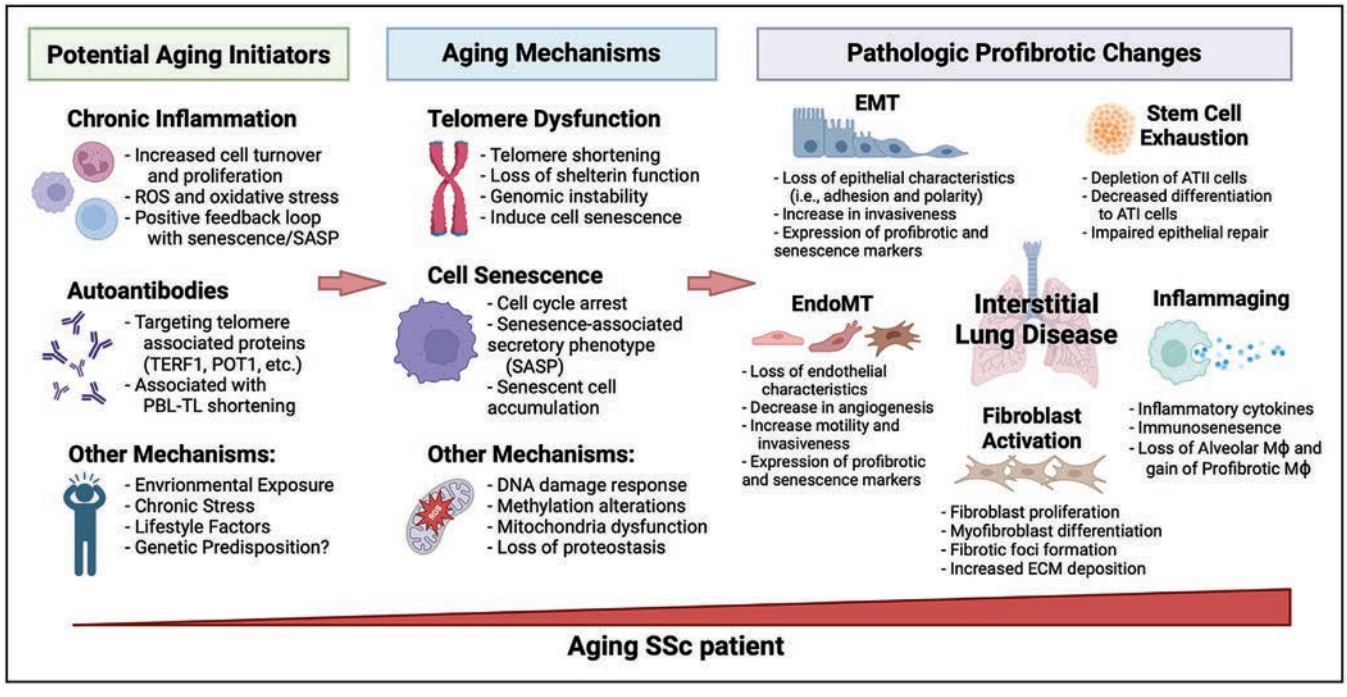

FIGURE 1. Scleroderma associated interstitial lung disease (SSc-ILD) as a model of aging mechanisms in SSc. Aberrant aging mechanisms are implicated in SSc pathogenesis. Although when in the disease course aberrant aging unfolds, potential initiators include chronic inflammation and autoantibodies targeting telomere associated proteins. Telomere dysfunction and cell senescence are present in SSc-ILD among other aging mechanisms. These likely contribute to the pathologic profibrotic changes that are present in SSc-ILD, some of which represent aging mechanisms that are amplified to a level that causes pathologic organ dysfunction. SSc, systemic sclerosis.

Telomere dysfunction in systemic sclerosis-interstitial lung disease

Telomere dysfunction and telomere shortening have been found across several forms of ILD, including SSc-ILD [40,41▪,42]. In idiopathic pulmonary fibrosis (IPF), the most common form of ILD, telomere dysfunction is an established driver of disease [43]. Mutations in TERC and TERT, telomere related proteins, are associated with familial IPF as well as a proportion of sporadic cases [44]. More commonly, a significant proportion of IPF patients are characterized by shortened telomeres in both the peripheral blood and affected tissue [7,8]. While SSc-ILD does not share the same genetic risk factors of IPF, there are overlapping features including older age, male predilection and for a subset of SSc-ILD patients, a shared histopathology [45]. SSc-ILD patients have shortened peripheral blood leukocyte telomere length (PBL-TL) compared to their non-ILD counterparts and degree of shortening is associated with ILD severity and overall survival [46–48]. Importantly, TL is also shortened in the lung epithelium, suggesting it may be mechanistically implicated in the disease pathogenesis [41▪]. PBL-TL has not been shown to be associated with other organ manifestations of SSc and therefore could represent an organ specific biomarker [42,48].

The cause of telomere shortening in SSc-ILD remains unknown. Unlike IPF where a genetic underpinning propagates telomere dysfunction, in SSc it is likely other disease-associated factors driving telomere attrition. Chronic inflammation is one possibility as the increased immune cell turnover can accelerate telomere shortening through replication. Sustained ROS production and oxidative stress associated with inflammation can also induce telomere attrition in a replication-independent manner [49,50]. Another possibility is 'acquired' telomere dysfunction from autoantibodies targeting telomere associated proteins. Adler *et al.* found a subset of SSc patients characterized by autoantibodies targeting TERF1 and other proteins involved in telomere maintenance, which associated with the presence and severity of ILD [51]. Patients with these antibodies were also found to have shortened PBL-TL. Whether these autoantibodies exert a direct pathologic function on the telomere is still to be determined.

Cellular senescence in systemic sclerosis-interstitial lung disease

Cellular senescence is also a key contributor to pulmonary fibrosis. Like in aged lung, senescent cells accumulate in lung tissue and acquire a SASP, including IL6 and TGFβ. These contribute to a proinflammatory and profibrotic environment promoting cellular changes including stem cell dysfunction, epithelial mesenchymal transition (EMT), and fibroblast activation [52]. Using bulk RNA-seq of in vitro senescent cells, DePianto *et al.* defined an epithelial, fibroblast, and consensus senescence signature that was recapitulated in scRNA-seq data from IPF and SSc-ILD lung [53]. When these signatures were probed in gene expression meta-analysis of SSc-ILD lung tissue, there was a 3-fold increase in senescence signatures in SSc-ILD compared to healthy controls [41▪]. Senescence related pathways including EMT, P53, and DNA repair pathways were also found to be increased in lung tissue from SSc-ILD [41▪,54].

What remains to be determined is whether specific cell subsets are disproportionately affected by senescence and thus play a more critical role in driving the dysregulated aging process in SSc-ILD. In IPF, AT2 cells, a progenitor cell of the lung epithelium, are depleted in IPF, exhibit shortened telomeres and have increased expression of p16, p21 and beta-galactosidase [55]. Similarly, AT2 cells are decreased in SSc-ILD, lose AT2 cell characteristics, and have telomere shortening [41▪,56]. Importantly pathologic AT2 cells are thought to be the progenitors of the aberrant basaloid population, a senescent epithelial cell with profibrotic phenotype, found uniquely in fibrotic lung disease initially in IPF and more recently in SSc-ILD [57–59]. Fibroblast senescence, although poorly understood in the lung has also been implicated in pulmonary remodeling. Cultured IPF fibroblasts demonstrate increased senescence markers and an activated phenotype, consistent with myofibroblasts with increased expression of α-SMA [60]. However, with the advent of single cell technology understanding of fibroblast heterogeneity is evolving and it will be valuable to elucidate which cells undergo senescence reprogramming in SSc-ILD in the context of this new information [61].

OTHER SYSTEMIC SCLEROSIS MANIFESTATIONS AND AGING

Skin fibrosis

Senescent cells are also detected in SSc skin [62▪]. Bulk gene expression analysis has shown upregulation of senescence signatures in affected skin with senescence genes including GDF15, COMP, and CDKN2A upregulated in diffuse vs. limited cutaneous SSc subjects [41▪]. At a single cell level, senescence signatures appear upregulated across many cell types in SSc skin, with highest enrichment in profibrotic fibroblasts located in areas of high ECM

deposition [62▪]. Furthermore whole exome sequencing of affected skin from early SSc subjects demonstrated increased burden of somatic mutations [63]. However unlike the lung, which often parallels and amplifies the mechanisms of 'normal' lung aging, skin involvement of SSc appears divergent from the intrinsic skin aging process [38]. Normal skin aging is characterized by increased ECM degradation, decrease in collagen synthesis and global fibroblast inactivity, mediated by decrease of profibrotic factors such as TGFβ, which is contrasting to what is seen in SSc skin. Furthermore, TL has not been found to associate with skin disease severity or be shortened in skin tissue [42,48]. This raises the possibility that the senescence present in SSc skin may arise from aging independent mechanisms and that the impact of aberrant aging may be tissue-specific.

Vasculopathy

Chronological aging is associated with increased risk of endothelial dysfunction and characterized by vascular stiffening, impaired neovascularization, and accumulation of senescent endothelial cells (EC) [64]. EC senescence can lead to decreased nitric oxide production, impaired vasodilation and impaired angiogenesis [65]. Senescent pulmonary artery smooth muscle cells (SMC) and pulmonary endothelial cells (PEC) accumulate in diseased vessels of subjects with idiopathic pulmonary arterial hypertension (PAH) and pulmonary hypertension (PH) in chronic lung disease [66,67]. In the lungs, senescent SMCs have been proposed to promote growth and migration of other SMCs, while senescent PECs recruit inflammatory cells through secretion of proinflammatory molecules [67,68]. In SSc, PBL-TL are shortened in SSc-PAH and associated with disease severity including lower DLCO%, elevated RVSP and increased hospitalization [48].

Senescent ECs can contribute to endothelial-mesenchymal transition (EndoMT), a process in which ECs undergo differentiation and adopt mesenchymal-like features including increased expression of pro-fibrotic markers (α-SMA, N-cadherin, type 1 collagen), ECM production, and migration and invasion capacities [69]. Cells in various stages of EndoMT have been identified in the dermal vessels of SSc patients and when cultured in SSc sera, dermal ECs undergo EndoMT and acquire fibroblast-like phenotype [70]. A recent scRNAseq study by Ma *et al.* showed EndoMT cells served as an additional source of pathologic ECM deposition in the skin and through their interaction with activated fibroblasts, served as a central communication hub to drive profibrotic signaling [71▪▪].

Immune aging in systemic sclerosis

The field of immune aging has grown over the last several decades with advances in RA, lupus and osteoarthritis, however, still much to be understood in the context of SSc. In natural aging, as the adaptive immune system wanes, a chronic overactivation of the innate immune system is observed, leading to a low-grade inflammatory state, which has been termed 'inflammaging' [72]. Within the T cell repertoire, there is a decline in naïve T cells with concurrent accumulation of terminally differentiated effector memory T cells, exhibiting a cytotoxic and NK-like phenotype, and senescent T cells [73,74]. Although T cell senescence is yet to be implicated in SSc, they are detected in the peripheral blood of early diffuse SSc subjects and their secretory phenotype, characterized by skewed cytokine production which sustains a persistent inflammatory environment, associates with disease severity in other autoimmune diseases such as RA [75,76]. For B cells, while there is an overall decrease in B cells with age, there is an accumulation of age associated B cells (ABCs), which are enriched for autoantibodies. These ABCs are expanded in several autoimmune diseases, and detected in a small cohort of SSc patients [77]. The innate system is increasingly active with age with increased bone marrow output with increased cytokine production. Cytokines and biomarkers, which we know are relevant to SSc including IL6, CRP, and IFN, are increasingly produced with age and contribute to the chronic inflammation of aging [78].

IMPLICATIONS AND INTERVENTIONS

Given the relevance of aging on SSc pathogenesis it is important to explore how these findings can be leveraged to advance the management of SSc patients. For example, molecular markers of aging could be used to stratify SSc patients in terms of their risk for a more severe or progressive phenotype. Multiple studies have shown shortened PBL-TL are clinically associated with worse survival in SSc and SSc-ILD, and likely serve as a surrogate for the molecular burden of senescent cells at the tissue level. While further validation is needed, these findings could potentially help support more aggressive screening or closer monitoring for patients at risk.

One question is whether as SSc patients age, their disease course shifts from an immune mediated phenotype to a more aging driven phenotype. Immune activation is prominent during early phases of SSc and likely the initiator of disease. However, later in the disease course, it is possible that aging and senescence mechanisms may become more critical drivers, leading to progressive fibrosis

and vasculopathy through sustained aging-related feed-forward loops that do not require high levels of inflammation or immune stimuli. For example, studies have shown that in ILD, patients with shortened telomeres have worse outcomes with immunosuppression, including SSc-ILD patients [79,80▪]. In these patients, aging and senescence mechanisms may be the predominate propagator of their lung fibrosis, and thus identifying these patients would provide the opportunity to reduce immunosuppression while targeting aging related mechanisms.

CONCLUSION

Overall, there is growing evidence that aberrant aging is implicated in SSc pathogenesis. Telomere dysfunction and cellular senescence, hallmarks of the aging process, are particularly relevant to the pathogenesis of SSc-ILD. Yet while there are clear links of aging and SSc, there are many questions that are left unanswered. Further work is needed to better understand the initiators of aberrant aging and to define when during the course of SSc do aging mechanisms become important drivers of the disease process. This could vary by tissue type and the specificity of organs involved, as different cellular subsets may have a variable susceptibility to aging and senescence. Overall, a deeper understanding of the aberrant aging processes in SSc will advance our knowledge of the underlying pathobiology and may allow identification of novel biomarkers and therapeutic targets to further improve management of patients with severe and progressive disease.

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Conflicts of interest

There are no conflicts of interest.

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This study shows that similar to IPF, non-IPF ILD patients with shortened telomeres may experience reduced survival when exposed to immunosuppression.

Neurovascular dysregulation in systemic sclerosis: novel insights into pathophysiology, diagnosis, and treatment utilizing invasive cardiopulmonary exercise testing

Elizabeth Tarras and Phillip Joseph

Purpose of review
Pathologic abnormalities in skeletal muscle and the systemic vasculature are common in patients with systemic sclerosis (SSc). These abnormalities may lead to impaired systemic peripheral oxygen extraction (EO_2), known as neurovascular dysregulation, which may be because of abnormal blood flow distribution in the vasculature, microvascular shunting, and/or skeletal muscle mitochondrial dysfunction. Findings from invasive cardiopulmonary exercising testing (iCPET) provide important insights and enable diagnosis and treatment of this SSc disease manifestation.

Recent findings
Recent findings from noninvasive cardiopulmonary exercise testing (niCPET) support the existence of neurovascular dysregulation in patients with SSc. Invasive cardiopulmonary exercise testing (iCPET) has pointed to reduced systemic vascular distensibility as a possible mechanism for neurovascular dysregulation in patients with connective tissue diseases, including SSc.

Summary
Neurovascular dysregulation is likely an underappreciated cause of exercise impairment and dyspnea in patients with SSc in the presence or absence of underlying cardiopulmonary disease. It is posited to be related to microcirculatory and muscle dysfunction. Further studies are needed to clarify the pathophysiology of neurovascular dysregulation in SSc and to identify novel treatment targets and additional therapies.

Keywords
dyspnea on exertion, impaired peripheral/systemic oxygen extraction, invasive cardiopulmonary exercise testing, neurovascular dysregulation, peripheral limitation to exercise, systemic sclerosis cardiovascular manifestations

INTRODUCTION

Systemic sclerosis (SSc), also known as scleroderma, is a rare, chronic connective tissue disease (CTD) characterized by a triad of immune system dysregulation, microvasculopathy, and tissue fibrosis [1]. SSc is defined by multisystem involvement and while its cause is unknown, SSc is posited to be initiated by environmental triggers in genetically susceptible hosts [2,3]. Despite recent advances in the pathobiological understanding, diagnosis and treatment of SSc, mortality remains high [4,5].

The pathogenesis of SSc is marked by endothelial cell dysfunction and microvascular injury [6]. This vasculopathy is related mechanistically to vessel fibrosis and ischemia in peripheral arteries and the microcirculation and is driven by the immune and inflammatory pathologies that underlie SSc [7,8]. Further, microvascular damage in SSc has heterogenous clinical manifestations and has been shown to include the skin, heart, lungs, gastrointestinal tract, kidneys, and coronary arteries [8–10].

Dysregulated circulation in skeletal muscle and the systemic vasculature has been implicated in SSc-related exercise impairment and dyspnea. This is observed in SSc patients even in the absence of abnormal breathing patterns or severe

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KEY POINTS

- Neurovascular dysregulation, or impaired systemic oxygen extraction (EO_2), can lead to impaired aerobic capacity (VO_2) in patients with systemic sclerosis (SSc).
- Neurovascular dysregulation in SSc is likely related to circulatory pathology in skeletal muscles and the systemic vasculature, which is common in patients with SSc.
- Invasive cardiopulmonary exercise testing (iCPET) can definitively diagnosis neurovascular dysregulation in SSc and can provide important data to identify novel treatment targets.
- Current treatment options for neurovascular dysregulation are limited but include slowly accelerated exercise training and off-label use of treatments such as pyridostigmine.

cardiopulmonary disease including interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH), though co-existing pathology is common [11,12]. This disease manifestation, herein referred to as neurovascular dysregulation, but also known as a peripheral limitation to exercise and/or impaired peripheral/systemic oxygen extraction (EO_2), is implicated in reduced skeletal muscle EO_2 during exercise and theorized to be due to abnormal blood flow distribution in systemic vasculature, shunting past muscle capillary beds, or intrinsic skeletal muscle mitochondrial dysfunction [13]. Neurovascular dysregulation disturbs the normal processes of aerobic respiration and cellular function and has been implicated in various diseases, including heart failure with preserved ejection fraction (HFpEF) [14], postacute sequelae of SARS-CoV-2 (PASC) [15], myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [13], and postural orthostatic tachycardia syndrome (POTS) [16]. We posit that neurovascular dysregulation likely represents an underdiagnosed cause of functional impairment in patients with SSc [17].

In this review, we discuss the pathophysiology of neurovascular dysregulation related to exercise impairment and dyspnea in patients with SSc with a focus on diagnostic evaluation using the invasive cardiopulmonary exercise test (iCPET). We highlight avenues for further research into therapeutic options for SSc-related neurovascular dysregulation.

NORMAL EXERCISE PHYSIOLOGY

Cellular metabolism is supported by the generation of ATP from ADP, which is produced by three distinct processes in striated muscle cells: aerobic oxidation of substrates (e.g. glycogen and fatty acids), anaerobic hydrolysis of phosphocreatine (PCr), and anaerobic catabolism leading to lactic acid production [18]. Sustaining a given level of exercise is contingent on the cardiopulmonary system's ability to persistently and efficiently deliver hemoglobin-bound oxygen to the microcirculation of contracting muscle cells, known as oxygen delivery (DO_2). This necessary increase in oxygen uptake (VO_2), is equal to the product of cardiac output (Q_c) and the arterial-mixed venous oxygen content difference [$C(a-v)O_2$], both of which must substantially increase during exercise [19]. Q_c increases through early augmentation of stroke volume (SV) followed by an increase in heart rate to ensure optimization of left ventricular contractility and preload [20]. This is coordinated with the regulation of arterial pH and pCO_2 in contracting muscles through control of ventilatory parameters, which respond to the distinct mechanisms of ATP production. As work in muscle cells rises, minute ventilation must increase sufficiently to compensate for lactic acid production. At moderate and prolonged submaximal exercise, these processes ensure that neither lactic acidosis nor respiratory alkalosis occur [21].

On a macrocirculatory level during exercise, oxygenated blood is distributed away from nonactive tissues including the splanchnic and renal vessels [22]. On a microcirculatory level, increased oxygen demand during exercise leads to increased production of vasodilatory substances, including histamine, adenosine, and nitric oxide (NO), which help to facilitate oxygen delivery across the capillary bed in muscles [23]. In healthy individuals, the rate of fall of capillary pO_2 depends on the ratio of muscle blood flow to muscle oxygen uptake. Oxygen becomes diffusion limited during exercise at the 'critical capillary pO_2'. This drives the initiation of anaerobic metabolism and a rightward shift of the oxygen–hemoglobin dissociation curve to the right to facilitate additional oxygen offloading, known as the Bohr effect (Fig. 1) [24].

While aerobic oxidation is the primary source of ATP regeneration, high intensity exercise requires anaerobic catabolism of glucose and glycogen leading to the production of lactic acid, referred to as the anaerobic threshold [18,20]. Lactic acid is buffered predominantly by bicarbonate (HCO_3^-), and minute ventilation must increase at a disproportionate rate relative to metabolic rate to compensate for the respiratory CO_2 load generated in the buffering process [21]. Ultimately, metabolic acidemia occurs that cannot be offset by buffering or the hyperventilatory response, which leads to exhaustion and the cessation of activity. This is referred to as the point of maximum oxygen uptake (VO_2 max), which is impacted by age, sex, fitness status, and disease state [25].

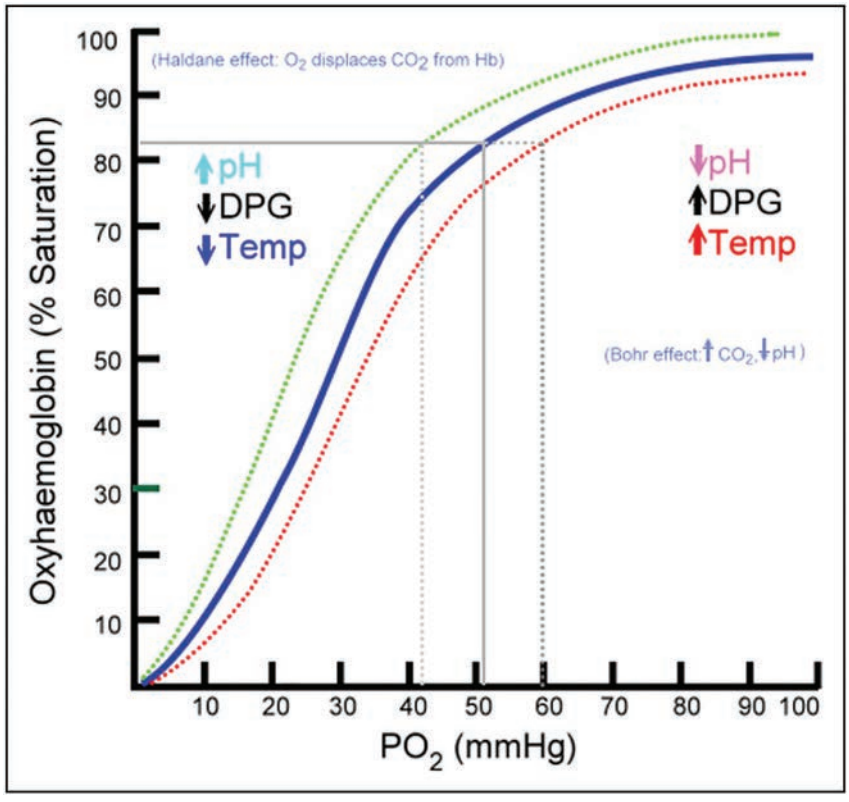

FIGURE 1. Bohr effect. Hemoglobin Dissociation Curve. Dotted red line corresponds with shift to the right caused by Bohr effect. CO2, carbon dioxide; DPG, 2,3-diphosphoglycerate; Hb, hemoglobin; PO2, partial pressure of oxygen; Temp, temperature. Contributed by Wikimedia Commons, Ratznium (Public Domain).

NEUROVASCULAR DYSREGULATION: PERIPHERAL LIMITATIONS

Exercise limitations can be due to pathophysiologic abnormalities in normal breathing mechanics, pulmonary mechanical function, cardiac function/Q_c, pulmonary vascular function, or EO_2, including in patients with SSc. Based on the Fick principle, which states that VO_2 max is equal to the product of Q_c and $C(a-v)O_2$, reduced VO_2 max should be due to a depressed Q_c or $C(a-v)O_2$, or both [23,26]. An impaired $C(a-v)O_2$ is commonly understood to represent a peripheral limitation to exercise, and can cause exertional symptoms such as fatigue and dyspnea. Impaired EO_2 leads to a low $C(a-v)O_2$ from limited oxygen utilization in the tissues and to an abnormally high mixed venous oxygen content at peak exercise [27]. Of note, Systrom *et al.* and Kisaka *et al.* have suggested that this may be too reductive a principle, overlooking the fact that muscle VO_2 is contingent on both convectional and diffusional transport of O_2 from muscle capillaries to mitochondria, but this is beyond the scope of this review [19,25].

Abnormal peripheral EO_2 has been described in patients with PAH, theorized to be related skeletal muscle perfusion heterogeneity or mitochondrial dysfunction [26]. It has also been demonstrated in patients with HFpEF [14,28] and in patients with primary mitochondrial myopathies [27,29]. Recently, this mechanism was found to account for depressed peak VO_2 in patients with PASC [30]. Poor systemic EO_2 has been found in up to 12.5% of patients with unexplained dyspnea in the absence of severe underlying cardiopulmonary pathology, here felt in part connected to the process of hyperventilation, which blunts the Bohr effect leading to poor oxygen offloading in exercising muscles [31].

Abnormal EO_2 coexists with other exercise-related pathology [32▪▪]. For instance, a subset of patients with undifferentiated exertional capacity with impaired EO_2 have been shown to have impaired exercise-related Q_c augmentation because of low biventricular filling pressures ('low flow'), suggesting that inadequate preload leads to suboptimal exercise response, perhaps due to a failure of normal vascular mechanisms to increase venous return to the cardiac system [16]. This seems to be the case for patients with POTS, where despite volume administration prior to testing, impairments in venous capacitance vessels may impair venoconstriction leading to low SV [16]. Additionally, in

patients with ME/CFS, a 'high flow' phenotype has been identified in patients with impaired EO_2, where this pathology corresponds to increased pulmonary blood flow, possibly related to extracardiac left-to-right shunting or a mitochondrial myopathy [13]. Interestingly, the latter group of patients has also been found to have a high prevalence of small fiber neuropathology, suggesting abnormalities in vascular tone, which can impair blood delivery to exercising muscles causing irregular metabolic function, early exertional intolerance, and muscle fatigue [13]. Taken together, peripheral limitations to exercise imply a more globally dysregulated neurovascular system, providing important context for understanding exercise limitations in SSc.

NEUROVASCULAR DYSREGULATION IN SYSTEMIC SCLEROSIS: PHYSIOLOGY AND PATHOGENESIS

In patients with SSc without overt cardiopulmonary disease or musculoskeletal involvement that would impede mobility (e.g. flexion contractures, myositis, inflammatory arthritis) exercise impairments are common [11]. Initial studies using conventional noninvasive cardiopulmonary exercise testing (niCPET) variably combined with transthoracic echocardiography (TTE), chest computed tomography (CT), and nonsimultaneous right heart catheterization (RHC) indirectly evaluated peripheral EO_2 and oxygen transport profiles through pattern-based approaches and found abnormalities in these processes to be common in patients with SSc with low aerobic capacity [12,33]. Combining CPET with invasive hemodynamic monitoring with a RHC (without an arterial line) has provided additional insight, with a study by Walkey *et al.* [34] demonstrating that up to 30% of SSc patients with low peak VO_2 lack evidence of significant ventilatory, pulmonary vascular, or left ventricular diastolic dysfunction limited exercise, thus suggesting the presence of a peripheral limitation in this population. Brown *et al.* utilized niCPET augmented by cardiovascular magnetic resonance (CMR-CPET) to simultaneously measure oxygen consumption and cardiac output to allow for indirect calculation of $C(a-v)O_2$, the most common marker of oxygen extraction. This study found that impaired $C(a-v)O_2$ was ubiquitous in SSc patients even in the absence of significant radiographic parenchymal lung disease or PAH [17]. Most recently, Ross *et al.* [11] paired niCPET with TTE and skeletal muscle MRI to show that SSc-related exercise impairment in the absence of cardiopulmonary disease is associated with skeletal muscle edema and fatty infiltration of muscle in the absence of clinically overt myositis.

In the only study to date to use invasive CPET (iCPET), and thus to definitively describe the contribution of circulatory dysregulation to whole body exercise impairments, Singh *et al.* found that patients with CTDs (including those with SSc) without cardiopulmonary disease had reduced peak VO_2 and reduced oxygen extraction ratio (calculated by $Ca\text{-}vO_2/CaO_2$) [23]. Of interest, this patient population also had evidence of reduced systemic vascular distensibility, which was associated with higher systemic vascular resistance (SVR) and impaired SV augmentation posited to be because of increased left ventricular afterload [23]. These findings provide novel insight into the mechanisms behind exertional tolerance in SSc, suggesting a global vasculopathy leading to systemic vascular stiffness and microvascular dysfunction contributing to impaired oxygen delivery to tissues, resulting in early AT, lactic acidemia, and depressed VO_2 max [23].

No prior data have elucidated direct mechanisms underlying abnormalities in peripheral EO_2 in SSc, but the pathophysiology of SSc provides important insights. Autonomic dysfunction has been identified as a cardinal feature of SSc. Dysautonomia related broadly to cardiovascular impairment has been described, suggesting sympathovagal imbalances due to a global reduction in parasympathetic tone and overactivity in the sympathetic system even in ostensibly asymptomatic patients with early SSc [35–37]. Vascular dysfunction in arterioles and capillary microcirculation is another hallmark of SSc, postulated to be an early phase in disease progression [6,38]. Microvascular endothelium injury leads to endothelial cell dysfunction characterized by disrupted cellular growth factors and immune mediators, disturbances of coagulation and fibrinolysis processes, and abnormalities in vasculogenesis [8]. Additionally, SSc-related vasculopathy is associated with an imbalance of vasoactive factors, including overproduction of endothelin-1, which is a vasoconstrictor-mediating smooth muscle proliferation, and underproduction of NO, a potent vasodilator [38]. Infections, reactive oxygen species, cytotoxic T cells and autoantibodies, including antiendothelial cell (AECA), antiangiotensin II, and antiendothelin type-A receptor (ETRA) antibodies are suspected to play a role, with the latter two antibodies thought to further mediate vasoconstriction and obliterative vasculopathy [38]. Histologically, SSc microcirculatory vessels have been shown to demonstrate neointimal lesions, adventitial fibrosis, perivascular mononuclear cell infiltration, and distortion of capillary beds [38].

Microcirculatory impairment in exercising muscles has also been associated with underlying

myopathies, which are common, heterogenous manifestations of SSc reported to affect 13–90% of patients [39] and associated with increased mortality [40]. This pathology is defined by histologic hallmarks of fibrosis and inflammation in muscles. Interestingly, microangiopathy with reduced vascularization of skeletal muscle fibers has also been described [39,41], along with neurogenic atrophy [40]. This process has been posited to be related to sarcopenia, defined as a progressive, diffuse disorder of skeletal muscles, characterized by progressive loss of muscle mass and function [42], and patients with SSc-related sarcopenia have been found to have a high prevalence of microvascular damage and decreased peripheral blood flow due to altered capillary density and distribution [43].

Taken together, we hypothesize that impaired EO_2 in SSc without cardiopulmonary disease or other explanatory factors may be related to impaired autonomic control because of overactive sympathetic activity blunting expected microcapillary dilation during exercise and impairing oxygen extraction [23]. This maladaptation is likely associated with the demonstrated imbalance between vasoactive substances in the vasculature (e.g. endothelin-1, NO). Whether this microcirculatory dysfunction is a cause or effect of common underlying myopathies or sarcopenia in SSc is yet to be determined. Finally, recent studies have pointed to the role of the mitochondria and metabolic dysfunction in the pathogenesis of SSc [44,45]. Future research is needed into whether primary mitochondrial defects or altered mitochondrial function because of immune and inflammatory dysregulation related to SSc is related to impaired peripheral EO_2 in this disease.

DIAGNOSIS

In patients with SSc who present with exercise limitations or dyspnea, it is critical to perform thorough noninvasive testing including, but not limited to, physical exam, serologic studies, cross-sectional chest imaging, electrocardiogram (ECG), TTE, 6-min walk test/ambulatory oximetry, and pulmonary function testing [46]. Additionally, based on the noninvasive findings, a resting supine RHC or left heart catheterization (LHC) may be indicated. These initial evaluations can aid in identifying SSc-related complications including PAH, ILD, and cardiomyopathies (i.e. left ventricular dysfunction or HFpEF), especially critical as these SSc-related syndromes underlie the majority of deaths in this population [47]. In the absence of any clear diagnosis, CPET can provide more physiologic insight into mechanisms, underlying exertional intolerance [48]. CPET is typically performed on a cycle ergometer with individualized, incremental increases in workload and can aid in determining the cause of exertional intolerance by distinguishing among cardiovascular, pulmonary, and skeletal muscle disorders. niCPET, which involves a metabolic cart, 12-lead ECG, and brachial sphygmomanometer, is an appropriate first step to evaluate exercise limitations in patients with SSc with presumed impaired EO_2. This test, however, is limited to discretely identifying the presence of decreased aerobic capacity (defined as a VO_2 max $<80\%$ predicted) and a pulmonary mechanical limit to exercise; whereas it can suggest central cardiovascular dysfunction (e.g. heart failure or pulmonary vascular disease) or impaired EO_2, it cannot reliably distinguish between these entities [20].

As such, in patients with SSc with exertional limitations found to have a depressed VO_2 max on niCPET with an otherwise unrevealing noninvasive evaluation, iCPET should be considered. iCPET can also be considered in absence of niCPET data in SSc patients with persistent dyspnea without a clear cardiopulmonary cause. iCPET protocols, which facilitate obtaining ventilatory parameters, intracardiac hemodynamics, and blood gas data, have been previously described in detail (Fig. 2) [20,32▪▪]. In brief, the procedure begins with ultrasound and fluoroscopy-guided placement of pulmonary and radial artery catheters followed by a standard, resting supine RHC. Subsequently, patients perform a maximal, incremental exercise test on an upright cycle ergometer with a mouthpiece that allows for the collection of gas exchange parameters. Hemodynamics, including mean systemic arterial pressure, right atrial pressure, right ventricle pressure, and pulmonary artery pressure, are collected concomitantly. Each minute, pulmonary artery wedge pressure and arterial and mixed-venous blood gases are measured [20,32▪▪]. Q_c is calculated using the direct Fick principle [32▪▪]. iCPET is able to evaluate complete cardiopulmonary hemodynamics and peripheral tissue EO_2 data and can diagnose early and rare causes of exertional intolerance such as exercise pulmonary hypertension [49], exercise HFpEF [50,51], and low biventricular filling pressures leading to depressed Q_c [16]. Additionally, it allows for analysis of peripheral tissue EO_2 data and for accurate diagnosis-related pathology (i.e. neurovascular dysregulation) in the presence or absence of other hemodynamic abnormalities.

iCPET is considered a safe and well tolerated procedure; however, specific concerns must be considered in patients with SSc. At the outset, patients must be able to exercise on a cycle ergometer, which may be a barrier for SSc patients with severe orthopedic pathology, including those with

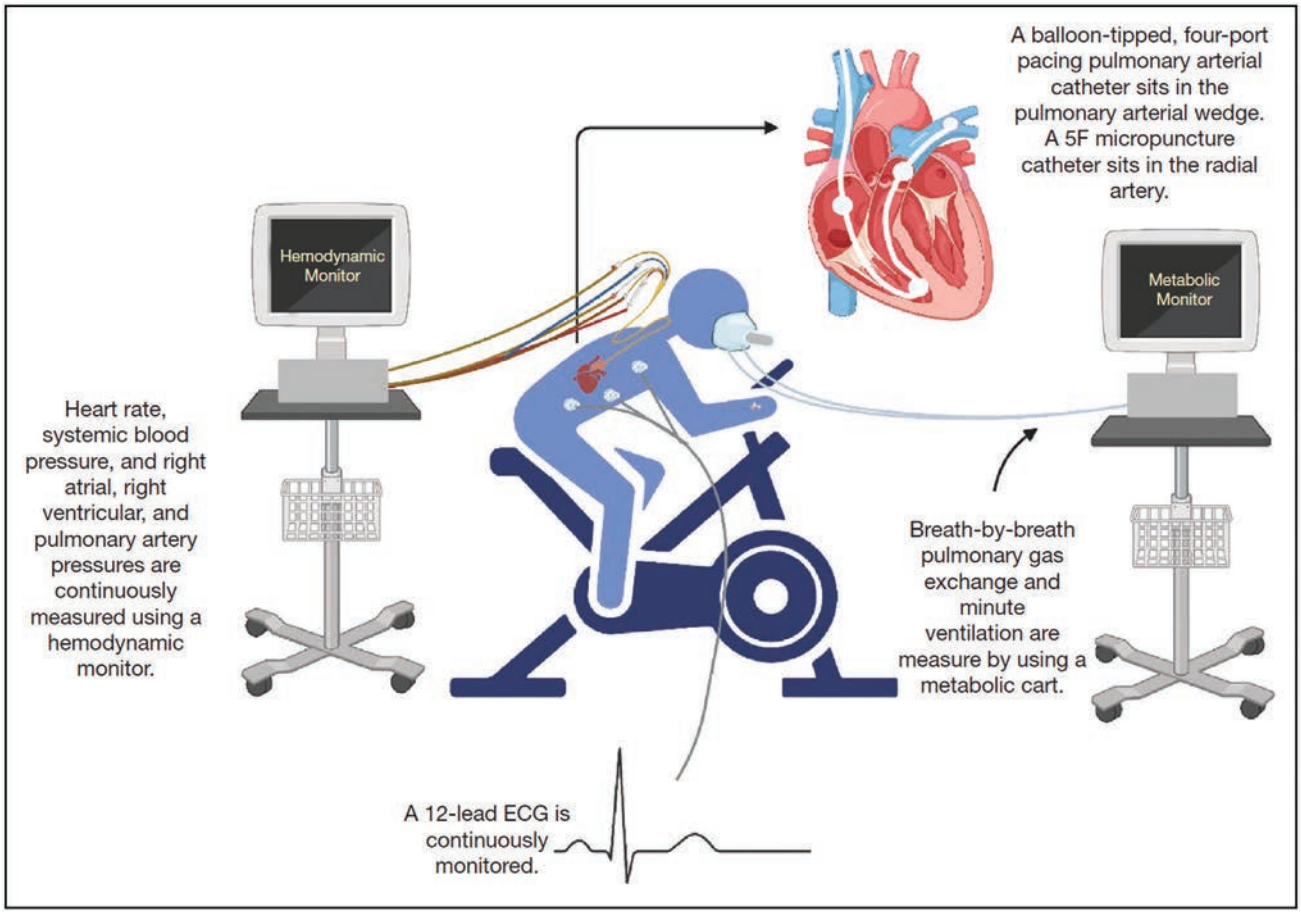

FIGURE 2. Invasive cardiopulmonary exercise test.

fixed contractures of the interphalangeal joints or lower extremities. Regarding procedural concerns, we generally prefer to perform iCPET with a radial artery catheter to directly measure radial artery saturation (SaO_2) given reliance on oxygen saturation from pulse oximetry (SpO_2) has been shown to lead to inaccurate data acquisition and thus misdiagnosis in patients with SSc and Raynaud's disease [52]. However, it is important to account for specific patient characteristics during procedural planning, given severe vaso-occlusive disease is considered a relative contraindication to radial artery access [53]. Additionally, though we routinely do not perform an Allen or Barbeau test to confirm patency of arterial flow to the hand in a general population per current consensus [53], we would consider doing so in patients with SSc given the high rates of ulnar artery occlusion in this population [54]. Finally, we may also alter our patient interface with the metabolic cart system in patients with SSc; while the gold standard is using a bite block mouthpiece, this may be unfeasible in patients with microstomia in which case we will opt to use a facemask instead [55]. With that said, given the general expertise of centers performing iCPET, we recommend referring all SSc patients in whom iCPET is indicated for direct consultation with the iCPET team for evaluation of procedural safety and feasibility.

As described earlier, in the case of SSc-related neurovascular dysfunction, expected iCPET findings include low VO_2 max (<80% predicted), representing decreased aerobic capacity in the setting of a peak exercise effort (respiratory exchange ratio >1.05 and peak heart rate >85% predicted) with no pulmonary mechanical limitation to exercise. DO_2 should be normal and accompanied by the main pathologic findings of elevated peak mixed venous oxygen saturation and peak venous oxygen content with depressed $C(a-v)O_2$ [$C(a-v)O_2$/(Hgb) <0.80] [32▪▪]. This finding in the absence of cardiopulmonary disease may be accompanied by the presence of low biventricular filling pressures [23], or by a high-flow state suggested by an elevated Q_c/VO_2 slope as in patients with ME/CFS and PASC; however, further studies are needed to fully elucidate distinct hemodynamic phenotypes [32▪▪].

Whether morphologic or functional analysis of the microcirculation should be pursued in the setting of iCPET confirmed SSc-related neurovascular dysregulation is not clear, though it may help

facilitate an approach to treatment. Historically, direct nailfold capillaroscopy (particularly nailfold videocapillaroscopy) has been utilized for morphological analysis of microvascular dysfunction in SSc. Contemporary technology has focused on dynamic analyses of microcirculatory blood flow to understand the functional implications of these changes (including laser doppler perfusion imaging and laser speckle contrast analysis) [7], whereas MRI-based Digital Artery Volume Index (DAVIX) is being explored as a surrogate outcome measure of vascular disease activity in SSc [56]. None of the aforementioned technologies, however, have been utilized to characterize microvascular dysfunction related to peripheral EO_2 during exercise and thus represent novel avenues of research. More recently, skin biopsies have been utilized to detect small fiber neuropathy (SFN) in patients with PASC and ME/CFS, which is felt to be related to the dysregulated small-vessel tone common in these syndromes and theorized to be related to impaired EO_2 [13,57]. Similarly, further research is needed to understand the utility of this testing in the SSc population.

TREATMENT AND KNOWLEDGE GAPS

There are no approved treatments for SSc-related exercise derangements because of impaired EO_2. Additionally, there is no evidence to currently suggest that traditional SSc treatments including biologic and immunosuppressive agents improve exercise capacity related to EO_2 in the absence of cardiopulmonary disease [58]. Instead, nonpharmacologic and pharmacologic treatment options for impaired EO_2 are largely based on extrapolated data from the POTS, ME/CFS, mitochondrial myopathy, and PASC literature, recognizing these are distinct conditions with heterogenous pathology. The most promising nonpharmacologic therapy is slowly accelerated exercise training, which has been shown to have significant benefit in patients with mitochondrial disease, leading to improved maximal oxygen uptake and without deleterious effect [59], and has been recommended in POTS patients to increase cardiovascular conditioning [60]. Although multiple vitamins and cofactors are commonly used to treat mitochondrial disease including CoQ_{10}, L-carnitine, creatine, α-lipoic acid, and various B vitamins, there is little evidence to support their benefit [61]. Related compounds aimed at supporting mitochondrial function, known as metabolic modulators, are currently being studied in small populations with PASC pointing to improved functional capacity, but results have yet to be replicated [62].

Regarding pharmacologic therapy, a small double-blind randomized controlled clinical trial (RCT) using iCPET showed that administration of pyridostigmine to patients with ME/CFS improved peak VO_2 by increasing Q_c and right ventricular filling pressures, potentially by targeting impaired venous return caused by autonomic dysfunction [63]. Another small study explored the use of rituximab to treat ME/CFS but showed no evidence of clinical utility [64]. Additionally, despite prior negative trials for using IVIG to treat SFN [65], there has been renewed interest in utilizing this treatment for PASC with promising results [66]. Finally, in SSc patients with impaired EO_2 with clinical overlap with POTS, data support use of medications such as midodrine, fludrocortisone, and droxidopa; however, these medications all lack robust RCTs to support their efficacy in an SSc population [67]. With that said, there is great need for RCTs in SSc-related neurovascular dysregulation to better characterize treatments to assess the efficacy and safety of the aforementioned interventions in this patient population.

SSc is associated with significant morbidity, including functional impairments related to exercise intolerance because of dysregulated circulatory biology. This has significant impacts on quality of life, necessitating further research into its underlying pathophysiology and treatment options. iCPETs offer substantial insight into the mechanisms underlying the process of neurovascular microcirculatory dysregulation in SSc above that which can be gleaned from noninvasive testing alone. Future diagnostic pathways, utilizing iCPET along with morphologic and functional assessment of muscles and their vasculature may be helpful. This could be further strengthened by genomic sequencing to uncover underlying mitochondria-related pathology [59], which is of strong interest in patients with SSc-related exercise impairment. Additionally, in recent years, there has been increasing interest in integrative multiomics approaches to understanding the pathogenic pathways of SSc, especially in the setting of the heterogeneity of the disease process [68]. Future directions should both seek to characterize the underlying molecular signatures of impaired EO_2 in patients with SSc and to utilize these markers to develop targeted treatments for this disease manifestation.

CONCLUSION

SSc is a complex disease with heterogenous manifestations causing significant morbidity. In patients with SSc without overt cardiopulmonary disease or significant musculoskeletal abnormalities, circulatory pathology in skeletal muscles and the systemic circulation can lead to neurovascular dysregulation,

which causes impaired systemic EO_2 leading to exercise impairment and dyspnea. iCPET can aid in clarifying this exercise-related physiologic derangement. Further research is needed into the cause of this condition and its relationship to other SSc-related vasculopathies, its long-term sequelae, and potential treatment options.

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Conflicts of interest

There are no conflicts of interest.

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

- ■ of special interest
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