

*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 and Section Editors for this issue.

## EDITOR IN CHIEF

### Steven B. Abramson

Steven B. Abramson, MD, is Senior Vice President and Vice Dean for education, faculty and academic affairs at NYU Langone Medical Center, USA. He is the Frederick H. King Professor and Chair of the Department of Medicine. As Vice Dean, he oversees the implementation of the medical school's nationally recognized curriculum for the twenty first century, including the country's first multispecialty three-year pathway to the MD degree.



A graduate of Dartmouth College, Dr Abramson earned his MD from Harvard Medical School and trained at NYU Medical Center and Bellevue Hospital, USA. He served as the Director of the Division of Rheumatology from 2000 to 2013, and has had numerous leadership positions in academic medicine. He has served on the Board of the National Arthritis Foundation, as Co-Editor of *Arthritis & Rheumatism*, a member of the Rheumatology Board of the American Board of Internal Medicine (ABIM), President of the Osteoarthritis Research Society International (OARSI), and former chairman of the Arthritis Advisory Committee of the Food and Drug Administration (FDA).

Dr Abramson has extensive experience in both basic science and clinical research in the field of inflammation and arthritis, and has published more than 300 papers on these and related topics. He received the prestigious American College of Rheumatology Distinguished Basic Investigator Award in 2011.

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### Hasan Yazici

Hasan Yazici, MD is a retired Professor of Medicine and Rheumatology. He currently practices Rheumatology, part time, at the Academic Hospital in Istanbul, Turkey. He still weekly attends the dedicated Behçet's syndrome outpatient clinic he has started with a group of his colleagues 40 years ago and co-edits the LER & CER - Letter to Editor Rheumatology: commentary and controversy in Rheumatology section in *Clinical and Experimental Rheumatology*.



After Dr Yazici received his MD from University of Istanbul, Turkey in 1969, he trained in internal medicine and rheumatology at the University of Nebraska and Creighton University (Metabolic Research Unit) in Omaha, Nebraska, USA, where his mentor was Paul D. Saville.

After returning to Turkey in 1974, he joined the Cerrahpasa Medical Faculty of University of Istanbul where he started both the multidisciplinary Behçet Disease Outpatient Clinic and the Division of Rheumatology, which he chaired until his retirement six years ago. His main research interests are Behçet's syndrome, clinical research methodology and ethics. He has published many original articles in peer reviewed journals in addition to his text book contributions, editorials, reviews and is the most cited author on Behçet's disease on Web of Science.

Dr Yazici has received a number of prestigious awards and has a long list of memberships in scientific societies including Science Academy Society of Turkey, European Academy of Sciences, Master of

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### Yusuf Yazici

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

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Dr Scher is founding member of Psoriasis and Psoriatic Arthritis Clinics Advancement Multi-center Network (PPACMAN), and also serves as in the NPF scientific committee, as an expert member for the development of the ACR guidelines for psoriatic arthritis (PsA), and as one of seven members of the FDAs Arthritis Advisory Committee. He is funded through the NIH to expand his studies on pharmacomicrobiomics in RA and PsA.

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# A review of vasculitis 2019: an introduction

Hasan Yazici<sup>a</sup> and Yusuf Yazici<sup>b,c</sup>

The year's review of vasculitides begins with two excellent articles on the recent advances in disease mechanisms of two nosologic entities rheumatologists have to recognize and manage. These entities both have a humble designation of uncertainty, antineutrophil cytoplasmic antibody (ANCA)-associated and IgG4-related. These, at the same time healthy, designations perhaps make them more open to scientifically fruitful investigation in contrast to other nosologic conditions of again undecided pathologic mechanisms many of us choose to call with an eponym or a phenotypic character. Examples are Behçet's disease and ankylosing spondylitis. In both cases the more humble designation of a syndrome would also make them more open to fruitful scientific query, with less lumping and more splitting. Furthermore, in the case of the eponym, it would show no historical disrespect to the original describer.

As Wester Trejo *et al.* (pp. 3–8) very clearly and intellectually entertainingly relate, the construct we call ANCA-associated vasculitis (AAV) might not be so pauci-immune, after all. The established understanding had been that there was very little or no immune-complex and complement deposition in the involved tissues in AAV. This also went along with a lack of hypocomplementemia, usually expected in our traditional immune-complex diseases. Based on data accumulating over a decade, this apparently had been an over simplification. It turns out that there are complement deposits, granted not as conspicuous as in immunofluorescence as in a patient with lupus nephritis, particularly made up of the elements of the alternative pathway. It seems the trigger of complement activation comes from neutrophils through NETOSIS. There is also evidence that agents that inhibit the complement cascade, like CCX168 molecule inhibiting C5aR, are effective in managing nephritis in AAV. As a final note, we find the recent nosologic splitting of ANCA negative AAV from AAV a stern reminder to the lumpers among us how science progresses mainly with splitting. It turns out that a complement defect, again in the alternative pathway, is probably responsible for the nephritis of ANCA negative AAV.

The construct of IgG4-related disease (IgG4-RD) is relatively new and that is perhaps why an IgG4

negative IgG4-RD has not yet been proposed. On the other hand, the mere fact that at least a dozen names had been assigned to this construct rather soon after its recognition [1] implies that such a proposal might not be very far-fetched. Meanwhile, its dedicated students (pp. 9–15) now summarize, analyze and give us important research clues about the types of immunologic/inflammatory cells involved in IgG4-RD. There seems to be not much debate about the involvement of CD4+ T cells in the pathogenesis of this condition, whereas the current research is about the roles played by the various subsets of these cells. There seems to be general agreement that the T follicular helper cells, with its five distinct subsets, are important members of the orchestra. The authors clearly describe how these subsets interact with each other in both causing tissue injury/fibrosis and tissue localization. At the end, we are advised about the important need for sound animal model(s) for this condition before we can have additional options, besides glucocorticoids, in managing IgG4-RD.

The last several decades saw an almost breathtaking progress in imaging modalities and an ever-increasing versatility in their clinical/research use. The chapter by Mavrogeni *et al.* (pp. 16–24) relates to one such use, cardiovascular MRI (CMR). The usefulness of this noninvasive/nonradiating imaging modality in visualizing the abnormality in the large vessels is widely appreciated. On the other hand, the authors importantly remind us that all systemic vasculitides can affect the heart at the microscopic level, whereas this becomes clinically apparent only in up to 10% of patients. CMR has the potential of closing this gap. We are also told that compared with the utilization of different imaging techniques, CMR has the superiority of showing us the wall details of the larger vessels. Two important limitations are a relative lack of availability and the

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rather long examination time required of this imaging modality.

Salvarani and Hatemi's chapter (pp. 25–31) on the management of giant cell and Takayasu arteritis (GCA, TAK) begins with reiterating that glucocorticoids are still the mainstay of treatment, whereas, in a perhaps older terminology, steroid-sparing agents are still much in need for effective management. The traditional immunosuppressive agents like azathioprine and methotrexate have limited usefulness. Adding tocilizumab, an IL-6 inhibitor, to glucocorticoids has been convincingly shown to be useful in managing GCA in two recent double blind trials. As such tocilizumab is becoming widely used in GCA, whereas issues like when and which patients it used to be used, whether it should be a first line agent to add in relapsed patients during glucocorticoids tapering and if and how it should be used for maintenance still remain. As for TAK, the use of biologics in addition to glucocorticoids is apparently more problematic. Two controlled trials, one with tocilizumab and another with abatacept, failed to show any efficacy, whereas beneficial effects in observational experience have been reported. The authors also point out that one important issue of debate is what best to use as progression/remission disease markers in TAK.

The next two articles in this update discuss current data on nervous system involvement in vasculitides. The first article is about central nervous system (CNS) disease in Behçet syndrome and the second is about involvement of peripheral nervous system in vasculitides in general. First, Uygunoğlu and Siva (pp. 32–39), from a dedicated Behçet center in Turkey give a very useful update of CNS Behçet based on their extensive and long-term experience in this condition. The two main highlights of their account are the Bagel Sign and the Motor Neuron types of spinal cord involvement and their less optimistic view of the usefulness of infliximab as a very successful agent in maintaining CNS remission. Surely a controlled study, unfortunately distinctly sparse in CNS Behçet, can potentially settle the second issue.

The chapter by Graf and Imboden (pp. 40–45) tells us about peripheral nerve involvement, a tissue localization distinctly rare in Behçet syndrome, in

vasculitides in general. We as, perhaps, many other rheumatologists will find their account of nonsystemic vasculitic neuropathies, which apparently can only be diagnosed by a biopsy, most useful.

The final chapter by Caproni and Verdelli (pp. 46–52) on the nomenclature for cutaneous vasculitis gives a detailed account of the trials and tribulations of finding a correct, descriptive name for different forms of skin vasculitis. It includes a discussion of the recent dermatology addendum [2] to the widely acknowledged 2012 Revised International Chapel Hill Consensus Conference of Nomenclature of Vasculitides. The authors underline that this addendum is an important landmark. They also underline this addendum is for nomenclature and not for diagnostic criteria. We find it interesting in this exercise that many well known students of vasculitis, the nomenclatura if you will, are leaving on the side the concept of classification which is nothing more than a quantitative expression of the astuteness of how we name what we observe, the nomenclature. We know of no other way than painstaking classification which always involves sensitivity, specificity and pretest probability in expression of the usefulness of any naming for any pathology in medicine. To this, of course, should also be added, a humble account of the probability of being wrong in any naming, surely including declaring a diagnosis [3].

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*There are no conflicts of interest.*

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# The role of complement in antineutrophil cytoplasmic antibody-associated vasculitis

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## Purpose of review

To provide a comprehensive overview of the current insight into the role of complement activation in antineutrophil cytoplasmic antibody-associated vasculitis (AAV). In addition, the therapeutic options targeting the complement system in AAV are discussed.

## Recent findings

It has become increasingly clear that complement, and more specifically signalling through the C5a receptor, contributes to the immunopathology of AAV. This has led to the design of clinical trials with a C5a receptor blocker. The first results show a reduction in tissue damage and a favourable safety profile, as other parts of the complement defence system are left intact.

## Summary

Although AAV was initially regarded as a pauci-immune disease, it is now well established that, in addition to autoantibodies, complement plays an essential role in the disease process. Animal models delivered the first insight, but the effective therapeutic interventions using complement inhibitors provided the proof that indeed complement activation contributes to disease activity and tissue damage in human AAV.

## Keywords

antineutrophil cytoplasmic antibody-associated vasculitis, complement, immunopathology, therapeutics

## INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) includes several conditions including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic GPA (EGPA) [1]. AAV can present with numerous signs and symptoms, ranging from a mild skin rash to life-threatening systemic disease [2] and recently novel diagnostic strategies have been proposed [3]. Clinical manifestations include ear, nose, and throat symptoms, upper respiratory tract problems (especially in GPA and EGPA) and renal involvement. These clinical phenotypes all have in common the presence of ANCA in around 90% of patients [4]. These antibodies target two distinct neutrophil antigens, namely, proteinase 3 (PR3) and myeloperoxidase (MPO) [5]. Although in resting neutrophils the ANCA antigens are expressed only intracellularly, the priming of neutrophils by, for example, cytokines or activated complement fragments leads to the increased expression of both ANCA antigens on the cell surface, allowing for the binding of ANCA [6]. Binding of ANCA to cell-associated antigens triggers further cellular activation leading to degranulation and migration of the neutrophils into the

vessel wall resulting in vasculitis [6]. The clinically most prominent manifestation of this vasculitis is glomerulonephritis [1]. Characteristic histologic lesions in the kidney include crescents and fibrinoid necrosis, with little to no immunofluorescent staining for immune complexes and complement [4]. Because of the pauci-immune nature of the immunohistochemistry of lesional tissue, a role for antibodies and complement in AAV was initially not expected. This idea was further supported by the lack of hypocomplementaemia, a sign frequently observed in other conditions involving complement activation and consumption. In addition, genetic screening did not identify complement variants as a risk for developing AAV [7], although a small

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## KEY POINTS

- In mouse models, activation of the complement system via the alternative pathway is necessary for the development of AAV.
- In the development of AAV, a complex interplay between complement, neutrophils, ANCA and the coagulation system causes an inflammatory amplification process that results in endothelial vascular injury.
- C5a and the C5a receptor play an important role in complement signalling in AAV.
- Inhibition of the complement system with C5a receptor blocker CCX168 (Avacopan) is a promising new therapy for AAV, currently being tested in the Phase III ADVOCATE trial.

number of studies did report on the possibility of a genetic background with regard to complement [8–10]. However, the notion that complement could be involved in the pathogenesis and could be a potential target for therapeutic intervention, was revived by mice experiments showing a role for the alternative complement pathway [11,12]. Since then, the evidence supporting complement involvement in AAV has accumulated. This review will explore current knowledge on the role of complement in AAV and the advances in treatment this has led to.

### The complement system

The complement system forms an important part of the innate immune system and plays a key role in inflammation [13]. It is involved in pathogen defence, but also in autoimmune diseases. It is made up of over 20 proteins that can be sequentially activated in an enzyme cascade. The complement cascade can be activated through three different pathways: the classical, lectin and alternative pathway. Activation of the classical pathway can occur through binding of antibody–antigen immune complexes to complement protein C1q. Activation of the system via the lectin pathway occurs via binding of mannose-binding lectin to mannose residues on the surface of microorganisms. Finally, the alternative pathway is initiated by spontaneous hydrolysis of C3, which can be further stimulated by the presence of antigens of, for example, pathogens. All activation pathways lead to the cleavage of complement factor C3, generating C3a and C3b. In turn, activated C3 triggers a common final pathway via activation and cleavage of C5. C5a is an important chemoattractant and recruits cells such as

neutrophils and macrophages, while also being involved in the activation of phagocytic cells. C5b leads to the formation of the membrane attack complex (MAC), which has the ability to cause cell lysis. In addition, the complement system can induce adaptive immune responses [14].

### INVOLVEMENT OF THE COMPLEMENT SYSTEM IN ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS PATHOGENESIS

The pathogenesis of AAV is not yet completely understood, but is of a multifactorial character. Genetic and environmental factors, infections and characteristics of the patient's immune system are all involved in AAV development [15]. Over the last 15 years, it has become increasingly clear that the complement system plays an important role at different stages in the disease process. First, C5a is one of the inflammatory proteins, in addition to cytokines such as tumour necrosis factor  $\alpha$ , that can prime neutrophils, making them susceptible to the binding of ANCAs to their cell surface [16].

Complement also plays a prominent role in the amplification loop that ensues neutrophil activation through the binding of ANCA. ANCA binding sets in motion a cascade of events including respiratory burst, degranulation and neutrophil extracellular trap (NET) formation, ultimately leading to vascular endothelial injury. The complement system plays a role in this process as activation of neutrophils can also lead to activation of the alternative complement pathway, causing increased leukocyte influx, neutrophil priming and vascular damage, thus further expanding the inflammatory process. As complement activation occurs after binding of ANCAs to their antigens, it would logically follow that the complement cascade becomes activated through the classical pathway (via immune complexes). However, the studies described below clearly point toward the alternative pathway as the main route of activation. Exactly which mechanism triggers the alternative pathway remains to be elucidated.

### The alternative pathway in antineutrophil cytoplasmic antibody-associated vasculitis

The first experiments showing evidence for complement involvement in AAV were performed in a mouse model for MPO-ANCA vasculitis. Xiao *et al.* [11,12] elegantly showed that mice deficient for alternative pathway activation were protected against the development of vasculitis and glomerulonephritis. However, mice deficient for C4 and

hence the classical and lectin pathways of complement, were equally susceptible to the disease as compared to wild type animals. These *in vivo* studies highlighted an important role for complement in murine AAV. In line with these results, a re-evaluation of previous human studies in pauci-immune vasculitis revealed that, in fact, there were several indications that complement activation could play a role in the disease process of human AAV [17]. Although the intensity of the staining is not as bright as in the case of, for example, lupus nephritis, kidney biopsies of AAV patients do stain positive for complement activation fragments [18]. More recent studies examining renal biopsies of patients with ANCA-associated glomerulonephritis found an association between those biopsies showing presence of immune deposits and proteinuria [19], as well as between alternative pathway proteins and crescent formation [18,20]. Other signs of complement activation in human AAV include increased levels of urinary alternative pathway activation products [21] and presence of alternative pathway activation fragments in the circulation [22]. Additionally, one study showed the presence of hypocomplementaemia in a small subset of patients [23]. As complement activation plays an important role in AAV, one might expect to detect complement consumption in a larger proportion of patients.

A small subset of patients with AAV are ANCA-negative [4,24], but are traditionally regarded to be part of the spectrum of patients with GPA, MPA and EGPA. In a recent study, it was suggested that the ANCA-negative patients may represent a separate disease entity: proteomic analysis of kidneys of AAV patients with ANCA-negative glomerulonephritis showed larger amounts of C3 and C9 compared with ANCA-positive patients. It was suggested that ANCA-negative AAV, seen as a separate disease entity, may be caused or promoted by a defect in the alternative pathway of complement activation [25].

### The neutrophil: a key player

A prominent role for neutrophils in the process of complement activation is likely as it has been reported that neutrophils can activate complement by expelling NETs, which are known to be able to activate both the classical pathway and alternative pathway [26,27]. Additionally, activated neutrophils can release alternative pathway components [28]. While most of the circulating complement proteins are synthesized in the liver, also leucocytes produce several complement proteins [29]. Although the local release of alternative pathway

proteins will not directly activate complement, it may provide a local reservoir of complement proteins that can fuel an already ongoing complement activation. Looking at AAV specifically, one study reported that neutrophils activated by MPO-ANCA released factors that had the capacity to activate the alternative pathway [12]. Although this mechanism is not completely understood, it may involve micro-particles [28] or NETs [27,30]. In addition, many neutrophils will die in this process, and apoptotic and necrotic cells are also known to activate complement [31–33]. A recent study showed that C1q binding was increased on apoptotic rat basophilic leukaemia cells that expressed PR3 and demonstrated their direct interaction [34]. Interestingly, the complement system may also play a protective role in neutrophil activation. Chen *et al.* [35] showed that complement factor H, an inhibitor of the alternative pathway, inhibits neutrophil activation by ANCA. However, factor H from patients with active AAV was deficient in its ability to bind neutrophils and inhibit their activation.

### Interaction between the complement and coagulation system

As reviewed previously [17], complement activation in AAV may not only result in neutrophil priming, activation and degranulation, but may also impact on the coagulation system and on pattern recognition receptor signalling. The effect of C5a on both neutrophils and endothelial cells to upregulate tissue factor and initiate the extrinsic pathway of coagulation is especially interesting [36]. Platelets express C5a receptors and their activation may result in granule release [37]. Activated platelets may in turn activate complement [38] and contribute to a local vicious circle of attraction of neutrophils, activation and degranulation, coagulation, triggering of platelets, additional complement activation and so on.

### CLINICAL APPLICATION

Future studies will reveal in even more detail in which ways complement activation is involved in AAV pathogenesis. For now, these human observational data and murine experimental data indicate that complement is involved in AAV in such a way that it is conceivable that inhibiting complement activation may have a therapeutic benefit. Our increasing understanding of the role of complement in AAV can be applied clinically in the form of providing new therapeutic targets, as well as aiding in patient prognosis and assessment of disease severity.

## Patient prognosis and assessment of disease severity

A small study showed that a minority of AAV patients (5%) have hypocomplementaemia, with lower overall and renal survival in this subgroup [23]. Similarly, Crnogorac *et al.* [39] found an association between lower serum C3 levels at diagnosis and poorer patient and renal survival in AAV patients. Complement depositions in renal biopsies can also give an indication of disease severity, as their presence has been correlated with poorer renal function at presentation, proteinuria, a higher proportion of glomerular crescents, a lower number of normal glomeruli as well as more severe tubulointerstitial lesions [18–20,40]. A study investigating the prognostic value of C3d- and C4d-positive glomerular staining in ANCA-associated renal vasculitis identified C3d-staining as an independent risk factor for the development of end-stage renal disease [41].

## Current therapeutic options for antineutrophil cytoplasmic antibody-associated vasculitis

Current treatment of AAV is based on general immune suppression including induction treatment with cyclophosphamide or rituximab and steroids. For maintenance treatment, azathioprine, methotrexate and rituximab are recommended [42]. Over the years, ongoing efforts to improve AAV management have transformed it from a universally fatal disease into a chronic condition [43]. However, relapse of the disease remains a significant problem and new and improved treatment modalities are called for. One possibility is targeting the complement system.

## Complement inhibitors in current clinical use

Because of the nature of the complement system with three different initiation pathways, a central activation protein (C3) and a common terminal pathway, the complement system provides many opportunities for inhibition [44]. In recent years, drugs have been designed that target specifically one of these components. In addition, it is possible to avoid blocking complement activation itself, by blocking the detection of activated complement fragments by cellular complement receptors instead. By choosing specific inhibitors that block one particular aspect of complement activation, a therapeutic benefit can be elicited, without affecting the other aspects of complement activation. As reviewed extensively elsewhere [44,45], there are several classes of complement-inhibiting drugs, including therapeutic blocking antibodies, purified human proteins, recombinant proteins from pathogens, peptides, small molecules, small interfering RNAs and spiegelmers.

Of all the complement-inhibiting drugs that are currently being developed, only two types are clinically available: the C5-blocking antibody eculizumab (Soliris, Alexion Pharmaceuticals, Boston, Massachusetts, USA) and several C1-inhibitor (C1-INH) preparations (Cinryze, Shire Pharmaceuticals, Dublin, Ireland; Berinert, CSL Behring, King of Prussia, Pennsylvania, USA and Ruconest, Pharming Group, Leiden, The Netherlands). The C1-INH preparations are mostly used for the treatment of angio-oedema [46]. Eculizumab has been in the clinic for the treatment of two rare diseases, paroxysmal nocturnal haematuria (PNH) and atypical haemolytic uremic syndrome (aHUS), with very favourable outcomes [47].

As this drug was approved for PNH in 2007 [48] and for aHUS in 2011 [49], substantial information is available regarding its use and safety. Strong therapeutic inhibition of complement results in a state that resembles complement deficiency. As is the case with naturally occurring genetic complement deficiency [50], drug-induced complement deficiency increases the risk of infections and indeed, several eculizumab-treated patients suffered from severe meningococcal infection [51]. However, nowadays, with appropriate precautions (e.g. vaccination) and monitoring, the risk of meningococcal infection is very low [44].

## Complement treatment in antineutrophil cytoplasmic antibody-associated vasculitis

In mice it has been observed that blocking C5 activation prior to disease induction could prevent MPO-ANCA-triggered vasculitis [52] and that most of the effect of complement activation was mediated via the receptor for the C5 split product C5a, the C5aR1 [16]. These observations identify C5 and C5aR as possible therapeutic targets. Although anecdotal case reports indicate that preventing C5 cleavage using eculizumab may be effective in the treatment of AAV [53], efforts for complement inhibition in AAV have been mainly focussed on inhibiting the signalling of the C5aR.

The rationale behind this may lie in differences between PNH and AAV pathogenesis. In PNH, cleavage of C5 into C5a and C5b leads to the incorporation of the MAC into erythrocyte membranes causing complement-mediated lysis. Eculizumab prevents this process by blocking the cleavage of C5. This treatment is effective, but inevitably also completely blocks MAC formation in pathogens, potentially leading to severe infections. In the setting of AAV, there is no major role for the MAC and only the effect of the released C5a, which attracts and activates neutrophils, has to be counteracted. In doing so, it is possible to leave the function



of complement regarding the formation of the MAC completely intact, allowing protection against infectious pathogens [54].

Murine studies clearly indicate that the C5a receptor [52,55], but not the C3a receptor [56], plays an important role in experimental AAV and that it is possible to dose-dependently inhibit MPO-ANCA-induced nephritis using a small compound inhibitor of the C5a receptor [57].

### Clinical trials with CCX168

As a result, clinical trials have been launched to test the safety and effectivity of an orally available human C5aR blocker, the small molecule CCX168 (Avacopan, ChemoCentryx, Mountain View, California, USA). The phase I studies revealed an excellent safety profile, while producing more than 90% receptor blockade in inflammatory cells in the blood throughout the day [58].

Next, the C5aR inhibitor on Leukocytes Exploratory ANCA-associated Renal Vasculitis (CLEAR) trial was performed using CCX168 [59<sup>\*\*\*</sup>]. In this randomized, double-blind, placebo-controlled phase II trial, CCX168 was orally administered to AAV patients. Three groups were compared: one receiving the standard treatment (cyclophosphamide/rituximab and high-dose prednisolone and placebo), one receiving CCX168 and prednisolone (cyclophosphamide/rituximab and low-dose prednisolone and 30 mg CCX168 twice daily) and the third group receiving CCX168 without prednisolone (cyclophosphamide/rituximab and no prednisolone and 30 mg CCX168 twice daily). At the first read-out of effectivity at 12 weeks, it was observed that CCX168 was well tolerated with no unexpected adverse events. It was concluded that CCX168 was at least equally effective as the standard of care in inhibiting nephritis [59<sup>\*\*\*</sup>].

The next trial that was performed was the Clinical ANCA Vasculitis Safety and Efficacy Study of Inhibitor of C5aR (CLASSIC). In this dose-ranging Phase II study of CCX168, no safety concerns were reported and a dose-response effect of the treatment was established [60]. With these encouraging results, a Phase III Clinical Trial of CCX168 in AAV (ADVOCATE) was initiated [61<sup>\*\*\*</sup>]. This ongoing study will assess the proportion of patients achieving remission at 26 and 52 weeks.

### CONCLUSION

In mouse models for MPO-AAV, activation of the complement system via the alternative pathway is necessary for the development of vasculitis. Subsequent human studies have led to the notion that complement also plays a role in human AAV and may be a relevant therapeutic target. Data from the

recent intervention studies clearly indicate that complement plays a key role via triggering of the C5a receptor. The use of C5aR blocker CCX168 (Avacopan) is a promising new drug for the treatment of AAV that is currently being tested in a Phase III trial.

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### Conflicts of interest

*There are no conflicts of interest.*

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# New insights into IgG4-related disease: emerging new CD4<sup>+</sup> T-cell subsets

Ryuta Kamekura<sup>a,b</sup>, Hiroki Takahashi<sup>c</sup>, and Shingo Ichimiya<sup>a</sup>

## Purpose of review

New insights into IgG4-related disease (IgG4-RD) have recently been obtained. A better understanding of the mechanisms underlying this disease is important for identification of therapeutic targets, which will lead to the development of specific strategies for treatment.

## Recent findings

Infiltration of activated T follicular helper (Tfh) cells is observed in affected tissues of IgG4-RD. Such Tfh cells have a greater capacity than tonsillar Tfh cells to help B cells produce IgG4. Circulating PD-1<sup>hi</sup>CXCR5<sup>+</sup> peripheral T helper (Tph)-like cells are also increased in patients with IgG4-RD. Because Tph-like cells express high levels of chemokine receptors and granzyme A, they have the capacity to infiltrate affected tissues and exert a cytotoxic function. Tph-like cells can also produce CXCL13, and CXCR5<sup>+</sup> Tfh cells and B cells are therefore preferentially recruited to form ectopic lymphoid structures in the sites. Tph cells may have a role to ignite inflammation and maintain persistent fibroinflammation in collaboration with Tfh cells in lesions of IgG4-RD.

## Summary

Recent advances in understanding the pathogenesis of IgG4-RD are remarkable. In this review, we summarize and discuss the possible pathologic role of CD4<sup>+</sup> T-cell subsets in IgG4-RD.

## Keywords

CD4<sup>+</sup> T-cell subsets, IgG4-related disease, Tfh cells, Tfr cells, Tph cells

## INTRODUCTION

IgG4-related disease (IgG4-RD) is a chronic fibroinflammatory disease characterized by a significant elevation of serum IgG4 concentration and marked infiltration of IgG4-positive plasma cells in an affected organ or affected organs such as lacrimal glands, salivary glands, lymph nodes, pancreas, retroperitoneum, and lungs [1]. A dense lymphoplasmacytic infiltrate and the formation of ectopic lymphoid structures (ELs) are characteristic histopathological findings in an IgG4-RD lesion [Figure 1]. These findings strongly suggest the preferential involvement of T and B lymphocytes in the development of this disease. Since the establishment of this disease entity, a number of studies have been performed for clarifying the immunological mechanisms of this disease. T cells and their subsets were focused on in the early period of IgG4-RD research. T helper 2 (Th2) cells [2–7] and regulatory T (Treg) cells [2,4,7–10] were considered as candidates of a main player in IgG4-RD. After such studies on CD4<sup>+</sup> T cells, T follicular helper (Tfh) cells [11,12<sup>•</sup>,13<sup>••</sup>,14–16] and CD4<sup>+</sup> cytotoxic T lymphocytes (CD4<sup>+</sup> CTLs) [17<sup>••</sup>,18<sup>•</sup>] were suggested to play a cardinal role in the immunological settings of IgG4-

RD. In addition to these CD4<sup>+</sup> T cells, we have recently identified other new CD4<sup>+</sup> T cell subsets including PD-1<sup>hi</sup>CXCR5<sup>+</sup> peripheral T helper (Tph)-like cells and T follicular regulatory (Tfr) cells in blood of patients with IgG4-RD and we found that they are significantly correlated with various clinical parameters [19<sup>••</sup>] (F Ito, R Kamekura, unpublished data). Because of their function for secretion of chemokines such as CXCL13, which is a ligand of CXCR5, Tph

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## KEY POINTS

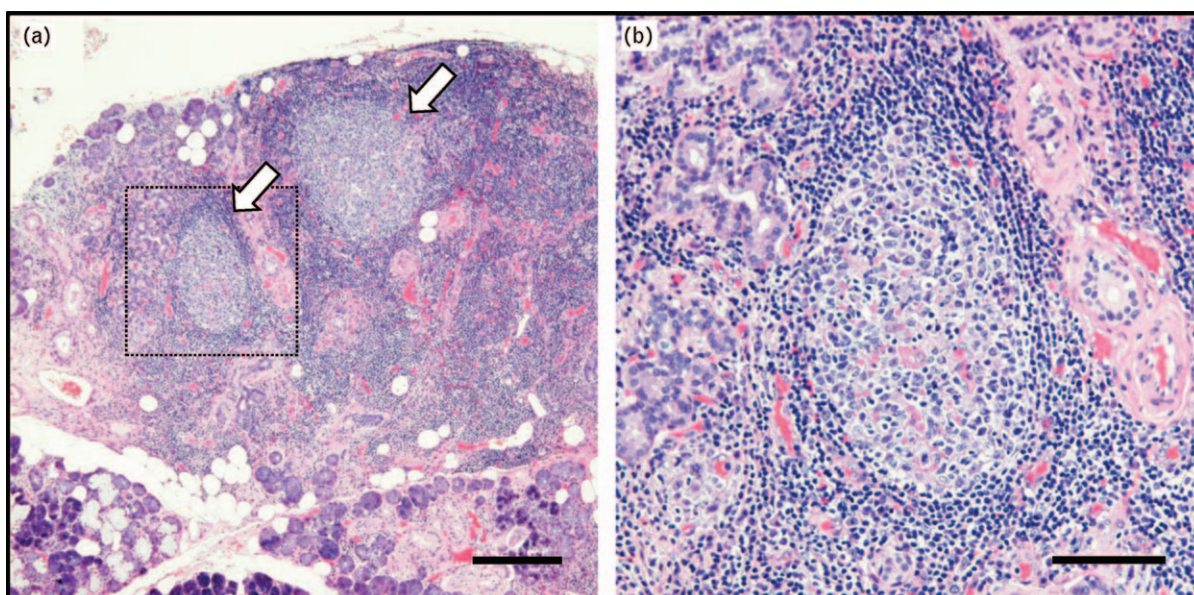
- There has been an accumulation of direct evidence of the involvement of new CD4<sup>+</sup> T-cell subsets such as CD4<sup>+</sup> CTL cells, Tfh cells, Tph cells, and Tfr cells in the pathogenesis of IgG4-RD.
- Functionally specialized Tfh cells infiltrate affected lesions of IgG4-RD and have a great capacity to help B cells produce IgG4.
- Tph cells have a potential role in the initiation of inflammation and maintenance of chronic fibroinflammation in collaboration with Tfh cells in lesions of IgG4-RD.
- In order to develop a new drug for IgG4-RD, studies using an animal model of this disease that can provide answers to all of the questions regarding immunological mechanisms are needed.

cells have a potential role in the initiation of inflammation and subsequently lead to the characteristic immune responses in collaboration with CXCR5-expressing lymphocytes such as Tfh cells and B cells in lesions of IgG4-RD. There has been an accumulation of direct evidence regarding the involvement of CD4<sup>+</sup> T-cell subsets in the immunological mechanisms of IgG4-RD [13<sup>22</sup>,20]. In this review, we summarize the roles of CD4<sup>+</sup> T-cell subsets and discuss the possible interplay between Tfh cells and Tph cells in the pathogenesis of IgG4-RD.

## Th2 CELLS AND ALLERGIES

Patients with IgG4-RD often have allergic disorders such as bronchial asthma and allergic rhinitis [21]. Indeed, levels of Th2 cells and Th2 cytokines including interleukin (IL)-4, IL-5, and IL-10 are frequently increased in affected tissues or peripheral blood of patients with IgG4-RD [2–5,7]. Of note, Culver *et al.* reported associations of IgG4-RD with allergy, atopy, eosinophilia, increased serum levels of IgE, and IgE-positive mast cells in lymphoid and affected tissues. They concluded that levels of IgE could be used for diagnosis and predicting relapse [6]. Taken together, the results suggest that Th2 cells and IgE-mediated allergic response play a role in the pathogenesis of IgG4-RD.

However, several recent studies have shown controversial results. Mattoo *et al.* reported that circulating memory Th2 cells in IgG4-RD are detected in a limited population of subjects with atopy [22]. They also showed that CD4<sup>+</sup>GATA3<sup>+</sup> Th2 cells were sparse in affected tissues of IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS), which is an archetype of IgG4-RD postulated as Mikulicz disease [17<sup>22</sup>,18<sup>23</sup>]. In addition, the percentage of tissue CD4<sup>+</sup>GATA3<sup>+</sup> Th2 cells in IgG4-RD does not seem to be correlated with clinical parameters such as serum IgG4 concentrations and the number of affected organs [17<sup>22</sup>]. Our group has also provided evidence suggesting that clinical values indicating allergic status such as specific IgE against allergens are not important in the pathological



**FIGURE 1.** Histopathology of IgG4-related disease. (a) Formation of ectopic lymphoid structures (arrows) in a submandibular gland from a patient with IgG4-related dacryoadenitis and sialoadenitis. (b) High magnification image of the dotted square area in (a). A section of submandibular glands was stained with hematoxylin and eosin. Scale bars are 500  $\mu$ m in (a) and 200  $\mu$ m in (b).



mechanism of IgG4-DS (M. Yamamoto, R. Kamekura, unpublished data). Therefore, it is still not clear how classic Th2 cells and IgE-mediated allergy are involved in the pathogenesis of IgG4-RD.

### Treg CELLS

Histopathologically, infiltration of IgG4-positive plasma cells accompanied by storiform fibrosis is usually observed in affected tissues of IgG4-RD [1,23]. It is well known that IL-10 and TGF- $\beta$  are key cytokines for IgG4 class-switching and fibrosis, respectively [24–26]. Therefore, regulatory T (Treg) cells have been focused on from the early period of IgG4-RD research as a pathognomonic source of IL-10 and TGF- $\beta$ . Indeed, several studies have shown an increased number of Treg cells and increased expression level of their master regulator, Foxp3, in both affected sites and circulating leukocytes in patients with IgG4-RD [2,4,7–10]. We also found increased levels of Treg cells in blood and affected tissues of patients with IgG4-RD (F. Ito, R. Kamekura, unpublished data). Taken together, the results of these studies suggest that Treg cells are preferentially involved in IgG4 class-switching and fibrosis in lesions of IgG4-RD; however, no direct evidence regarding the function of Treg cells in IgG4-RD was shown in those reports. Further studies are probably required to clarify IgG4 class-switching and fibrosis caused by Treg cells in IgG4-RD.

### CD4<sup>+</sup> CYTOTOXIC T LYMPHOCYTES

CD4<sup>+</sup> T cells with a cytotoxic function (named CD4<sup>+</sup> CTLs) have been observed in various immunological conditions such as virus infection, autoimmune diseases, and cancer [27,28]. CD4<sup>+</sup> CTLs are characterized by their unique function of secreting perforin, granzyme, and IFN- $\gamma$  for killing target cells in an MHC class II-restricted fashion [27,28]. Recently, there has been an accumulation of experimental evidence suggesting the involvement of CD4<sup>+</sup> CTLs in IgG4-RD. Mattoo *et al.* first reported the clonal expansion of CD4<sup>+</sup> CTLs in inflamed tissue sites of IgG4-RD. These cells presented SLAMF7, granzyme A (GZMA), IL-1 $\beta$ , and TGF- $\beta$ , suggesting their capacity related to tissue inflammation and fibrosis. Interestingly, clinical remission induced by rituximab-mediated B-cell depletion seems to be associated with a reduction in CD4<sup>+</sup> CTLs in IgG4-RD [17<sup>■</sup>]. In another report, the same group presented results showing an oligoclonal expansion of circulating plasmablasts (CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>CD38<sup>+</sup> cells) in patients with IgG4-RD [29]. These findings indicate that CD4<sup>+</sup> CTLs collaborate with activated plasmablasts and play an

important role in the pathogenesis of IgG4-RD. However, there has been no functional experiment on CD4<sup>+</sup> CTLs in IgG4-RD because of the minor population in CD4<sup>+</sup> T-cell subsets and the lack of specific surface markers for live cell sorting. Additional studies are required in the future to obtain direct evidence of cytotoxicity and fibrosis in affected tissues of IgG4-RD by these cells.

### Tfh CELLS

As mentioned above, abundant infiltration of IgG4-positive plasma cells is usually observed in tissue lesions of IgG4-RD [1]. This suggests that dysregulation of the IgG4 class-switch underlies the pathogenesis of IgG4-RD. Tfh cells, which are postulated as a specialized class of effector helper CD4<sup>+</sup> T cells, assist B cells to form germinal centers of lymphoid follicles, and Tfh cells thereby contribute to the class switch recombination of B cells and the selection of high-affinity B cells in germinal centers [30,31]. Importantly, Tfh cells have the capacity to secrete IL-4 and IL-10, which are key cytokines for IgG4 class-switching [24]. Tfh cells have been considered as a potential key player in the development of IgG4-RD.

It is well known that Tfh cells not only localize in lymphoid tissues but also exist in blood circulation and lesional sites of extra-lymphoid tissues [13<sup>■</sup>,31]. Because of accessibility of blood samples, accumulating evidence has shown the role of circulating Tfh cells in IgG4-RD. Circulating Tfh cells comprise three subsets, Tfh1 cells, Tfh2 cells and Tfh17 cells, that can secrete restricted repertoires of the cytokines IFN- $\gamma$ , IL-4 and IL-17, respectively, as seen in conventional helper T-cell subsets such as Th1 cells, Th2 cells, and Th17 cells [32–34]. In IgG4-RD, increased circulating Tfh2 cells and activated Tfh2 cells with a high expression level of programmed cell death 1 (PD-1, i.e., PD-1<sup>hi</sup> Tfh2 cells) are able to help naïve B cells differentiate into plasmablasts and produce IgG4 [11,12<sup>■</sup>,14]. Circulating activated Tfh1 cells were also shown to be increased in IgG4-RD and to be correlated with disease activity but not with serum IgG4 levels [12<sup>■</sup>].

Recently, our group and others have demonstrated abundant infiltration of Tfh cells in affected submandibular glands of patients with IgG4-RD [13<sup>■</sup>,14,15]. We further reported that lesional Tfh cells isolated from submandibular glands of patients with IgG4-DS showed high expression levels of B-cell lymphoma (Bcl) 6 and activation markers such as PD-1 and ICOS and had a greater capacity than tonsillar Tfh cells to help B cells produce IgG4 [13<sup>■</sup>]. Moreover, Maehara *et al.* [16] recently reported that

the expansion of IL-4<sup>+</sup>BAFF<sup>+</sup> Tfh cells in lymphoid organs is linked to IgG4 class switching. Taken together, the results indicate that activated Tfh cells possessing unique functions abundantly infiltrate affected lesions of IgG4-RD and play an important role in the pathogenesis of IgG4-RD. Fundamental questions that remain to be answered are whether circulating Tfh cells and resident Tfh cells in affected tissues of patients with IgG4-RD have the same origin and, if so, how circulating Tfh cells migrate from or into the affected tissues. Further studies on Tfh cells in IgG4-RD are needed to answer these questions.

### Tfr CELLS

Tfr cells have recently been characterized as a unique CD4<sup>+</sup> T-cell subset that participates in the control of germinal center formation and class switch recombination of B cells in collaboration with Tfh cells [35–37]. Tfr cells express CXCR5, which is also shared by B cells and Tfh cells. Tfr cells are regulated by Bcl6, PD-1, and ICOS as well as forkhead box P3 (Foxp3), as observed in Treg cells [31,36]. To exert germinal center responses, Tfr cells produce IL-10 and TGF- $\beta$  for the direct regulation of B cells and Tfh cells. Because most of the studies regarding Tfr cells in disease have mainly been performed in mouse models [38,39], functional roles of Tfr cells in human diseases are not fully understood. Recent studies have shown that Tfr cells proportionately and numerically proliferate during HIV infection and contribute to inefficient germinal center responses and then inhibit HIV clearance [40]. Other studies have demonstrated a decreased number of circulating Tfr cells and a significant correlation between the percentage of Tfr cells and clinical parameters in patients with systemic lupus erythematosus or multiple sclerosis [41]. In contrast, the pathological significance of Tfr cells in IgG4-RD has not been investigated. Therefore, we examined Tfr cells using clinical specimens to address the question of whether Tfr cells are associated with the pathogenesis of IgG4-RD. Our results showed that the number of Tfr cells was increased in blood and inflamed submandibular glands from patients with IgG4-DS (F. Ito, R. Kamekura, unpublished data). The percentage of Tfr cells was positively correlated with clinical parameters including serum level of IgG4 and number of involved organs in patients with IgG4-RD. Interestingly, the number of IL-10-producing circulating Tfr cells in patients with IgG4-RD was increased compared with that in healthy elderly patients, indicating the possible involvement of Tfr cells in IgG4-specific class-switch recombination in lesions of IgG4-RD. Collectively,

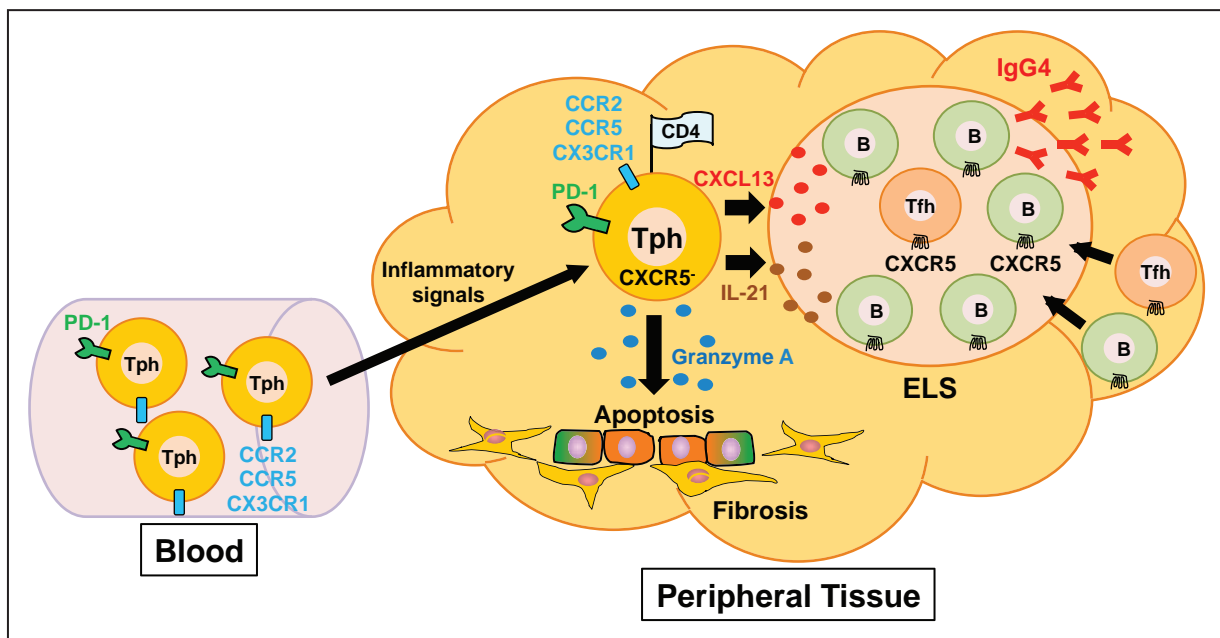
these findings seem provide a novel insight into the role of Tfr cells in the disease pathogenesis.

### Tph CELLS

A more recent study on rheumatoid arthritis (RA) has revealed an unidentified subset of CD4<sup>+</sup> T cells named Tph cells (PD-1<sup>hi</sup>CXCR5<sup>+</sup>CD4<sup>+</sup> cells) [42<sup>■</sup>]. Tph cells present Tfh cell-like features to produce factors associated with B-cell help, including IL-21 and CXCL13 in the inflamed synovium of RA. Unlike Tfh cells, Tph cells in the synovium of RA do not express high levels of Bcl6 and instead show elevated levels of Blimp1, which opposes the actions of Bcl6 as a counter-regulator [43]. Tph cells also have a unique expression profile of chemokine receptors, such as CCR2, CCR5, and CX3CR1 (a fractalkine receptor), that ignite their migration to inflamed sites [42<sup>■</sup>]. Thus, Tph cells show substantial differences from Tfh cells in their surface phenotypes, migratory capacity, and transcriptional regulation [42<sup>■</sup>]. Recent studies have shown an increased percentage of Tph cells in blood from patients with primary Sjögren's syndrome [44]. In addition, Gu-Trantien *et al.* [45] reported that CD4<sup>+</sup> T cells with a Tph cell-like phenotype were found in breast cancer tissues and that they have a possible regulatory function in immune responses against tumor cells. Based on these observations, we have first reported a possible pathological role of Tph cells in IgG4-RD [19<sup>■</sup>]. Our results have shown that circulating PD-1<sup>+</sup>CXCR5<sup>+</sup> cells (including PD-1<sup>hi</sup>CXCR5<sup>+</sup> cells, thus collectively named Tph-like cells here) were significantly increased within CD4<sup>+</sup> T cells in patients with IgG4-RD compared to those in healthy volunteers. We also found that their percentage was positively correlated with serum levels of IgG4 and soluble IL-2 receptor and with the number of involved organs in IgG4-RD patients. In addition, we found that such Tph-like cells frequently expressed GZMA, which is related to a cytotoxic property. Clinical remission achieved by treatment with glucocorticoids clearly led to a numerical reduction of Tph-like cells [19<sup>■</sup>]. Taken together, our findings strongly suggest that circulating Tph-like cells play a pivotal role in the pathogenesis of IgG4-RD.

### COLLABORATION OF Tph CELLS AND Tfh CELLS IN THE FORMATION OF ECTOPIC LYMPHOID STRUCTURES

In peripheral tissues of chronic inflammation such as IgG4-RD and RA, aggregations of T cells and B cells (so-called ectopic lymphoid structures, ELSs) frequently develop [46] [Figure 1]. In ELSs, T cell–B cell interactions result in uncontrolled somatic



**FIGURE 2.** A model for the functional role of PD-1<sup>hi</sup>CXCR5<sup>-</sup> peripheral T helper (Tph) cells and T follicular helper (Tfh) cells in affected organs of IgG4-RD. Because Tph cells express high levels of chemokine receptors including CCR2, CCR5, and CX3CR1, Tph cells rapidly infiltrate inflamed tissues of IgG4-RD. Production of CXCL13 by Tph cells induces the recruitment of CXCR5<sup>+</sup> Tfh cells and B cells. As a result, it is possible that the interaction of Tfh cells with B cells that subsequently accumulate and form ectopic lymphoid structures (ELSs) in the lesions provides an immune microenvironment in which production of IgG4 is induced. Tph cells also express a high level of cytotoxic granules such as granzyme A and exert cytotoxic activity in inflamed tissues.

hypermutation, class switch recombination, and differentiation of plasma cells [46], the functional interplay of which accelerates the development of the disease. Our previous observations revealed abundant infiltration of PD-1<sup>hi</sup>ICOS<sup>hi</sup> Tfh cells in lesional ELSs of IgG4-DS [13<sup>\*\*\*</sup>], suggesting that activated Tfh cells interact and strongly induce B cells to produce IgG4 in ELSs of IgG4-RD. The appearance of a new player, Tph cells, in the research field of chronic inflammation, might lead to a deeper understanding of the immunological mechanisms of ELS formation in lesions of chronic inflammation including IgG4-RD. Based on results of previous studies and our recent findings regarding IgG4-RD, we presume the following relationship between Tph cells and Tfh cells in the pathogenesis of IgG4-RD. Owing to the high expression levels of chemokine receptors including CCR2, CCR5, and CX3CR1, Tph cells are preferentially prone to infiltration of inflamed tissues in patients with IgG4-RD. Production of CXCL13 by Tph cells may subsequently provide early stimuli for the recruitment of CXCR5<sup>+</sup> immune cells including both Tfh cells and B cells. As a result, it is possible that Tfh cells and B cells accumulate to form ELSs in the lesions and eventually provide an immune microenvironment in which the production of IgG4 is further induced. Because fractalkine, which is a ligand of CX3CR1, is

highly expressed by endothelial cells in submandibular glands of IgG4-DS (H. Yabe, R. Kamekura R, unpublished data), Tph cells might have a role in the initiation of inflammation and maintenance of chronic fibroinflammation in IgG4-RD, although influencing vascularities in the lesions [47]. Given that destructive inflammation is observed in IgG4-RD [Figure 1], our experimental evidence indicates that Tph-like (PD-1<sup>+</sup>CXCR5<sup>-</sup>CD4<sup>+</sup>) cells, which preferentially contain cytotoxic granules of GZMA, are responsible for such pathological changes in IgG4-RD [19<sup>\*\*\*</sup>]. In this study, we could not see abundant expression of *IL21* mRNAs in Tph-like cells compared with those in Tfh cells from patients with IgG4-RD [19<sup>\*\*\*</sup>]. Taken together, the results suggest that Tph-like cells in IgG4-RD play a pathological role as CD4<sup>+</sup> CTLs rather than as B cell helpers such as Tfh cells [Figure 2].

Several questions remain to be answered to understand the mechanisms of the origin and differentiation of Tfh cell in lesions of IgG4-RD and the developmental relationship between Tfh cells and Tph cells.

## CONCLUSION

Following the recent advances of our knowledge of human CD4<sup>+</sup> T cells, IgG4-RD research has

progressed remarkably. The appearance of Tph cells and Tfh cells as key players in the pathogenesis of IgG4-RD is one example of the progress in research. Accumulating evidence may lead to the development of specific therapeutic targets and a new strategy for IgG4-RD treatment. Treatment with glucocorticoids is still effective for IgG4-RD; however, it often causes side effects and relapse frequently occurs after tapering or discontinuing administration of glucocorticoids [48]. A new strategy needs to be developed to overcome the problem of relapse and refractory cases of IgG4-RD. Thus, it is important to consider experimental findings and knowledge regarding the pathogenesis of IgG4-RD from direct examination of inflamed tissues from patients with IgG4-RD. Because of the limited availability of clinical specimens, studies using lesional tissues and blood from patients with IgG4-RD are not always straightforward. Evidence obtained from animal models of this disease may also help to address the questions regarding immunological mechanisms.

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## Conflicts of interest

There are no conflicts of interest.

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# Cardiovascular magnetic resonance in the diagnosis and management of cardiac and vascular involvement in the systemic vasculitides

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## Purpose of review

Cardiac manifestations in systemic vasculitides, either primary or secondary due to infection, malignancy or autoimmune rheumatic diseases may be life-threatening. Cardiovascular (CVD) magnetic resonance (CMR) has been recently proposed as an ideal noninvasive tool to evaluate systemic vasculitides. In the present article, we present an overview of CMR in the diagnosis and follow-up of cardiac involvement in systemic vasculitides.

## Recent findings

CMR is a noninvasive, nonradiating modality, capable to assess cardiac function, perfusion and tissue characterization that can be of great diagnostic value in both primary and secondary systemic vasculitides. It has been already documented that CMR is superior to other imaging modalities, because it has great versatility and higher spatial resolution that allows the detection of early CVD phenomena occurring during systemic vasculitides. Magnetic resonance angiography and oedema-fibrosis imaging detect early CVD involvement such as acute and/or chronic inflammation, coronary macro-micro-circulation abnormalities and/or small vessel vasculitis.

## Summary

CMR due to its great versatility gives valuable information about cardiac function, perfusion, type of fibrosis and vascular integrity that may significantly contribute to treatment decisions beyond vascular scores, other disease activity or severity indices or the acute phase response.

## Keywords

cardiovascular magnetic resonance, large, small, medium vessel vasculitides, myocardial fibrosis, myocardial oedema, systemic vasculitides

## INTRODUCTION

The systemic vasculitides constitute a group of heterogeneous, rare diseases characterized by inflammation and fibrinoid necrosis of blood vessel wall; they may be either primary or associated with other autoimmune disorders, infection or malignancy. Vascular wall inflammation may lead to serious cardiovascular (CVD) lesions in most rheumatic conditions, including rheumatoid arthritis (RA), systemic sclerosis and systemic lupus erythematosus (SLE). CVD accounts for significantly increased morbidity and mortality, observed in systemic inflammatory diseases [1]. In systemic vasculitides, a bimodal pattern of mortality has been demonstrated during the course of the disease with infections and active vasculitis representing the leading causes of death in the first year after the diagnosis. CVD events and malignancies alongside infections reported as the main contributors to the excess mortality risk in later years of systemic vasculitides [2].

The classification of systemic vasculitides according to Chapel Hill Consensus Conference [1] depends on the predominant type of vessels affected. They can involve the aorta and its major branches, as in giant cell arteritis (GCA) and Takayasu arteritis, medium-sized vessels, as in polyarteritis nodosa (PAN) and Kawasaki disease and small-sized vessels (arterioles, capillaries and venules), as in granulomatosis with polyangiitis (GPA), formerly known as Wegener

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## KEY POINTS

- Vascular and myocardial involvement can be observed in systemic vasculitides.
- CMR using oedema-fibrosis imaging can detect CVD disease acuity and fibrosis in systemic vasculitides.
- CMR is the best noninvasive modality for early diagnosis of both vascular and myocardial involvement in systemic vasculitides.
- CMR allows treatment guidance beyond vasculitis scores and acute phase reactant indices.

granulomatosis, microscopic polyangiitis (MPA), eosinophilic GPA (EGPA) – traditionally termed Churg–Strauss syndrome, and in mixed cryoglobulinemic vasculitis, amongst several other syndromes. GPA, MPA and EGPA share a common pathophysiologic lesion with necrotizing granulomatous lesions in different organs without immune deposits; they are characterized by the presence of antineutrophil cytoplasmic antibodies (ANCA) and are grouped as ANCA-associated systemic vasculitides [3].

Almost all systemic vasculitides can target the heart with frequencies widely ranging between 6 and 10% in clinical studies to 70–90% in histopathology investigations. Certain entities such as EGPA and Takayasu arteritis cause cardiac complications in up to 60% of patients [4]. Heart involvement in systemic vasculitide encompasses different pathophysiologic mechanisms such as systemic inflammation, endothelial activation as well as accelerated atherosclerosis [5]. The mode and incidence of cardiac involvement however vary amongst the different vasculitic syndromes with large vessel vasculitis affecting mainly the aorta and the valves. Medium and small vessel vasculitis manifest with a more widespread pattern involving any structure of the cardiac tissue including myocardium, pericardium, valves and coronary arteries. This results in diverse clinical entities namely myocarditis, pericarditis, valvulopathy, conduction system disorders, coronary arteritis and acute ischemic coronary events [6,7]. In addition, the severity of cardiac disease varies from mild cases to mostly (sub)clinical and occasionally even clinically overt life-threatening conditions. Given the remitting-relapsing pattern and the chronic nature of systemic vasculitides, cardiac manifestations may present with varying severity and activity over the years. They may also lead to congestive heart failure and atherosclerotic coronary artery disease (CAD), conferring an unfavourable impact on overall survival [8,9]. On top of disease-related cardiac

complications, additional CVD abnormalities may arise either as treatment adverse effects or as a result of traditional CVD risk factors. These include left heart hypertrophy due to hypertension and old age, toxic effects of antirheumatic treatments on the myocardium, uremic pericarditis or infective endocarditis owing to immunosuppressive therapy. They can all contribute to the increased CVD mortality and should be considered in the overall CVD evaluation of individuals with any systemic vasculitides [10<sup>¶</sup>,11<sup>¶</sup>].

Specific cardiac involvement in systemic vasculitides has been considered as a rare complication due to the subtle clinical signs and the lack of specific manifestations. In addition to subclinical presentation, ECG and echocardiography are not completely sensitive or specific for diagnosing vasculitic injury. This makes the diagnostic assessment problematic especially in patients without overt clinical symptoms. However, such individuals may be at risk of developing severe, life-threatening arrhythmias and end-stage heart failure [12,13].

Endomyocardial biopsy – the gold standard for the diagnosis of myocardial damage – is not always diagnostic due to patchy distribution of the inflammation/fibrosis, and is therefore rarely performed. Considering that immunosuppressive treatment improves survival [14<sup>¶</sup>] but may also attenuate or resolve vasculitic heart lesions, if administered promptly [15<sup>¶</sup>], the early detection of occult myocardial involvement in systemic vasculitides may be crucial for improving the long-term outcome in these conditions.

CVD magnetic resonance (CMR), a noninvasive, nonradiating modality, capable to perform tissue characterization. It can be of great diagnostic value, not only in primary but also in secondary systemic vasculitides [16,17,18<sup>¶¶</sup>,19,20]. Compared with other noninvasive modalities, CMR presents great versatility and higher spatial resolution that allows the detection of early CVD phenomena occurring during systemic vasculitides. A detailed comparison of the performance of CMR against the other noninvasive CVD diagnostic modalities in systemic vasculitides is shown in Table 1. CMR has been already used in the evaluation of systemic rheumatic diseases [21,22<sup>¶¶</sup>,23]. In the context of systemic vasculitides, Raman *et al.* [21] described the typical CMR pattern of heart involvement in systemic vasculitides involving small-size and medium-size vessels. Recently, Fayad *et al.* [23] also suggested that subclinical coronary small vessel vasculitis secondary to RA can be a potential mechanism for the increased CVD risk in patients with this condition.

In this review, we present the role of CMR in the diagnosis of CVD involvement in systemic

**Table 1.** Diagnostic performance of various noninvasive imaging modalities in the evaluation of systemic vasculitides

Modality	Ventricular function	Myocardial perfusion	Myocardial inflammation	Myocardial fibrosis	Vascular lesions
Echo	++	+	–	+	+
Nuclear	++	+++	–	++	+
CT	++	++	–	++	+++
CMR	++++	++++	++++	++++	++++

CMR, cardiovascular magnetic resonance; CT, computed tomography.

vasculitides and discuss how such information can impact upon appropriate and timely therapeutic decisions.

### HOW CAN CARDIOVASCULAR MAGNETIC RESONANCE CONTRIBUTE TO THE DIAGNOSIS, RISK STRATIFICATION AND TREATMENT EVALUATION IN SYSTEMIC VASCULITIDES?

CMR can offer a CVD ‘portrait’ of systemic vasculitides patients by providing useful information about the acuity of vascular/myocardial inflammation and fibrosis, before any vascular aneurysm/stenosis or myocardial dysfunction occur. Furthermore, some studies suggest a role for CMR in the risk stratification of systemic vasculitides and demonstrate that oedema/fibrosis visualization with CMR may have the potential to inform treatment modifications in systemic vasculitides with or without abnormal routine cardiac evaluation. These modifications involve strategies/drugs aimed at providing both better CVD support (mainly cardiac medications) and better control of the vasculitic process (mainly immunomodulatory medications) [24<sup>22</sup>]. This can be achieved using T2 imaging for oedema and T1 imaging post gadolinium for perfusion and fibrosis detection [11<sup>2</sup>]. Recently, the application of T2 mapping, native T1, post contrast T1 mapping and extracellular volume fraction (ECV) gives quantitative information about oedema and diffuse myocardial fibrosis, respectively, missed by the classic late gadolinium enhanced (LGE) images [25]. The CMR sequences that should be used for the evaluation of CVD involvement in systemic vasculitides are shown in Table 2.

### LARGE-VESSEL VASCULITIDES

Takayasu arteritis typically involves proximal aorta and systemic inflammation may weaken the vascular wall predisposing to aneurysm formation and dissection. Thus Takayasu arteritis can easily disturb cardiac function through different mechanisms such as aortic valve abnormalities owing to aortitis and vascular

wall dilatation, myocarditis, coronary arteritis and myocardial ischemia as well as impairment of myocardial contractility ranging from asymptomatic disease to rapidly progressing heart failure [26]. Heart involvement in GCA remains an issue of debate [27,28] with the exception of the well documented 17-fold and four-fold increased risk for thoracic/abdominal aortic aneurysm [29].

Although tools such as the Birmingham Vasculitis Activity Score [30] and Vasculitis Damage Index [31] are helpful research and clinical practice for the evaluation of disease activity and severity in primary systemic vasculitis, they lack sensitivity in the assessment of large vessel vasculitides [30]. This poses difficulties in the evaluation of overall disease activity and cardiac involvement in particular. The acute phase reactants also lack sensitivity for the detection of acute and chronic vascular lesions [31], as vascular wall inflammation is persistent and the arterial lesions may progress over time, even in patients, who appear to have clinically quiescent disease and normal biomarkers [32].

**Table 2.** Cardiovascular magnetic resonance sequences that should be used for the evaluation of cardiovascular involvement in systemic vasculitides

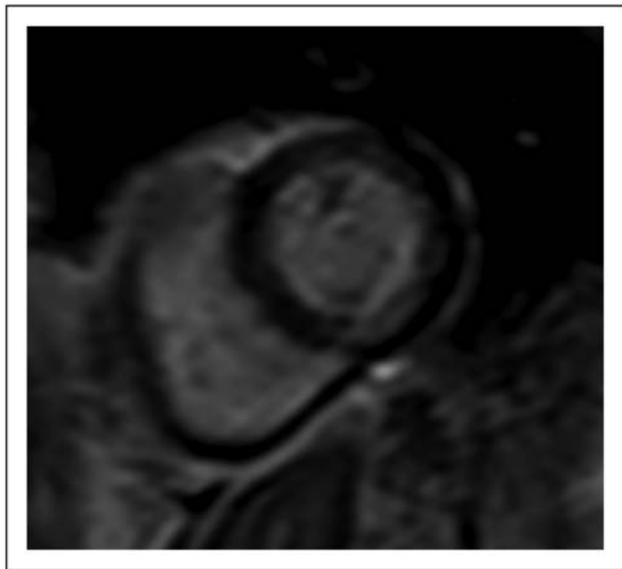
CMR sequences	Information provided
SSFP	LV–RV function
STIR2	Qualitative assessment of vascular/myocardial oedema
T2 mapping	Quantitative assessment of myocardial oedema
EGE	Myocardial capillary permeability
LGE	Myocardial replacement fibrosis
MPRI	Myocardial perfusion index
Native T1 mapping	Myocardial diffuse oedema/fibrosis
ECV	Myocardial diffuse fibrosis
MRA with or without contrast	Noninvasive angiography of great vessels

CMR, cardiovascular magnetic resonance; ECV, extracellular volume fraction; EGE, early gadolinium enhancement; LGE, late gadolinium enhancement; LV, left ventricular; MPRI, myocardial perfusion rate index; MRA, magnetic resonance angiography; RV, right ventricular; STIR2, short tau inversion recovery; SSFP, steady-state free precession.



CMR has some important inherent advantages against other imaging modalities for the imaging of large vessel vasculitis. Arterial wall imaging without the limitations of ultrasonography and information beyond luminography can be provided [33]. This is of great value in the early assessment of the disease process, because vessel stenosis/aneurysm is a relatively late phenomenon. Furthermore, cardiac anatomy/function can also be evaluated with better accuracy and reproducibility and this is of great significance in cases with coexistent aortic regurgitation, as in Takayasu arteritis. Finally, CMR is the ideal technique for myocardial tissue characterization, identifying the presence and acuity of myocardial infarction (MI) and/or inflammation [34]. The use of T1-weighted, T2-weighted and steady-state free precession sequences, combined with contrast-enhanced three dimensional magnetic resonance angiography (MRA) has equivalent diagnostic accuracy with radiograph angiography [35].

In large-vessel vasculitides, CMR provides high-resolution imaging of wall thickening, luminal and aneurysmal changes without the risk of an invasive procedure, the use of iodinated contrast agents and radiation exposure (Fig. 1). In addition, its sensitivity to diagnose subtle vessel wall thickening during the early inflammatory stage of Takayasu arteritis, using LGE images, allows the detection of Takayasu arteritis at a potentially reversible stage [36]. An additional advantage of CMR is its capability to assess disease activity and response to treatment noninvasively and without radiation. Tso *et al.* reported on 16 patients who, after repeated scans, were documented as



**FIGURE 1.** Subendocardial vasculitis presented as diffuse subendocardial fibrosis in eosinophilic granulomatosis with polyangiitis.

having four new occlusions, seven stenoses and one dilatation. The same investigators also identified new lesions in three patients, in the absence of concurrent arterial wall oedema and in five patients following the appearance of oedema [36]. In addition CMR may reveal autoimmune myocarditis that may coexist with Takayasu arteritis [37].

The use of intravascular contrast medium can improve significantly the capacity of CMR angiography in the differentiation between active and inactive disease [35]. In addition, the application of whole-body MRA combined with vessel wall imaging provided better evaluation of disease extent in Takayasu arteritis [36]. The recent application of an MRI-based scoring system for lumen stenosis, wall thickness and wall enhancement could also be a noninvasive approach to assess Takayasu arteritis activity [36].

### MEDIUM-VESSEL VASCULITIDES

Medium-vessel systemic vasculitides include PAN and Kawasaki disease. Cardiac involvement includes coronary artery aneurysms/ectasia, myo-pericarditis and MI with consequent heart failure. Kawasaki disease may involve the coronary arteries leading to stenosis and ectatic or aneurysmal lesions potentially leading to unstable angina and/or MI. Furthermore, it may primarily affect the heart leading to myo-pericarditis that may occur either simultaneously or independently from the coronary artery lesions [38]. All these entities, if left untreated, may finally lead to heart failure [38].

CMR is the ideal modality to evaluate coronary artery anatomy, myocardial inflammation and/or infarction in Kawasaki disease [38]. It is also of great value to detect stress perfusion defects during the follow-up evaluation of children and adolescents [19]. The myocardial perfusion rate index (MPRI) may show impaired myocardial perfusion in Kawasaki disease. MPRI can change over time, suggestive of progressive coronary artery changes, which may precede fibrosis and therefore, it should be included in the routine CMR evaluation of Kawasaki disease [38].

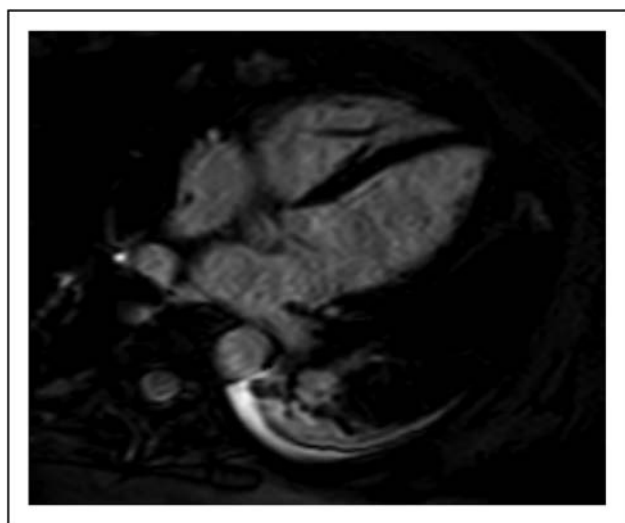
### ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIDES

ANCA-associated vasculitides (AAV) comprise one of the most difficult clinical problems in daily clinical practice both in terms of diagnosis and treatment. Particularly in view of potential myocardial involvement, the early identification of patients at high risk is of great importance as cardiac disease is an independent predictor of mortality with half of EGPA patients dying due to cardiac reasons. Most

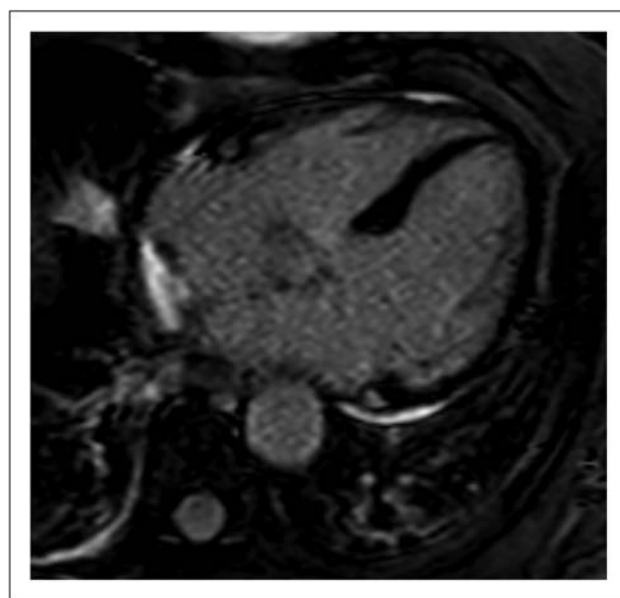
importantly, CVD involvement in AAV may run an asymptomatic period without any echocardiographic or ECG abnormalities before it manifests clinically [39].

In this regard, CMR can help identify the different phases of the vasculitic process in the heart and blood vessels, using different types of sequences. The vasculitic process in the myocardium can present either as myocardial inflammation (myocarditis) or as diffuse subendocardial vasculitis. CMR, due to its high spatial resolution, is the only noninvasive modality that can give early, reliable and reproducible information about myocarditis and subendocardial vasculitis, commonly found in the small-vessel vasculitides [15].

The most important information that CMR can provide in small-vessel systemic vasculitides is the assessment of disease acuity. CMR using the combination of T2-weighted, early (early gadolinium enhancement) and LGE images can distinguish acute from chronic inflammation [16]. In addition, the LGE pattern can provide information about the pathophysiologic background of the lesion. In this context, subendocardial vasculitis presents with diffuse subendocardial fibrosis (Fig. 1), myocarditis with intramyocardial or subepicardial LGE lesions not following the distribution of coronary arteries (Fig. 2), whereas MI usually presents with subendocardial or transmural LGE lesions following the distribution of coronary arteries (Fig. 3) [16]. We should clarify that LGE provides information only about the presence of replacement fibrosis in the myocardium. However, in cases with diffuse myocardial fibrosis, T1 mapping and more specifically ECV are the ideal indices to detect the presence of



**FIGURE 2.** Myocarditis presented as subepicardial lesion not following the distribution of coronary arteries in polyarteritis nodosa.



**FIGURE 3.** Subendocardial myocardial infarction in the basal part of lateral wall, following the distribution of left circumflex artery in granulomatosis with polyangiitis.

diffuse myocardial fibrosis, missed by LGE [25]. In patients with increased creatinine where contrast medium is contraindicated, native T1 mapping can give information about diffuse myocardial oedema/fibrosis without the use of contrast agent [25].

With regards to clinical use of CMR-derived information, a recent study indicated that cardiac tissue catheterization with T1, T2 mapping and EVC in 37 AAV patients with high disease activity [Birmingham Vasculitis Activity Score (BVAS) > 5] revealed higher rates of cardiac disease compared with healthy controls [40]. Significantly most of these AAV individuals were non or oligosymptomatic from the cardiac point of view, with normal ECG and well preserved left ventricular (LV) injection fraction in echocardiography. This suggests that mapping techniques can detect subtle diffuse myocardial fibrosis in patients with otherwise normal cardiac evaluation in line with previous observations in the setting of AAV or other systemic inflammatory diseases [41,42,43]. It is worth noting that BVAS was not correlated with mapping measurements indicating a disassociation between disease activity and myocardial fibrosis/oedema and highlighting the emerging need for comprehensive and accurate assessment of myocardial performance irrespective of symptoms and overall inflammatory burden in systemic vasculitides.

An important contributor to CVD morbidity and mortality in AAV is accelerated atherosclerosis and heart failure. Patients with AAV have a two-fold to four-fold increased risk of coronary heart disease

compared with controls as the chronic inflammatory state has proatherogenic effects on the vascular wall, leading initially to endothelial dysfunction and then to subclinical plaque formation similarly to what occurs to other systemic inflammatory disorders [44,45].

In addition, heart failure is not uncommon in people with AAV. However global indices of cardiac function such as LV ejection fraction, cannot precisely assess the extent of myocardial dysfunction, which usually presents with diastolic dysfunction and a relatively low prevalence of systolic abnormalities [39]. Such changes may reflect the effect of cumulative inflammation-driven patchy or diffuse myocardial fibrosis on cardiac remodeling and function. Last but not least, fluid overload, hypertension and hypoxia caused by severe organ involvement such as glomerulonephritis and interstitial lung disease are also superimposed myocardial stressors contributing to the derangement of myocardial function and heart failure. CMR is extremely sensitive in detecting ischemic changes associated with atherosclerotic CAD as well as the slight myocardial changes related to myocardial impairment and more importantly differentiating them from cardiac damage due to acute inflammatory and autoimmune process [46,47].

## **VARIABLE VESSEL-SIZE SYSTEMIC VASCULITIDES**

Adamantiades-Behçet disease (ABD) and Cogan syndrome are included in this subgroup. Cardiac lesions in ABD include pericarditis, endocarditis, intracardiac thrombosis, valvular disease, MI, endomyocardial fibrosis and coronary artery aneurysms. Different types of cardiac disorders may coexist in the same patient [48]. Cogan syndrome has a mortality rate of approximately 10% and between causes of death are systemic vasculitis, ruptured aortic aneurysms, MI and heart failure [49]. In all these entities CMR can provide assessment of vascular/myocardial disease acuity and myocardial fibrosis [24<sup>22</sup>], as described in earlier sections.

## **VASCULITIDES ASSOCIATED WITH SYSTEMIC AUTOIMMUNE DISEASE**

SLE, Sjögren syndrome, RA, antiphospholipid syndrome (APS), scleroderma (SSc) and sarcoidosis (SRC) are included in this subgroup.

The commonest cardiac diseases in SLE are perimyocarditis (usually silent) and endocarditis (usually nonbacterial Libman–Sacks endocarditis), found in more than 40% of hearts at autopsy, and vasculitis leading to myocardial fibrosis. In addition,

severe coronary atherosclerosis and coronary arteritis may lead to MI. Heart failure can develop as a result of any of the above mentioned pathological processes, carries an ominous prognosis and is responsible for the high CVD morbidity and mortality in SLE [50]. Primary Sjögren syndrome is rarely associated with heart disease. However, if it occurs, valvular involvement is the commonest cause. Recently, myocarditis, related to leukocytoclastic vasculitis, has been described in Sjögren syndrome [51]. RA patients are twice more likely to develop MI irrespective of age, history of prior CVD events and traditional cardiovascular risk factors. It has been shown that atherosclerotic CVD in RA is of similar magnitude to the CVD observed in diabetes mellitus. RA also increases the risk of nonischemic heart failure, valvular disease and myo-pericarditis [52]. Cardiac involvement in APS may be presented as heart valve disease affecting approximately a third of patients. It can be also presented as intracardiac thrombosis, pulmonary hypertension, right ventricular (RV) or LV dysfunction, microvascular thrombosis, coronary artery or microvascular disease with overt or silent clinical presentation. Cardiac involvement in SSc can present with systolic and/or diastolic heart failure, myo-pericarditis, pulmonary hypertension, rhythm disturbances and valvular disease, usually with nonspecific clinical signs and symptoms. In all of these conditions, evaluation by CMR can identify oedema, microvascular disease and localised or diffuse fibrosis, assessing in parallel LV and RV function as well as heart valve morphology and function [22<sup>22</sup>].

Finally, CMR in SRC may detect myocardial inflammation, fibrosis and perfusion defects. Of all cardiac tests, CMR is the most accurate for both diagnosis and prognosis of cardiac SRC [53<sup>22</sup>], with myocardial scar, identified by LGE, being a strong independent predictor of death and other adverse cardiac events [53<sup>22</sup>] in these patients.

## **CARDIOVASCULAR MAGNETIC RESONANCE LIMITATIONS**

There are several limitations of CMR as a routine tool for the assessment of CVD involvement in the vasculitides and other clinical entities, including that:

- (1) It is a time consuming modality, not widely available;
- (2) It has a high cost and needs high level of expertise;
- (3) It cannot be used to scan patients with metallic clips, pacemakers and other implantable devices;
- (4) Renal function should be carefully monitored to avoid nephrogenic fibrotic sclerosis syndrome, due to contrast agent, in patients with

compromised renal function [22<sup>22</sup>]. However, the recent application of noncontrast native T1-mapping techniques can give useful information about oedema/fibrosis without the use of contrast agents [22<sup>22</sup>];

- (5) Although CMR may reveal signs of vascular inflammation, including arterial wall thickening and arterial wall oedema, no clear correlation of these findings with disease activity or progression has yet been demonstrated [23];
- (6) CMR may overestimate the stenosis in branch arteries, whereas limitations in resolution may result in relatively poor image quality of distal aortic branches [24<sup>24</sup>].

### **PUBLISHED CARDIOVASCULAR MAGNETIC RESONANCE EXPERIENCE EXISTS FOR THE FOLLOWING VASCULITIDES**

- (1) Takayasu arteritis (vascular and myocardial inflammation/fibrosis, ventricular function and valvular assessment).
- (2) Kawasaki disease (coronary arteries, peripheral vessels, myocardial inflammation, ischemia, fibrosis and ventricular function assessment).
- (3) Churg–Strauss syndrome (microvascular ischemia/fibrosis and ventricular function assessment).

### **HOW CARDIOVASCULAR MAGNETIC RESONANCE FINDINGS COULD GUIDE CARDIAC AND ANTIRHEUMATIC TREATMENTS/STRATEGIES**

There are only a few studies supporting a role for CMR in the risk stratification of CVD in patients with systemic vasculitides. According to a recent study, for systemic vasculitides patients with cardiomyopathy, CMR reassessment is promising in detecting those with a less favourable cardiac outcome [54]. CMR has also documented that the lack of or inadequate duration of noncorticosteroid immunosuppressive treatment was an independent factor of cardiac involvement in EGPA and the extent of myocardial damage was associated with shorter duration of noncorticosteroid immunosuppressive treatment [15<sup>15</sup>]. A CMR study targeting to detect cardiac lesions and monitor of treatment efficacy in EGPA with cardiac involvement revealed myocardial oedema in 87.8%, perfusion defects in 54.5% and LGE indicative of replacement fibrosis in all. Improvement after treatment was observed in 81% of them (in 11% completely remission and in 35% evolution to global fibrosis) [55<sup>55</sup>]. Furthermore, patients with EGPA in clinical remission

showed increased incidence of cardiovascular involvement, demonstrated by lower left ventricular ejection fraction, signs of active inflammation, presence of interstitial and replacement fibrosis and intraventricular thrombosis [56<sup>56</sup>]. In addition, in patients with active EGPA, CMR enabled the detection of cardiac involvement when cardiac symptoms were not present [57<sup>57</sup>]. Another study recommended that CMR evaluation should be performed in all antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides with sustained remission, even if symptoms are absent and ECG is normal, for treatment risk stratification [41]. In Takayasu arteritis, CMR can identify patients most at risk for complications, prompting the initiation of early preventive therapy [33,58] and in GCA the presence of myocarditis that needs aggressive immunosuppressive therapy to avoid LV dysfunction [59<sup>59</sup>]. In Kawasaki disease, CMR offers important clinical information during both the acute and chronic phase of Kawasaki disease. In the acute phase, it can identify myocardial inflammation, microvascular disease, MI, LV dysfunction, changes of the coronary artery lumen and wall, which may in turn lead to cardiac and/or autoimmune treatment modifications. During the chronic phase, CMR is of value for ischemia detection with consequent changes in risk stratification and treatment, if myocardial ischemia is detected [40<sup>40</sup>].

CMR can reliably assess myocardial ischemia and fibrosis due to either CAD, coronary microvascular disease or myocarditis, CVD disease acuity and the pathophysiologic background behind silent/overt heart failure or rhythm disturbances in systemic vasculitides patients [24<sup>24</sup>]. The response to these queries can significantly influence both cardiac and antirheumatic treatment. European Society of Cardiology guidelines propose that every morphologic or functional change in myocardium, detected by any diagnostic technique including CMR should motivate early start of cardiac treatment either pharmaceutical or interventional [60]. On the other hand, the early detection of myocardial inflammation, even if the underlying disease seems quiescent, gives to rheumatology a powerful tool to directly intervene on myocardial/vascular inflammation using new powerful immunosuppressive anti-inflammatory strategies and follow-up their direct effect on myocardium. However, at the moment, evidence-based results, established through short-term and long-term multicenter studies are still missing. Therefore, we need at least 3 levels of evidence to document the necessity of additive antirheumatic treatment in systemic vasculitides patients with CMR evidence of myocardial inflammation including studies from registries with



adequate phenotype, treatment and outcome data, longitudinal long-term observational studies of systemic vasculitides patients who have been/have not been treated with additive antirheumatic medication, based on CMR, randomized controlled trials of antirheumatic treatment/not treatment, based on CMR alone, with long-term outcomes [24<sup>■</sup>].

## CONCLUSION

Vascular and myocardial lesions although rare, maybe be life-threatening in systemic vasculitides. CMR using MRA and oedema-fibrosis imaging can detect early CVD involvement and guide treatment beyond vascular scores and/or acute phase reactant indices. Whether treatment changes informed by CMR findings would result in better long-term CVD and overall outcomes in patients with systemic vasculitides remains to be formally assessed in studies designed specifically for the purpose.

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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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# Management of large-vessel vasculitis

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## Purpose of review

Glucocorticoids are the mainstay of therapy for large-vessel vasculitis, but potential toxicity and frequent relapses led to studies with nonbiologic and biologic glucocorticoid-sparing agents. The aim of this review is to discuss the recent evidence for the management of giant cell arteritis (GCA) and Takayasu arteritis (TAK).

## Recent findings

Tocilizumab proved to be a powerful glucocorticoid-sparing agent for GCA in a randomized placebo-controlled trial, whereas the trials with tocilizumab and abatacept failed to show a significant difference from placebo in relapse-free survival rate in TAK. Further trials are awaiting for establishing the role of abatacept and ustekinumab for GCA, and rituximab and tumor necrosis factor inhibitors, including certolizumab for TAK, as well as nonbiologic agents for both indications.

## Summary

Despite recent randomized controlled trials with biologic agents, management of large-vessel vasculitis largely depends on observational studies. Well designed controlled trials using validated outcome measures in large number of patients, identification of biologic markers that could guide the choice of targeted treatments, and standardization of disease assessment including imaging modalities are unmet needs for the management of large-vessel vasculitis.

## Keywords

giant cell arteritis, large-vessel vasculitis, management, Takayasu arteritis

## INTRODUCTION

Large-vessel vasculitis includes giant cell arteritis (GCA) and Takayasu arteritis (TAK) that are both granulomatous vasculitides affecting the aorta and its major branches and share certain clinical, radiologic, and histologic findings but show differences in patient demographics, epidemiology, pathogenesis, and response to some treatment modalities [1]. GCA is a vasculitis involving large and medium-sized vessels predominantly affecting patients aged 50 years or older [2]. GCA therapy is still largely based on glucocorticoids, but recently, other therapeutic agents have been proposed. TAK, on the other hand, is more common among women below the age of 40. Current management usually comprises immunosuppressives in addition to glucocorticoids right from the beginning [3]. This review will focus on the recent advances for the treatment of GCA and TAK.

## MANAGEMENT OF GIANT CELL ARTERITIS

### Traditional glucocorticoid-sparing agent

#### Leflunomide

Two small case series reported that leflunomide may be an effective and well-tolerated glucocorticoid-sparing

agent in GCA [4,5]. In a recent prospective observational study, 76 consecutive newly diagnosed GCA patients were treated with a fixed glucocorticoid regimen; at week 12, leflunomide 10 mg daily was recommended as an add-on therapy to 30 GCA patients, whereas the others continued with glucocorticoid only [6]. The patients had a follow-up period of at least 48 weeks. Four patients in the leflunomide group (13.3%) and 18 (39.1%) in glucocorticoid-only group ( $P = 0.02$ ) flared during the follow-up. Furthermore, 56.7% of the patients treated with leflunomide were able to stop glucocorticoids at week 48, but none in glucocorticoid-only group. Leflunomide was well tolerated. Leflunomide can be a less expensive alternative to biological agents; however, randomized controlled trials (RCTs) are needed to confirm the utility of this drug.

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## KEY POINTS

- Glucocorticoids remain to be the mainstay of treatment for giant cell arteritis and Takayasu arteritis. Glucocorticoid-sparing drugs may be required to prevent toxicity with long-term use and relapses during glucocorticoid tapering.
- Tocilizumab is an effective and well-tolerated glucocorticoid-sparing agent for giant cell arteritis, whereas abatacept seems to have a moderate effect.
- Upfront use of immunosuppressives in addition to glucocorticoids may be preferred in Takayasu arteritis, as this strategy seems to improve relapse-free survival rate.

## Biologic agents

### Tocilizumab

Interleukin-6 (IL-6) has a key role in the pathogenesis of GCA [7–10]. Elevated levels of IL-6 are present and correlate with disease activity. Tocilizumab (TCZ) is a humanized monoclonal antibody that blocks signaling by binding to the  $\alpha$ -chain of the human IL-6 receptor [11<sup>¶</sup>].

Case reports, observational studies, and a randomized placebo-controlled phase 2 trial (Swiss study) showed that TCZ was an effective treatment for GCA and glucocorticoid sparing [12–14].

More definitive results on the safety and efficacy of TCZ in GCA have been acquired with the recently published multicenter, randomized, double-blind, placebo-controlled, phase 3 trial, the Giant-Cell Arteritis Actemra, which represents the largest prospective study evaluating treatment efficacy in GCA [15<sup>¶¶</sup>]. A total of 251 patients (119 newly diagnosed and 132 with relapsing disease) were enrolled and randomly assigned, in a 2:1:1:1 ratio, to receive subcutaneous TCZ (at a dose of 162 mg) weekly or every other week, combined with a 26-week prednisone taper, or placebo combined with a prednisone taper over a period of either 26 weeks or 52 weeks. TCZ combined with a 26-week prednisone taper was superior to either a 26-week or 52-week prednisone taper and placebo with regard to the sustained remission at week 52, the primary outcome, which occurred in 56% of the patients treated with TCZ weekly, 53% of those treated with TCZ every other week, 14% of those in the placebo group that underwent the 26-week prednisone taper, and 18% of those in the placebo group that underwent the 52-week prednisone taper ( $P < 0.001$  for the comparisons of either active treatment with placebo). TCZ treatment was also associated with a highly significant reduction in the cumulative prednisone dose

over 52 weeks (1862 mg in each TCZ group, 3296 mg in the placebo group and a 26-week prednisone taper and 3818 mg in the placebo group and a 52-week prednisone taper,  $P < 0.001$  for all comparisons). In addition, among the 131 patients with relapsing disease, the risk of flare was significantly lower in the group that received weekly TCZ than in the two placebo groups (HR, 0.23;  $P < 0.001$ ; and HR, 0.36;  $P = 0.01$ ), although not in the group treated with TCZ every other week (HR, 0.42;  $P = 0.05$ ; and HR, 0.67;  $P = 0.37$ ). This different outcome between the two TCZ dose regimens was not seen in patients with newly diagnosed disease at baseline. Fewer patients reported serious adverse events in the group that received TCZ weekly (15%) or every other week (14%) than in the two placebo groups (22 and 25%, respectively). This study clearly demonstrated that TCZ is highly effective in GCA, has a powerful steroid sparing effect, and is well tolerated.

However, some open questions remain, the first regarding which patients should be treated. Should all patients with GCA be treated with TCZ at diagnosis or should we reserve TCZ only for patients at high risk for serious glucocorticoid side-effects and for those with flaring disease resistant to glucocorticoid treatment? Considering the high prevalence of glucocorticoid-related side-effects (86% of patients) in GCA and the correlation between the cumulative glucocorticoid dose and the development of side-effects [16], an early initiation of TCZ therapy in all new GCA patients could represent a reasonable option. Results from RCTs are assuring regarding its safety, but a close follow-up is still required for infections, neutropenia, thrombopenia, and increased transaminase and cholesterol levels. Three other unanswered questions are how long TCZ treatment should be continued, and if long-term treatment with TCZ is effective and well tolerated and can prevent the life-threatening vascular complications caused by GCA. It is also unknown whether maintenance therapy with a conventional immunosuppressive agent should be initiated once discontinuation of TCZ is attempted. In the Swiss trial, GCA patients received TCZ for 52 weeks; thereafter, TCZ was stopped and the patients were followed up [17]. After the last infusion of TCZ, 11/20 (55%) patients relapsed with a median time to relapse of 5 months. These data clearly indicate that in more than half of the patients in clinical remission with TCZ, arteritis still persists. Other data from the Swiss trial support this evidence. TCZ did not completely suppress immune-inflammatory markers [18] and signals of vessel inflammation at magnetic resonance angiography (MRA) normalized in only one-third of patients at week 52 [19<sup>¶</sup>]. Similarly, a PET/CT study and pathological data have confirmed



the presence of active arteritis in patients treated with TCZ, apparently in remission [13,20]. Therefore, it cannot be excluded that the effect of TCZ could be symptomatic rather than curative.

Both the T-helper 17 (T<sub>H</sub>17) cells and T-helper 1 (T<sub>H</sub>1) cells have a key role in the pathogenesis of GCA [21]. Although TCZ effectively blocks the T<sub>H</sub>17 cell pathways, probably this drug has only limited effect on the chronic vasculitis characterized by a predominant T<sub>H</sub>1 signature which is IL-6-independent and unaffected by glucocorticoids.

A recent multicenter prospective French study has evaluated TCZ as add-on therapy to glucocorticoids during the first 3 months of GCA treatment [22<sup>■</sup>]. All 20 enrolled patients received four infusions of TCZ (8 mg/kg/4 weeks) in association with a standardized prednisone regimen. About 75% of patients were in remission with 0.1 mg/kg/day or less of prednisone at week 26; however, 50% of patients experienced relapses during the 9 months following TCZ discontinuation.

TCZ interferes with the hepatic synthesis of acute-phase reactants; therefore, C-reactive protein (CRP) and erythrocyte sedimentation rate are unreliable for monitoring GCA patients treated with this drug. A recent study suggested that serum osteopontin might be a suitable biomarker for disease activity in TCZ-treated patients [23].

### Ustekinumab

Ustekinumab is a human immunoglobulin G1 $\kappa$  monoclonal antibody which blocks both interleukin-12 (IL-12) and IL-23 activity by binding to the common p40 subunit. The key role of both the T<sub>H</sub>1/IL-12/interferon gamma and T<sub>H</sub>17/IL-23/IL-7 pathways in the pathogenesis of GCA makes ustekinumab an attractive therapeutic option for GCA [21]. In a recent prospective open-label study, ustekinumab 90 mg was administered subcutaneously every 12 weeks to 25 patients with refractory GCA [24]. At week 52, the median (interquartile range) daily prednisolone dose decreased significantly from 20 mg [15<sup>■</sup>,25] to 5 mg (2.5, 5) ( $P < 0.001$ ), CRP decreased significantly from 12.9 mg/l (5.3, 42) to 6 (2.6, 12.5) mg/l ( $P = 0.006$ ), and 6 (24%) patients were able to stop prednisolone completely. No patients had a relapse of GCA while receiving ustekinumab. Computerized tomography angiography demonstrated improvement of large-vessel vasculitis (improvement/resolution of the wall thickening) in the eight patients studied with repeat imaging. Only three patients discontinued ustekinumab for adverse events. Therefore, ustekinumab seems to be effective and well tolerated for GCA; however, experience remains at the moment too limited to draw definitive conclusions.

### Abatacept

GCA is probably an antigen-driven disease in which activated T lymphocytes, macrophages, and dendritic cells play a key role in the disease pathogenesis [25]. Abatacept is a fusion protein, which links the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 to the modified Fc portion of human immunoglobulin G1. This drug prevents CD80/CD86 from binding to CD28 on the surface of the T cell, resulting in failure of the costimulatory signal required for T-cell activation.

In a multicenter, randomized, double-blind trial, 49 patients with newly diagnosed or relapsing GCA were treated with abatacept 10 mg/kg intravenously on days 1, 15, and 29 and week 8, together with prednisone administered daily [26<sup>■</sup>]. At week 12, 41 patients in remission underwent a double-blinded randomization to continue to receive abatacept monthly or switch to placebo. A standardized prednisone taper with suspension at week 28 was administered. The relapse-free survival rate at 12 months, the primary end point, was 48% for those receiving abatacept and 31% for those receiving placebo ( $P = 0.049$ ). There was no difference in the frequency of adverse events between the two treatment arms. This study shows that abatacept is moderately effective in the treatment of GCA. Replication in larger cohorts is necessary.

### Future therapies

Although B cells have received little attention as putative players in the immunopathology of GCA, recent observations, showing the presence of artery tertiary lymphoid organs (ATLOs) in the inflamed temporal artery, suggest a role for B cells in the GCA pathogenesis [27<sup>■</sup>,28<sup>■</sup>]. Further studies are needed to define the role of rituximab, a chimeric anti-CD20 monoclonal antibody, in GCA.

Circulating IL-1 levels and IL-1 $\beta$  mRNA expression in temporal arteries are increased in GCA [29,30]. Anakinra (IL-1Ra), an IL-1 $\beta$  antagonist, and gevokizumab, a recombinant humanized anti-IL-1 $\beta$  antibody, are currently being tested in GCA (NCT02902731 and European Clinical Trials Database identifier 2013-002778-38, respectively).

Zhang *et al.* [31<sup>■</sup>] examined inhibition of Janus Kinase (JAK)-signal transducer and activator of transcription protein (STAT) signaling in medium and large-vessel vasculitis. They studied vascular inflammation induced in human arteries engrafted into immunodeficient mice reconstituted with monocytes and T cells from patients with GCA. The mice were treated with tofacitinib, a JAK inhibitor targeting JAK3 and JAK1. Tofacitinib effectively suppressed innate and adaptive immunity in the

vessel wall, suggesting that tofacitinib could be a potential treatment for GCA. An open-label pilot study on baricitinib (JAK1 and JAK2 inhibitor) in relapsing GCA is ongoing (NCT03026504).

Blocking vascular remodeling may be a therapeutic target in addition to anti-inflammatory therapies [32]. Imatinib mesylate, which strongly inhibits platelet derived growth factor-mediated responses and endothelin receptor antagonists (endothelin-1 has a crucial role in inducing vascular remodeling in GCA), may represent a promising future treatment for GCA [33,34<sup>\*\*\*</sup>].

## MANAGEMENT OF TAKAYASU ARTERITIS

### Traditional glucocorticoid-sparing agents

Glucocorticoids are the mainstay for induction of remission in TAK, but relapses are frequent with the solo use of glucocorticoids, reported as high as 93% within 2 years in a recent population-based cohort study from Norway [35<sup>\*</sup>]. Upfront use of immunosuppressives in addition to glucocorticoids for preventing relapses and for glucocorticoid sparing seems to be a preferable management strategy in TAK patients. A recent single-center large cohort from India supports this contention. Among 251 patients with TAK, 235 (94%) were treated with immunosuppressives (MMF 64%, azathioprine 22%, and methotrexate 8%) together with high-dose glucocorticoids right from the beginning [36], providing high cumulative relapse-free survival rates of 93, 73, 66, and 52% at 1, 3, 5, and 10 years, respectively. Among these, 87% showed no or minimal damage progression. Early response and relapse rates were similar with prednisolone 1 mg/kg/day and 0.5 mg/kg/day. Immunosuppressives may also help control hypertension as observed in a retrospective series of 381 TAK patients [odds ratio (OR), 2.4; 95% confidence interval (CI) 1.25–4.6;  $P=0.008$ ] [37<sup>\*</sup>].

The choice of immunosuppressives for TAK shows variation according to experience of each center, cost, and level of disease severity perceived by the physician. A recent meta-analysis of non-glucocorticoid therapies for TAK indicated similar relapse rates between cyclophosphamide, MMF, azathioprine, methotrexate, and leflunomide [38].

Only two studies that compared nonbiologic agents were published until now and one of them was a recent study comparing the outcome of 46 patients treated with cyclophosphamide and 12 treated with methotrexate [39]. Clinical remission (Kerr score  $\leq 1$ ) rates were similar with cyclophosphamide (72%) and methotrexate (75%) despite more active disease in the cyclophosphamide group

at baseline. The authors suggested that cyclophosphamide may be a useful alternative in relapsing patients or patients with severe disease at baseline. There were no serious adverse events in this study. However, long-term adverse events and infertility are important concerns for cyclophosphamide use in a group of relatively young patients.

### Biologic agents

The recent meta-analysis identified 12 studies with nonbiologic and 23 with biologic agents [tumor necrosis factor  $\alpha$ -inhibitors (TNFi), rituximab, TCZ, and abatacept] [38]. Biologic agents were almost always used in refractory patients, after at least one nonbiologic immunosuppressive. Pooled proportion of patients achieving remission was similar with biologics and nonbiologics (0.64, 95% CI 0.56–0.72; and 0.58, 95% CI 0.40–0.74, respectively), whereas relapse rate was lower with biologics (0.54, 95% CI 0.39–0.68 versus 0.31, 95% CI 0.22–0.41). Serious adverse events occurred in 34/363 with nonbiologics and 41/208 with biologics and were mostly infections. The results of this meta-analysis may be biased by small sample sizes, lack of control groups, and heterogeneity regarding patient selection and definition of outcomes of the included studies.

### Tumor necrosis factor $\alpha$ -inhibitors

There are no controlled studies with TNFi in TAK. Observational data, mostly with infliximab, suggest that TNFi improve event-free survival, decrease disease flares, and damage [40]. A recent population-based cohort study from Norway showed that 10% of patients treated with TNFi developed new lesions within 2 years compared with 40% with disease modifying antirheumatic agents (DMARDs) (OR, 0.13) and 93% with glucocorticoid monotherapy (OR, 0.02) [35<sup>\*</sup>]. Moreover, the sustained remission rate was higher with TNFi (42%) compared with DMARDs (20%,  $P=0.03$ ). A comparison of their treatment strategies before and after the year 2000 showed that upfront immunosuppressive use in addition to glucocorticoids increased from 4 to 51% and TNFi use increased from 13 to 44%. These changes were associated with a decrease in arterial damage incidence rate from 19.4 to 10.4 per 100 patient-years ( $P=0.004$ ).

A recent multicenter retrospective case series of 10 patients treated with certolizumab showed a rapid response with decreased CRP levels and Indian Takayasu Activity Score 2010 scores and prednisolone dose could be tapered [41<sup>\*\*\*</sup>]. An initial remission was obtained at median 4 months in all patients, whereas one patient experienced relapse after 2 years. Imaging showed no progression in the

6 patients who were properly evaluated. One of the advantages of certolizumab in TAK patients who are more frequently female is its low potential to cross the placenta. One of the patients gave birth to a healthy child. Certolizumab was well tolerated with mild infections.

TNFi use may be associated with an increased risk of infections. Caution is required especially for tuberculosis that may have a relatively high background prevalence in countries where TAK is more common. High-dose corticosteroids that are used together with TNFi in these patients may increase the risk of tuberculosis.

### **Tocilizumab**

Based on the potential role of IL-6 in the pathogenesis of TAK and beneficial results from open-label studies, a 12-week randomized placebo-controlled phase 3 trial was conducted [42]. A total of 36 patients who experienced a relapse within the last 12 weeks while using at least 0.2 mg/kg/day prednisolone equivalent, and who obtained remission with glucocorticoids were randomized to TCZ 162 mg or placebo. The steroid dose was tapered by 10% per week from week 4 to a minimum of 0.1 mg/kg/day. The primary endpoint was time to relapse and despite a trend for longer relapse-free survival with TCZ, the difference was not statistically significant [hazard ratio (HR), 0.41; 95% CI 0.15–1.10;  $P=0.0596$ ]. The rapid glucocorticoid taper that was mandated led to a high relapse rate, 8/18 (44.4%) with TCZ and 11/18 (61.1%) with placebo arm. Adverse events were compatible with the known safety profile of TCZ.

Despite the failure to meet the primary endpoint in the RCT, a multicenter retrospective study of 46 patients treated with TCZ (39 refractory to glucocorticoid and DMARDs) showed a decrease in the median National Institute of Health scale from 3 to 0 ( $P<0.0001$ ), radiological activity from 83 to 20% at month 6 and 17% at month 12 ( $P<0.001$ ), and the daily prednisone dose from 15 mg to 4 mg at month 3 and to 5 mg at month 6 ( $P<0.0001$ ) [43]. The event-free survival rate was 81% at month 12, 72% at month 24, and 48% at month 48, significantly higher than the event-free survival rate at 3 years in a similar cohort from the French Takayasu network treated with DMARDs ( $P=0.02$ ). A systematic review of case reports and series of TCZ for the treatment of TAK in 105 patients also showed beneficial results with an initial clinical response within 3 months in 43%, radiological improvement in 65%, and glucocorticoid reduction in 90%. Relapses were observed in 9% during treatment with TCZ, and in 46% after discontinuation of TCZ [44]. Adverse events were mainly infections.

The lack of imaging in many of these reports and lack of widely accepted outcome measures for TAK as well as the unreliability of acute phase reactants for indicating vascular inflammation, disease progression, and predicting damage in TAK make it difficult to interpret these results.

### **Abatacept**

An RCT was conducted with abatacept on the basis of a similar rationale to GCA and with an identical protocol [45]. However, this study did not meet the primary endpoint which was relapse-free survival. Among the 34 patients who were enrolled, 26 were randomized. Relapse-free survival rate at 12 months was 22% for the abatacept group and 40% for placebo ( $P=0.853$ ). The median duration of remission was 5.5 months in the abatacept group and 5.7 months in the placebo group ( $P=0.125$ ). Adverse events were comparable between the groups.

It is not possible to comment on the steroid-sparing potential of abatacept or TCZ on the basis of these RCTs because of the standard prednisolone taper in the active treatment and placebo arms.

### **Rituximab**

The potential role of B cells in TAK based on B-cell infiltrates in the affected vessel wall and high levels of B-cell subsets, particularly plasmablasts in the peripheral blood, encouraged the use of rituximab [45,46]. In a recent case series, seven patients refractory to glucocorticoid and immunosuppressives except for one were treated with rituximab with 12–72-month follow-up [47]. Four of the patients were unresponsive with persistent disease activity and/or radiographic disease progression. This finding contrasted with previous case reports that showed clinical and laboratory remission in 8/9 patients treated with rituximab and a recent multicenter case series of eight patients that showed a significant decrease in CRP levels, prednisolone dose, and Kerr index score with 12-month follow-up [48]. Differences in disease assessment methods including imaging, relatively short duration of follow-up in the previous reports, and publication bias because of more frequent publication of improved cases may explain the difference in the results.

### **Surgical interventions**

Despite intensive medical treatment, surgical interventions may be necessary in patients with TAK. The main two options are endovascular interventions including percutaneous transluminal angioplasty, stent insertion, and stent graft placement, and open surgery including bypass grafting, patch angioplasty, and endarterectomy.



A recent meta-analysis compared endovascular and open surgical interventions in 770 patients in 19 observational studies [49]. Restenosis was more common after endovascular procedures (OR, 5.18; 95% CI 2.8–9.6), especially for coronary, supraaortic branches, and renal arteries. Although not formally analyzed, restenosis was not always correlated with long-term prognosis. However, stroke was more common with open surgery (OR, 0.33; 95% CI 0.12–0.90) when the supraaortic branches were involved. Mortality rate was not different between the groups.

Another meta-analysis comparing two endovascular procedures revealed a similar risk of restenosis with percutaneous transluminal balloon angioplasty and stenting except for renal arteries, in which balloon angioplasty performed better (OR, 4.40; 95% CI 2.14–9.02;  $P < 0.001$ ) [50]. However, the risk of acute vascular complications, most commonly dissection, was higher with balloon angioplasty (OR, 0.07; 95% CI 0.02–0.29;  $P < 0.001$ ).

None of the studies included in these meta-analyses was randomized. The length and degree of stenosis, the vessel that was involved, and whether the procedure was performed for restenosis would affect the choice of procedure and thus the results of these studies. Moreover, having active disease during surgery would negatively impact the outcome.

## CONCLUSION

Glucocorticoids remain the cornerstone of GCA therapy. The introduction of TCZ as a powerful glucocorticoid-sparing agent is a major therapeutic advance in GCA treatment. More data are needed to define the role of abatacept and ustekinumab. Future effective treatment could suppress not only the vascular inflammation but also pathways of vascular remodeling and these two modalities of treatment might be combined. Individualized treatment of GCA patients remains a far-off prospect for now, and collaborative efforts are needed to identify biologic markers that could guide the choice of targeted treatments. Observational studies suggest that upfront use of immunosuppressives together with glucocorticoids improve the relapse-free survival for TAK. Controlled evidence with biologic and nonbiologic agents and standardization of disease assessment including imaging modalities are unmet needs for identifying ideal management strategies for TAK.

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# Nervous system involvement in Behçet's syndrome

Uğur Uygunoğlu and Aksel Siva

## Purpose of review

Neurological involvement in Behçet's syndrome is defined as 'the occurrence of neurological symptoms and signs in a patient who meets the International Diagnostic Criteria for BS not otherwise explained by any other known systemic or neurological disease or treatment, and in whom objective abnormalities consistent with neuro-Behçet's syndrome (NBS) are detected either on neurological examination, neuroimaging studies (magnetic resonance imaging [MRI]), and/or on cerebrospinal fluid (CSF) examination'. Given that the neurological involvement of Behçet's syndrome carries a poor prognosis, we aimed to describe the differential diagnosis of NBS and highlight the different radiological patterns together with the treatment options.

## Recent findings

Two distinct MRI patterns of spinal cord involvement in Behçet's syndrome according to T2-weighted axial images were described: 'Bagel Sign' pattern: a central lesion with hypointense core and hyperintense rim with or without contrast enhancement; and 'Motor Neuron' pattern: a symmetric involvement of the anterior horn cells. Infliximab prevents patients from having further attacks and even led to improvement in the neurological examination.

## Summary

As the treatment options completely differ, a NBS diagnosis should be carefully made in patients with clinical and MRI features mimicking other central nervous system inflammatory disorders.

## Keywords

bagel sign, Behçet's disease, Behçet's syndrome, differential diagnosis, infliximab, neuro-Behçet syndrome, uveo-meningeal syndromes

## INTRODUCTION

The uveo-meningeal syndromes are a group of disorders that present with intraocular and neurologic findings. Inflammatory and/or autoimmune disorders are the most common clinical causes of uveo-meningeal syndromes, and along the Silk Road, Behçet syndrome is the most prevalent uveo-meningeal syndrome with the prevalence of 20–421/100 000 [1,2]. Neurological involvement in Behçet's syndrome is named as neuro-Behçet syndrome (NBS). In terms of the differential diagnosis of NBS, Figure 1 reveals the uveo-meningeal syndromes [1,3,4]. Given the several clinical subsets and geographical variation indicating different disease mechanisms, Yazici *et al.* [5<sup>••</sup>] proposed using the term Behçet's Syndrome rather than Behçet's disease for this disorder, and therefore we will favor this terminology within this review.

Given that there is no existing laboratory marker, the diagnosis of NBS depends on the clinical history, neurological examination, and radiological patterns. In 2014, a consensus report was published regarding the recommendations using a nine-point

Likert scale. The median value for all answers was eight or nine points; however, the scores ranged from four to nine for questions regarding 'how to differentiate neuro-Behçet's disease', 'the role of disease modification treatment', 'the type of disease modification treatment', 'the role of biological agents', 'the role of cyclosporine', and 'headache in Behçet's syndrome' [6]. Considering that even experts hold different opinions in this regard and given the broad spectrum of Behçet's syndrome clinical phenotypes, patients should be very carefully evaluated using a multidisciplinary approach that includes a rheumatologist, an ophthalmologist experienced in uveal diseases, a dermatologist, and a

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## KEY POINTS

- NBS is included in the differential diagnosis of uveo-meningeal syndromes.
- Bagel sign is the distinctive MRI pattern observed in the spinal cord involvement of Behçet's syndrome.
- Infliximab has significant efficacy in the treatment of NBS.

neuroradiologist in addition to the neurologist who is experienced in the field of clinical neuro-immunology.

In this context and in light of the published data and our experiences, this review was developed to describe the following:

- (1) When we should suspect NBS?
- (2) Radiological patterns of NBS.
- (3) Laboratory findings supporting the NBS diagnosis.
- (4) How to treat the patients in the acute and chronic stages?

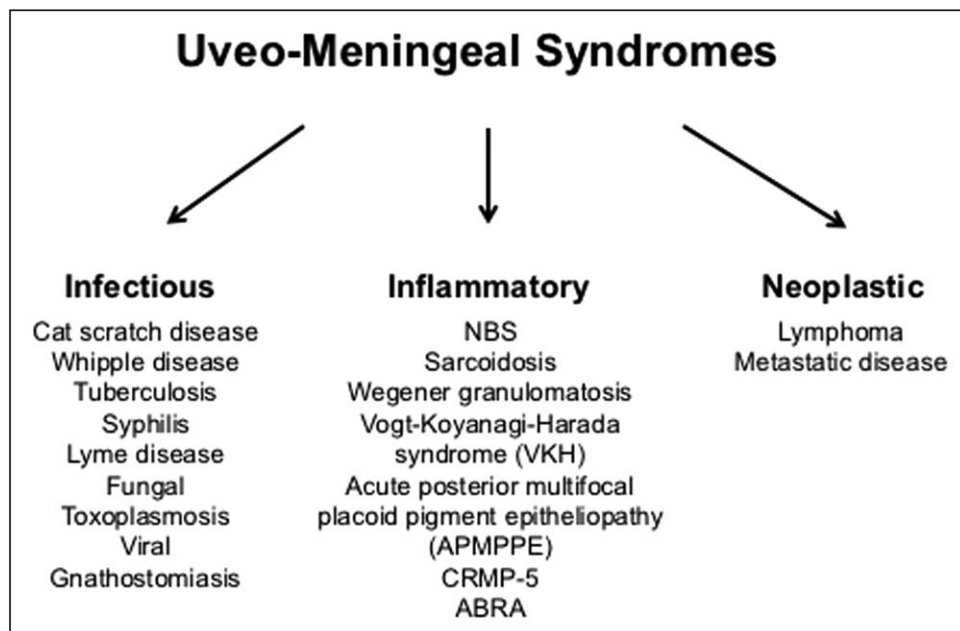
## DIAGNOSIS OF NEURO-BEHÇET'S SYNDROME

Although it has not been validated, we currently use the international consensus recommendation criteria, a slightly modified version of our Cerrahpaşa diagnostic criteria for NBS diagnosis, which we have introduced in 2001 [7]. These can be summarized as

'the occurrence of neurological symptoms and signs in a patient who meets the International Diagnostic Criteria for BS not otherwise explained by any other known systemic or neurological disease or treatment, and in whom objective abnormalities consistent with NBS are detected either on neurological examination, neuroimaging studies (magnetic resonance imaging [MRI]), or abnormal cerebrospinal fluid (CSF) examination'.

Neurological involvement in Behçet's syndrome can be classified as primary or secondary. Primary neurological involvement occurs in approximately 5–10% of all patients with Behçet's syndrome [8]. The age of onset of NBS, excluding pediatric cases, is usually later within the third decade, with a mean duration between the onset of Behçet's syndrome and NBS of about 5 years [9,10]. NBS is almost three times more frequent in males than females. The frequency of neurologic involvement increases to 13% in males and 5.6% in females when Behçet's syndrome patients are followed for up to 2 decades [11]. In addition, 6% of patients may present with neurological involvement without fulfilling the International Study Group's (ISG) classification criteria for Behçet's syndrome, which is the most challenging factor for clinicians who are trying to make an accurate diagnosis and decide to start long-term treatment [8,12].

Approximately 75–80% of NBS cases present with central nervous system (CNS) involvement, which is called 'parenchymal NBS' (p-NBS) or intra-axial NBS and usually affects the telencephalic–diencephalic junction, brainstem, and spinal cord. These patients present with a subacute



**FIGURE 1.** The uveo-meningeal syndromes.

(or rarely acute) onset of severe headache, cranial nerve palsy, dysarthria, ataxia, and hemiparesis. The presentation may include all or some of these symptoms and signs. During the acute stage, mild confusion may also be seen. p-NBS is an important cause of morbidity and mortality [13].

The second most common form of neurological involvement is cerebral venous sinus thrombosis (CVST), which is also called vascular NBS or extra-axial NBS occurring in up to 20% of patients with neurological involvement. In these patients, the prominent clinical feature is severe headache usually developing over a few weeks. Typically, papilledema and, occasionally, sixth nerve palsy are observed. This form occurs more commonly in the pediatric population, suggesting that age may influence the form of neurological involvement [13]. The most commonly affected sinuses are the superior and sagittal sinuses [14]. In contrast to other causes of CVST, hemiparesis, impaired consciousness, and epileptic seizures are uncommon clinical features of CVST in patients with NBS, and venous infarcts are rare in NBS-CVST [14]. This may be related to the time frame of thrombosis, which occurs more slowly than other causes of CVST. Another interesting finding about NBS-CVST is the time of diagnosis. Yesilot *et al.* [15] found a median delay of 61 days from the onset of symptoms to diagnosis in the Behçet's syndrome group and 6 days in the non-Behçet's syndrome group, which also supports that thrombosis develops slowly in Behçet's syndrome-CVST. In addition to evaluating the hereditary and acquired risk factors for CVST cause, clinicians should evaluate the symptoms of Behçet's syndrome particularly in the patients living on the Silk Road or immigrated from countries where Behçet's syndrome is prevalent. The two types, intracranial and extracranial NBS, very rarely occur in the same individual, have a tendency to develop in different age groups, and are associated with different systemic manifestations of Behçet's syndrome, and therefore presumably have a different pathogenesis [16]. Neuro-psycho-Behçet syndrome, headache (migraine-like, nonstructural), peripheral nervous system involvement, and sub-clinical NBS are the primary neurological involvement patterns other than p-NBS and CVST [10].

Regardless of the type of NBS whether CVST or p-NBS, headache is the most common clinical feature in both types. However, headache occurring during the course of the disease should not be classified as NBS when the clinical history, neurological examination, MRI patterns, and CSF findings do not suggest NBS. When the headache type and characteristics were evaluated by Saip *et al.* [17], paroxysmal migraine like pain occurring with flares

of Behçet's syndrome's systemic features was described. In another study regarding with headache Vishwanath *et al.* [18] found that most patients having a history of migraine reported that their headache worsened during the disease activation. In the light of these studies, similar to other rheumatologic disorders, headaches in Behçet's syndrome may imply a manifestation of the disease, progression, or complication [19].

Anxiety and depression are the most common psychosomatic symptoms in Behçet's syndrome. However, some patients with Behçet's syndrome develop a neurobehavioral syndrome, which consists of euphoria, loss of insight/disinhibition, indifference to their disease, and psychomotor agitation or retardation, with paranoid attitudes and obsessive concerns. We have observed the development of these psychiatric symptoms either at the onset of other neurological symptoms of NBS or as independent phenomena that are unrelated to the use of glucocorticosteroids or any other therapy. We have named this syndrome 'neuro-psycho-Behçet syndrome' [20]. In a recent study, a reduced quality of sleep in patients with Behçet's syndrome was observed, and sleep disorders, such as sleep apnea and restless leg syndrome, were found to be more common in Behçet's syndrome patients [21]. This observation emphasizes the importance of addressing the quality of the sleep of and the presence of sleep disorders in patients with Behçet's syndrome to better manage the common somatic complaints in these patients, such as fatigue or daytime sleepiness. There are few studies on cognitive impairment in NBS [22,23]. Oktem-Tanör *et al.* [22] indicated that the most severely affected memory process was delayed recall, which was impaired in all patients in the verbal and/or visual modalities.

Neurologic complications secondary to the systemic involvement of Behçet's syndrome, such as cerebral emboli from the cardiac complications of Behçet's syndrome or increased intracranial pressure secondary to superior vena cava syndrome, may occur. The neurologic complications of Behçet's syndrome treatments, such as CNS neurotoxicity with cyclosporine and peripheral neuropathy secondary to thalidomide or colchicine, may also occur [8].

## RADIOLOGICAL PATTERNS OF NEURO-BEHÇET'S SYNDROME

As there is no marker for the diagnosis of Behçet's syndrome and NBS, cranial MRI and magnetic resonance venography (MRV) are of utmost importance for diagnosis and for differentiating NBS from other disorders that mimic NBS patterns in MRI.



Figure 2 illustrates the following classical patterns of neurological involvement in Behçet's syndrome [24]:

- (1) Telencephalon
- (2) Diencephalic
- (3) Brainstem
- (4) Spinal cord

In p-NBS, cranial MRI shows almost stereotypical lesions involving the brainstem, mainly the midbrain and upper pons, and extending to the diencephalon and basal ganglia; a caudal extension is also observed in some patients. The lesions are hyperintense on T2 images and hypo/isohypointense on T1. Usually, there is a much smaller area of enhancement and, occasionally, small hemorrhages can be seen within the lesions. After steroid treatment, the lesion(s) regress to punctate T2 hyperintense areas, and brainstem atrophy may develop [25,26<sup>22</sup>]. In addition to the characteristic findings of NBS, significantly more lesions were detected with susceptibility-weighted imaging than with conventional T2\*GE. Most of the lesions in intra-axial NBS were found to be hemorrhagic, supporting the proposed venous theory as the pathophysiology of NBS [27].

When there is spinal cord involvement, it may tend to be longitudinally extensive [26<sup>22</sup>,28,29]. We described two distinct MRI patterns of spinal cord involvement in Behçet's syndrome according to T2-weighted axial images: 'Bagel Sign' pattern: a central lesion with hypointense core and hyperintense rim with or without contrast enhancement; and 'Motor Neuron' pattern: a symmetric involvement of the anterior horn cells [26<sup>22</sup>].

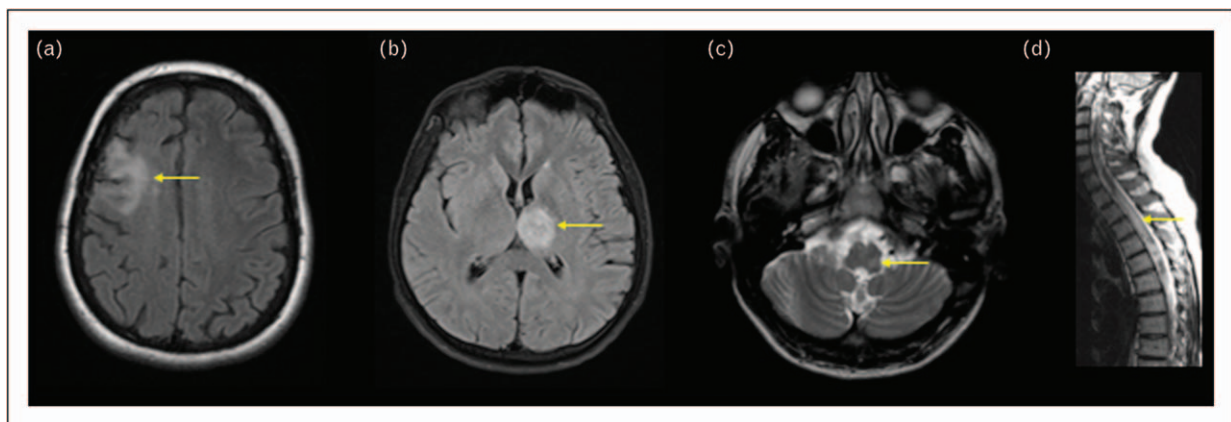
The parenchymal distribution of NBS lesions seems to support the hypothesis of small vessel vasculitis, mainly venular involvement. The known anatomic arrangement of CNS intra-axial veins explains the predominant involvement of brainstem structures. This pattern of lesion distribution might help to differentiate NBS from other types of vasculitis as well as from inflammatory-demyelinating diseases of the CNS, such as multiple sclerosis (MS) [25,26<sup>22</sup>,30]. Autopsy studies and biopsy specimens of the CNS lesions are consistent with vascular inflammation as well, and they show a clear venous predominance [31]. Radiologic studies also support this finding, in that the lesions seen in NBS are not compatible with arterial territories [25]. Furthermore, significant perilesional edema with a tendency to disappear or to leave disproportionately small residues has been reported in follow-up studies [25,26<sup>22</sup>]. This feature is consistent with venous infarction, as not all signal intensity changes seen in venous occlusive disease necessarily represent infarction, but rather an accumulation of water within interstitial spaces. All this information, together with the above mentioned observations, supports the probable inflammatory-venous pathogenesis for the CNS lesions seen in Behçet's syndrome.

Rarely, instead of the typical brainstem-diencephalic lesion(s), atypical patterns and involvement at other parenchymal sites may occur (Fig. 3) [32].

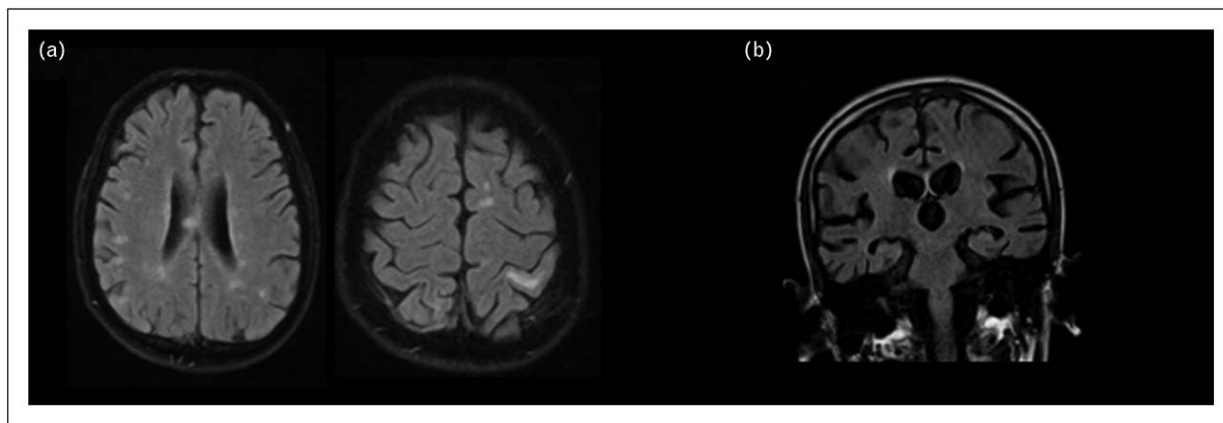
According to the MRI features two different patterns are described as:

- (1) Bilateral cortical-subcortical lesions
- (2) Regional or global atrophy

As the treatment options completely differ, a NBS diagnosis should be carefully made in patients



**FIGURE 2.** Brain magnetic resonance and spinal cord MRI of typical parenchymal patterns during the parenchymal neuro-Behçet's syndrome episodes are shown. (a) Axial FLAIR image shows the telencephalic involvement. (b) Axial FLAIR image shows the diencephalic involvement. (c) Axial T2-weighted image shows the brainstem involvement. (d) Sagittal T2-weighted image shows the longitudinally extensive ( $\geq 3$  sagittal spinal cord segments) involvement.



**FIGURE 3.** Brain MRI of atypical parenchymal patterns during the parenchymal neuro-Behçet's syndrome episodes are shown. (a) Axial FLAIR images are not typical for parenchymal neuro-Behçet's syndrome showing bilateral periventricular, subcortical and juxtacortical lesions – multiple sclerosis needs to be ruled out in such patient. (b) Coronal FLAIR image shows significant global cerebral atrophy.

whose MRIs show lesions at sites other than the brainstem–diencephalic region. For instance, in our clinical experience, we have seen many patients without any significant neurological symptoms suggestive of NBS who fulfill the Behçet's syndrome criteria and have MRIs showing bilateral cortical–subcortical lesions similar to lesions seen in multiple sclerosis [30]. The imaging findings are actually rather different between NBS and MS:

- (1) The posterior fossa lesions of MS are small and discrete, whereas p-NBS brainstem lesions are large and diffuse; in addition, posterior fossa lesions of p-NBS may have a mass effect and extend toward the diencephalic, thalamic, and basal ganglia regions and only rarely to the optic nerve regions. Cerebellar lesions are uncommon in NBS.
- (2) Periventricular, juxtacortical, and corpus callosum lesions are common in MS but rare in p-NBS.
- (3) Hemispheric subcortical regions are rare in NBS and, when present, are usually small and asymptomatic, whereas coalescent periventricular lesions are more supportive of MS.
- (4) Spinal cord involvement rarely extends more than a few vertebral segments in MS.

In addition to these differences, Maggi *et al.* [33] recently published an article comparing the frequency of perivenular lesions between MS and other inflammatory vasculopathies. Although they found that NBS patients had a higher frequency of lesions with the central vein sign (CVS) when compared with patients with other inflammatory vasculopathies, the CVS was significant to differentiate MS

lesions than other inflammatory neurologic disorders including NBS. However, a subgroup of Behçet's syndrome patients with neurological complaints may have MS-like lesions on MRI, and their MRI images may even fulfill the radiological criteria for MS or some may have both Behçet's syndrome and MS as comorbid disorders [30].

There are also a number of reports of NBS cases whose MRIs showed mass lesions that mimicked brain tumors, some necessitating histological diagnosis [34]. Although the inflammatory nature could not be shown in all cases, these lesions are likely to be acute inflammatory edematous lesions that show significant resolution after treatment with intravenous methylprednisolone (IVMP). Brainstem and global atrophy may provide an important clue of a progressive NBS even if the patient does not fulfill the ISG criteria for Behçet's syndrome. Cerebellar involvement may rarely happen in the acute phase, but although not common cerebellar atrophy has been reported during the progressive phase of NBS [35].

Although uncommon in systemic Behçet's syndrome, major vessel involvement, including arterial occlusion and arterial aneurysms may be seen in NBS. The high frequency of multiple aneurysms, the fusiform nature and peripheral location of the aneurysms, the presence of vasculitis signs on the parent artery, the atypical morphology of the aneurysm, and the predominantly male sex of patients suggest that pathogenetic mechanisms in Behçet's syndrome play a major role in the development of these aneurysms. The sites of involvement include the common carotid, internal carotid, middle cerebral, superior cerebellar, anterior cerebral, anterior communicating, and vertebral arteries. Immunosuppressive treatments may be effective for some

unruptured aneurysms, whereas surgical or endovascular treatment should be considered in patients with ruptured aneurysms [36].

## LABORATORY FINDINGS

Although most studies did not find any association between positive human leukocyte antigen (HLA)-B51 status and the frequency of CNS involvement, Demirseren *et al.* [37] found that subgroup HLA-B5103 had a significantly higher frequency in patients with neurological involvement. When considering that HLA-B51 positivity is strongly associated with frequent relapses, patients not fulfilling the ISG criteria with clinical and MRI features suggestive of NBS should be examined for HLA-B51 [38].

CSF findings may help to discriminate NBS from other inflammatory diseases, particularly from MS. Although oligoclonal bands (OCB) are detected in 95–100% of MS patients, less than 15% OCB positivity is seen in NBS [39]. In addition, up to 100 or more cells, predominantly neutrophils, are observed in NBS, whereas such a pleocytosis is an unexpected feature of MS. In terms of CSF findings not regularly studied in clinical practice, Hirohata *et al.* [40] reported that the increment of IL-6 in the CSF may be related to disease activity; Aldinucci *et al.* [41] found matrix metalloproteinase-9 increased in NBS serum compared with MS serum and decreased in CSF; and Belghith *et al.* [42] found a significant increase in CSF IL-10 in NBS in comparison with MS.

As Behçet's syndrome myelopathy involving more than three segments is likely to be neuromyelitis optica spectrum disorders (NMOSD), we check the NMO-IgG and anti-myelin oligodendrocyte glycoprotein (MOG) Ab status even of patients fulfilling Behçet's syndrome criteria. However, thus far, we have not observed any NMO-IgG and MOG antibody-positive patients (article in preparation).

In contrast to the CSF findings of p-NBS, CSF is usually normal in CVST except for increased pressure [10].

## TREATMENT AND PROGNOSIS

Due to the lack of randomized control trials, treatment of NBS still remains empirical and based on clinical experience. Therefore, individualized treatments should be sought, and a multidisciplinary approach is required for decisions about long-term treatment as 6% of NBS patients do not fulfill the ISG criteria, and mortality and morbidity are very high. Given the high disability rates in young populations, aggressive treatments should be promptly started.

IVMP for 5–10 days, followed by a slow oral tapering, is the first choice in the relapse. We tend

to use IVMP for 7–10 days, followed by gradual oral tapering over 3–6 months, depending on the relapse severity. Given that the neurological involvement is associated with high morbidity and mortality rates, we start immunosuppressive treatment at the time of steroid initiation. As randomized controlled trials are lacking, the question arises as to which immunosuppressive should be started. Prognostic factors may help clinicians choose the optimum long-term therapy for the patient.

Although no outcome measures have been validated for determining the NBS prognosis, we use Kurtzke's Expanded Disability Status Scale (EDSS), which was originally devised for MS-associated disability, to assess disability in NBS [43]. We exclude the visual function score to avoid the contribution of uveitis to the visual score [7]. Severe relapses with high EDSSs, frequent relapses, extensive brain stem involvement in MRI, spinal cord involvement, early disease progression, and high CSF pleocytosis are the poor prognostic features for NBS. Initiation with severe disability, a primary or secondary progressive course, fever at onset, relapse during steroid tapering, meningeal signs, and bladder involvement may also be associated with poor outcome. Sex, accompanying systemic features, and age of onset do not change the prognosis of NBS [7,44]. In addition, HLA-B51 antigen was found to be independently associated with NBS relapse [38]. Considering all prognostic factors, especially a higher EDSS score at relapse and the severity of the systemic features, we choose either azathioprine or infliximab after the first NBS relapse.

Although that the efficacy of azathioprine in p-NBS presenting with severe attacks didn't have any evidence base background, it seemed to be the best option available by experience. Therefore, we were using azathioprine (2–2.5 mg/day) in both forms of NBS (p-NBS and CVST) due to the absence of sufficient evidence about biologic agents and the relative safety and tolerability of azathioprine compared with other agents, such as cyclophosphamide. However, after the experience of ours and others revealing that infliximab had prevented patients from having further attacks and even led to improvement in the neurological examination, we tended to start infliximab even after the first attack if patients had the aforementioned poor prognostic features [7,45]. In addition, our study also revealed that none of the Behçet's syndrome patients who were treated with infliximab for reasons other than neurological involvement developed NBS with this treatment.

As with other inflammatory disorders (i.e., neurosarcoidosis) in which infliximab is commonly used, the time of discontinuation of infliximab



therapy remains controversial. Although Sfrikakis *et al.* [46] reported that long-term remission was achieved after discontinuation of infliximab at the end of the second year, we have observed that some patients developed new p-NBS attacks after stopping treatment after 2 years or even with the extension of infliximab administration beyond 2 years. The most frequent adverse event in patients using infliximab is tuberculosis (TB). Therefore, TB screening should be performed before initiating infliximab, and isoniazid prophylaxis (300mg/day) should be prescribed for 6 or 9 months in patients diagnosed with latent TB.

In some case reports, adalimumab, tocilizumab, cyclophosphamide, methotrexate, and IFN $\alpha$  were also found to be preventive in the long term; however, these observational findings need to be confirmed in larger case series with proper methodology. Cyclosporine is an effective treatment in Behçet's syndrome patients who have eye involvement. However, the higher risk of developing CNS disease under cyclosporine treatment should be kept in mind, and this drug should be avoided in patients with established NBS [47].

Given that CVST carries a better prognosis than p-NBS, azathioprine may be considered a first-line treatment in CVST after high-dose IVMP administration in the acute phase [13]. The addition of anticoagulant medication to steroids is controversial, as Behçet's syndrome patients with CVST are more likely to have systemic large vessel disease, including pulmonary and peripheral aneurysms that carry a high risk of bleeding [48]. In addition to the complication rate with warfarin and that some studies regarding anticoagulation treatment in Behçet's syndrome being controversial in CVST, and that the recurrence rate of deep-vein thrombosis is more likely to decrease with an immunosuppressants, the use of immunosuppressants in the treatment of CVST should be the priority.

## CONCLUSION

Clinical findings and neuroimaging demonstrate that there are two major forms of NBS: first, CNS parenchymal involvement and second, CVST. MRI and MRV are the primary modalities for diagnosing NBS. Parenchymal lesions are generally located within the brainstem, occasionally extending to the diencephalon, and less often they are within the periventricular and subcortical white matter. When NBS is considered to have a poor prognosis, azathioprine and/or infliximab should be initiated immediately after the NBS diagnosis.

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There are no conflicts of interest.

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# Vasculitis and peripheral neuropathy

*Jonathan Graf and John Imboden*

## Purpose of review

Vasculitis of medium-sized and small vessels commonly affects peripheral nerves and can occur in context of a systemic vasculitis with multiorgan involvement or a nonsystemic vasculitis limited to the peripheral nervous system. This review summarizes the clinical and pathological features of systemic and nonsystemic vasculitis of the peripheral nervous system.

## Recent findings

Vasculitis of peripheral nerves is a diffuse process that affects the vasa nervorum along the entire length of affected nerves but appears to cause injury primarily in a zone in the proximal-middle of the nerve that is particularly susceptible to ischemic injury. Nerve biopsy can help establish the diagnosis of a systemic vasculitis, particularly when other organ involvement is not clinically apparent, and is required for diagnosis of nonsystemic vasculitic neuropathy. Observational studies suggest that nonsystemic vasculitic neuropathy responds to immunosuppressive therapy but conclusive data are lacking.

## Summary

The current review summarizes the clinical and pathological features of both systemic and nonsystemic vasculitis of the peripheral nervous system so that clinicians can better recognize, make a more timely diagnosis, and thus treat this condition more effectively in their patients.

## Keywords

antineutrophil cytoplasmic antibody vasculitis, nonsystemic vasculitic neuropathy, polyarteritis nodosa, vasculitic neuropathy

## INTRODUCTION

Ischemic neuropathy is a common complication of the primary systemic vasculitides, such as polyarteritis nodosa (PAN) and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, that affect medium-sized and small vessels [1–11]. The shared pathogenic mechanism is vasculitis of the vasa nervorum, the small arteries and vessels that supply blood to the peripheral nerves, leading to nerve ischemia [12]. The classic clinical presentation is an acute or subacute painful multifocal neuropathy that has a predilection for the lower extremities, affects two or more named nerves, and progresses in a step wise manner [1,4,8,10,11]. However, vasculitic neuropathy can manifest in a variety of ways, including asymmetric polyneuropathies and distal symmetric sensory neuropathies, and it also can be slowly progressive, particularly in cases of nonsystemic vasculitic neuropathy (NSVN), a form of vasculitis that clinically remains restricted to peripheral nerves [13–15,16].

## OVERVIEW

The current review will focus largely on the involvement of the peripheral nervous system by the

primary systemic vasculitides and by NSVN. Vasculitic neuropathy is prevalent among patients with PAN and ANCA-associated vasculitis, and peripheral nerves can be the first organ system affected by these disorders. The majority of patients (65–85%) with PAN have involvement of the peripheral nervous system [4,5,9,11,17,18]. Among the ANCA-associated vasculitides, peripheral neuropathy is more common in eosinophilic granulomatosis with polyangiitis (EGPA) (60–80%) than microscopic polyangiitis (MPA) (40–50%) or granulomatosis with polyangiitis (GPA) (20–25%) [1–5,7,11,19,20,21]. Vasculitic neuropathy also is prevalent in cryoglobulinemic vasculitis associated with chronic hepatitis C virus (HCV) (60%) [4]. In contrast, IgA vasculitis (Henoch Schonlein purpura) and

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## KEY POINTS

- Peripheral nerve vasculitis can occur in context of a systemic vasculitis with multiorgan involvement or a nonsystemic vasculitis limited to the peripheral nervous system.
- Vasculitis of peripheral nerves is a diffuse process that affects the vasa nervorum along the entire length of affected nerves but appears to cause injury primarily in a zone in the proximal-middle of the nerve that is particularly susceptible to ischemic injury.
- Clinically, vasculitic neuropathies cause pain, weakness, and sensory loss in the distribution of a named nerve followed by involvement of additional nerves in a stepwise fashion over weeks to months.
- Diagnosis of systemic vasculitis is often suggested by the presence of other organ-specific manifestations, but in cases in which vasculitis initially manifests in or is limited to peripheral nerves, biopsy is essential to establish a diagnosis.
- Prompt recognition of these clinical and pathological features is important to better recognize and more effectively treat patients with peripheral nerve vasculitis.

hypersensitivity vasculitis rarely affect the peripheral nervous system despite their predilection for small vessels, and vasculitic neuropathy also is rare in Behcet disease [4,5,22]. Among large vessel vasculitides, involvement of the peripheral nervous system occurs uncommonly (giant cell arteritis) or not at all (Takayasu arteritis) [22]. Vasculitic neuropathy can be a complication of systemic autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus, and primary Sjogren syndrome [1,4]. Diabetic radiculoplexus neuropathy, which is due to ischemic injury from a microvasculitis, has been the subject of several excellent recent reviews [15,16\*].

## HISTOPATHOLOGY

The vasculature of peripheral nerves – the vasa nervorum – consists of extrinsic nutrient arteries that course along the exterior of the nerve and feed a complex system of small arteries, arterioles, and capillaries within the nerve. The vessels of this intrinsic system run longitudinally within the epineurium (the connective tissue that surrounds the nerve and also occupies the interfascicular space), the perineurium (the connective tissue surrounding the nerve fascicles), and the endoneurium (the intrafascicular connective tissue) [23]. The epineurial, perineurial, and endoneurial vessels are highly anastomotic, creating a plexus-like system that

provides the nerve with a rich blood supply and protects it from ischemic injury and infarction (i.e., these intrinsic vessels are not typical ‘end arteries’) [23].

The histologic findings of affected vessels in nerve biopsies include changes typical of vasculitis in other tissues, including transmural inflammation with infiltrates that can include mononuclear cells, neutrophils, or both; leukocytoclasia; focal fibrinoid necrosis; disruption of the internal elastic lamina of arteries and larger arterioles; perivascular hemorrhage; and luminal occlusion and recanalization [1,4,8,15]. Vasculitis in nerve biopsies is often segmental, with involved segments as short as 50  $\mu\text{m}$ , and only a small percentage of vessels may be involved in a biopsy specimen [12]. PAN, the ANCA vasculitides, and HCV-associated cryoglobulinemic vasculitis primarily affect large arterioles (75–300  $\mu\text{m}$  in diameter) in the epineurium and perineurium [1,4,8,12]. NSVN often involves the endoneurial small vessels but also can affect epineurial and perineurial arterioles [15]. In all forms of vasculitic neuropathy, true nerve infarction is an uncommon finding on biopsy. Usually, however, there is histologic evidence of ischemic neuropathy in the form of axonal degeneration that is predominantly within the central regions of the fascicles and that is asymmetrically distributed between fascicles [1,4,8,12].

A detailed autopsy study of nerve pathology in MPA provides interesting insights into the mechanism of nerve injury in vasculitic neuropathy. Morozumi *et al.* [12] dissected the median nerves (from axilla to wrist) and the sciatic/tibial nerves (from gluteal fold to ankle) from eight individuals with MPA who had multifocal neuropathies documented prior to death. Each nerve was cut into consecutive 4 cm segments that were then sectioned and analyzed histologically for the frequency of vasculitis (defined as the proportion of vessels with vasculitis in each segment) and the severity of nerve fiber loss. Vasculitis was found in the epineurium of the median and sciatic/tibial nerves of all patients [12]. The vasculitis was present diffusely in the vasa nervorum along the course of each nerve, and the proportion of affected vessels (generally in the range of 1–8%) was uniform from proximal to distal segments [12]. In contrast to this uniform distribution of vasculitis along the nerve trunks, loss of nerve fibers was not uniform, with little or no fiber damage in the proximal segments of the nerves followed a sharp increase in fiber loss from the middle to the distal segments [12]. Moreover, central fascicular degeneration – a pattern indicative of nerve ischemia – was found only in the proximal-middle segments of the nerves [12]. Therefore, MPA produces a diffuse vasculitis of the vasa nervorum along the entire course of affected nerves but causes

actual nerve damage primarily in a zone that is located in the proximal-middle portions of peripheral nerves and evidently is predisposed to ischemia. Significantly, analysis of peripheral nerve pathology in an autopsy case of arteritis associated with RA led to a similar conclusion, suggesting that this may be a general mechanism of nerve injury in systemic vasculitis [24].

### CLINICAL PRESENTATIONS OF VASCULITIC NEUROPATHY

The peripheral nervous system can be the first organ system involved in systemic vasculitis, particularly in cases of PAN, EGPA, and MPA. The classic presentation is the acute onset of pain, weakness, and sensory loss in the distribution of a named nerve (mononeuritis) followed by involvement of additional nerves in a stepwise fashion over weeks to months (multifocal neuropathy or mononeuritis multiplex) [1–10]. The pain can be severe and is more often described as throbbing and aching than as burning. Vasculitic neuropathies tend to be lower extremity predominant and to cause distal symptoms and signs [1–10]. The most frequently involved peripheral nerve is the deep peroneal nerve leading to foot drop [1–10]. In the upper extremities, the ulnar nerve is more often affected than the radial and median nerves. Asymmetry is a hallmark of multifocal neuropathy. Occasionally, however, rapid progression of multifocal neuropathy or nearly simultaneous involvement of multiple nerves can lead to a generalized sensorimotor neuropathy that requires careful examination to reveal its asymmetry [1–10]. Progression to involve contiguous distal nerves in a single extremity can mimic a plexopathy or polyradiculopathy [23]. Uncommon peripheral nervous system manifestations of vasculitic neuropathy include stocking-glove sensory neuropathies and pure motor neuropathies [1–10,23]. In cases of systemic vasculitis, involvement of the peripheral nervous system almost always occurs in the context of antecedent constitutional symptoms of weight loss, fatigue, malaise, and low-grade fever for weeks to months [5]. In NSVN, however, constitutional symptoms are usually absent, and the disease course is more indolent, adding to diagnostic difficulty [15,16].

### DIAGNOSIS OF VASCULITIC NEUROPATHY

The evaluation of a patient with possible vasculitis of the peripheral nervous system begins with a good history, thorough physical exam, and diagnostic evaluation that focus on signs and symptoms

indicative of an underlying systemic vasculitis. The demonstration of vasculitis in other tissues (e.g., evidence of arteritis on visceral angiography in cases of PAN) is often sufficient to establish the diagnosis of vasculitic neuropathy [5]. However, when neuropathy is the initial manifestation of the vasculitis and there is no definitive evidence of vasculitis elsewhere, then nerve biopsy is needed for diagnosis. Electrodiagnostic studies can guide the selection of a nerve for biopsy and also can point to the presence of a multifocal neuropathy when the nature of the neuropathy is uncertain.

When the diagnosis is not clear, it is common practice to biopsy either the sural or superficial peroneal nerves to establish the presence of vasculitic neuropathy [1,10,11]. Absent a true gold standard, the actual sensitivity of nerve biopsy is not certain, but estimates range from 45 to 70% [1,10,11,25,26]. Therefore, the absence of vasculitis on a nerve biopsy should not be the sole basis to exclude vasculitic neuropathy. Concomitantly sampling of tissue from the neighboring gastrocnemius (with sural nerve biopsies) or peroneus brevis muscles (superficial peroneal nerve biopsies) may enhance the sensitivity of biopsies by up to 15% [1,10,11,25,26]. The examination of nerve biopsies for evidence of vasculitis is not always straightforward; the Peripheral Nerve Society has published diagnostic criteria for pathologically definite, probable, or possible vasculitic neuropathy [15].

### POLYARTERITIS NODOSA

PAN is a systemic necrotizing arteritis that involves medium-sized muscular and occasionally smaller arteries but rarely arterioles or capillaries. It can be associated with hepatitis B infection, but most cases are idiopathic. Patients usually experience constitutional symptoms that include fever, fatigue, anorexia, and weight loss prior to the development of target damage from arteritis [17,18]. Apart from the lung, PAN can affect almost any visceral organ, but the extent of organ involvement varies widely among individual patients, ranging from single-organ to multiorgan disease. PAN targets the peripheral nerves more often than other organ systems [9,11]. Involvement of the peripheral nervous system by PAN typically presents as a mononeuritis or multifocal neuropathy (mononeuritis multiplex) [5,9]. The rapid involvement of multiple nerves can lead to the appearance of a generalized polyneuropathy [5]. Occasionally PAN causes distal sensory polyneuropathies, polyradiculopathies, plexopathies, or pure motor neuropathies [5].

Greater than 90% of patients with PAN have laboratory evidence of systemic inflammation in the



form of elevations in the erythrocyte sedimentation rate, serum C-reactive protein, or platelet count [17]. Serum complement levels can be either normal or low [5]. Patients with hepatitis B-associated PAN always have detectable serum levels of hepatitis B surface antigen [5]. When there is involvement of abdominal viscera, conventional angiography can detect saccular or fusiform microaneurysms and stenosis in the medium sized vessels of the renal, hepatic, and mesenteric circulations that, in the proper clinical context, are diagnostic of PAN [18]. These vascular abnormalities usually are below the resolution of computed tomographic and magnetic resonance angiography.

### **ANTINEUTROPHIL CYTOPLASMIC ANTIBODY VASCULITIS**

The ANCA-associated vasculitides can affect medium-sized arteries, arterioles, capillaries, and venules in a range of target tissues [7,19<sup>■</sup>,21,27,28]. Antecedent or concomitant systemic symptoms of fever, night sweats, fatigue, and weight loss are common [2,19<sup>■</sup>,20,29]. Patients with EGPA often have a long history of asthma, and, in some cases, may have started a leukotriene receptor antagonist while tapering systemic glucocorticoids [19<sup>■</sup>,27]. GPA can manifest initially as prolonged upper airway involvement in the form of chronic sinus inflammation before disseminating [29]. Scleritis, serous otitis media, and cavitary pulmonary nodules are common findings in GPA [29]. Both GPA and MPA confer a high risk for diffuse alveolar hemorrhage and rapidly progressive glomerulonephritis [29]. Fleeting pulmonary infiltrates are characteristic of lung involvement in EGPA [27]. Peripheral nerve involvement is prevalent in the ANCA-associated vasculitis, ranging from 80% in EGPA to 25% of patients with GPA [5]. Vasculitic neuropathy is a common presenting manifestation of EGPA [19<sup>■</sup>,27].

All patients with suspected GPA, MPA, or EGPA should be tested for ANCA using two complementary techniques: by indirect immunofluorescence on fixed neutrophils and by immunoassay for antibodies to the proteinase-3 (PR3) and myeloperoxidase (MPO) [30]. Antibodies to PR3 are characteristic of GPA and produce a cytoplasmic pattern (cANCA) on immunofluorescence, whereas antibodies to MPO are characteristic of MPA and EGPA and produce a perinuclear pattern (pANCA) on immunofluorescence [30]. ANCA immunofluorescence testing also can detect antibodies to other neutrophil components, such as elastase, that produce an 'atypical pANCA' pattern, are characteristic of drug-induced ANCA vasculitis, and also can be seen in other autoimmune diseases such as RA and inflammatory

bowel disease [31–33]. ANCA testing is positive in 78–96% of patients with MPA or with disseminated GPA but has lower sensitivity (less than 50%) for EGPA [30].

### **HEPATITIS C VIRUS-ASSOCIATED CRYOGLOBULINEMIC VASCULITIS**

Cryoglobulinemic vasculitis associated with chronic HCV infection is an immune-complex mediated inflammatory arteritis of small and medium-sized vessels [34–36]. The pattern of the cryoglobulin is usually type II (cryoprecipitable immune complexes consisting of a monoclonal rheumatoid factor, usually IgM kappa, and polyclonal IgG) and less often type III (cryoprecipitable immune complexes of polyclonal rheumatoid factor and polyclonal IgG) [34–36]. Types II and III cryoglobulins usually precipitate only at nonphysiological temperatures (e.g., 4 °C) and cause immune complex-mediated vascular damage rather than vascular occlusion by cryoprecipitated protein as occurs with type I cryoglobulins (cryoprecipitable monoclonal gammopathies) [34,35]. The corresponding clinical picture therefore reflects inflammation of small arteries, arterioles, and capillary beds with a predilection for the skin, vasa nervorum, and kidney.

Peripheral nerve involvement is common in HCV-associated cryoglobulinemic vasculitis (up to 68%) and manifests as neuropathic pain and a distal sensory neuropathy [35,37]. Others can present with a mixed polyneuropathy or multifocal neuropathy with sensory-motor deficits similar to those seen in PAN and ANCA-associated vasculitis [36]. Most patients have palpable purpura [34–36]. Serum levels of complement, especially C4, are often very low, and all patients have rheumatoid factor activity when serum samples are processed at 37 °C to avoid false negative results from loss of the rheumatoid factor through cryoprecipitation [34,35].

### **NONSYSTEMIC VASCULITIC NEUROPATHY**

NSVN is a vasculitis of the vasa nervorum that behaves clinically like an organ-specific vasculitis limited to the peripheral nervous system [15,16<sup>■</sup>,38]. It accounts for approximately 25% of all vasculitic neuropathies, which is comparable with the combined contributions of MPA and PAN [15,16<sup>■</sup>,38]. The mean age of onset for NSVN is 60 years, and there appears to be a slight female predominance [15,16<sup>■</sup>,38]. Constitutional symptoms are usually absent. Only a minority of patients (30%) have weight loss, fatigue, myalgias, and arthralgias, and fewer than 15% have low grade fever [15,16<sup>■</sup>,38,39]. The peripheral nerve manifestations

of NSVN are similar to those of systemic vasculitis but have a more indolent, slowly progressive course with a longer delay (typically 6 months or more) between symptom onset and initial presentation [15,16<sup>■</sup>,38]. Extremity pain is a common symptom, and many patients present with some degree of gait impairment [15,16<sup>■</sup>,38,39]. Most patients have a multifocal neuropathy or an asymmetric polyneuropathy, but 25% have a distal symmetrical polyneuropathy and 15% have a sensory neuropathy [38,39]. Laboratory studies are unrevealing; the erythrocyte sedimentation rate and the serum C-reactive protein level are usually within normal limits [15,16<sup>■</sup>,38,39]. The diagnosis of NSVN requires a nerve biopsy demonstrating definite or probable vasculitic neuropathy by established criteria as well as exclusion of alternative explanations – that is, the absence of clinical, imaging, laboratory, or biopsy evidence of systemic vasculitis, autoimmune conditions associated with vasculitis, or specific infections associated with vasculitis [15]. Despite these exclusions, approximately 10% of individuals with apparent NSVN will progress to a systemic vasculitis, raising the possibility that NSVN is part of the spectrum of an MPA-like systemic vasculitis [16<sup>■</sup>,38]. For most patients, however, NSVN behaves like a separate disease: a nonfatal condition that, unlike MPA, does not disseminate to other organ systems [16<sup>■</sup>,38]. Indeed, there are documented cases of patients with untreated NSVN who survived for decades without spread of the vasculitis to other systems [16<sup>■</sup>,38].

The treatment recommendations in the Peripheral Nerve Society Guidelines on NSVN are level U Good Practice Points (insufficient data to judge efficacy) and are based on observational studies of NSVN and extrapolation from studies of systemic vasculitis [15]. The recommendations endorse treatment of all patients with progressive NSVN. First-line is monotherapy with glucocorticoids (e.g., prednisone 1 mg/kg/day tapered over 6 months to 10 mg daily, followed by maintenance therapy with 5–7.5 mg daily for an additional 6–18 months) [15]. Some centers initiate treatment with intravenous pulses with methylprednisolone [15,39]. Combination therapy either with cyclophosphamide, methotrexate, or azathioprine is recommended for those with rapidly progressive NSVN or for patients who progress despite glucocorticoid monotherapy [15]. In one series of 46 patients, most of whom were treated with a form of combination therapy, NSVN had a monophasic disease course without relapses on immunosuppressant treatment, and the 21 patients who had discontinued treatment (after a median of 3 years) remained stable without recurrences [39].

## RHEUMATOID ARTHRITIS

Necrotizing arteritis is a serious, but now very rare, complication of long-standing, erosive, seropositive RA [40–43]. The arteritis shares clinical and histological features with PAN and can affect the vasa nervorum, leading to mononeuritis, multifocal neuropathy, and other manifestations of ischemic neuropathy [24,40–43]. Virtually all patients have rheumatoid factor, and 50% have a positive test for ANCA, usually in an atypical perinuclear pattern [43,44]. This necrotizing arteritis should not be confused with the still common and more benign form of rheumatoid vasculitis that manifests as nail-fold infarcts and does not affect the peripheral nervous system or other organ systems.

## SUMMARY AND CONCLUSION

Vasculitis of medium-sized and small vessels commonly affects peripheral nerves and can occur in context of a systemic vasculitis with multiorgan involvement or a nonsystemic vasculitis limited to the peripheral nervous system. Typically, vasculitic neuropathies tend to be lower extremity predominant and to cause distal symptoms and signs that include pain, weakness, and sensory loss in the distribution of a named nerve followed by involvement of additional nerves in a stepwise fashion over weeks to months. Diagnostic evaluation should focus on signs and symptoms indicative of an underlying systemic vasculitis, although when neuropathy is the initial manifestation of the vasculitis and/or there is no definitive evidence of vasculitis elsewhere, then nerve biopsy is needed for diagnosis. Prompt recognition of these clinical and pathological features is important to better recognize and more effectively treat patients with peripheral nerve vasculitis.

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## Conflicts of interest

*There are no conflicts of interest.*

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# An update on the nomenclature for cutaneous vasculitis

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## Purpose of review

Cutaneous vasculitis reflects a spectrum ranging from skin limited to severe systemic forms. To date, there is still no generally acknowledged nomenclature for cutaneous vasculitis. This review aims to summarize the recent advances in the nomenclature of cutaneous vasculitis.

## Recent findings

The most widely adopted vasculitis classification system is the one of 2012 Revised Chapel Hill Consensus Conference (CHCC) which represent not such a classification but a nomenclature system that name vasculitis on the basis of the size of the vessel affected. The CHCC 2012 did not deal with the special features of cutaneous vasculitis and did not explicitly discuss the presence of skin-limited or skin-dominant forms of vasculitis. Therefore, a consensus group was formed to propose an Addendum to CHCC 2012, focusing on cutaneous vasculitis. The Addendum better clarify the main aspects of some single-organ vasculitis, including IgM/IgG vasculitis, nodular vasculitis, erythema elevatum et diutinum and recurrent macular vasculitis in hypergammaglobulinemia. Moreover, it differentiated normocomplementemic from hypocomplementemic urticarial vasculitis. Finally, it recognized cutaneous polyarteritis nodosa as a distinct subtype of polyarteritis nodosa.

## Summary

Classification criteria are useful tools to standardize names and definitions for cutaneous vasculitis; however, they do not represent diagnostic criteria. Collaborative efforts are still needed to get a shared classification and valid diagnostic criteria for cutaneous vasculitis.

## Keywords

classification, cutaneous vasculitis, polyarteritis nodosa, single-organ vasculitis, urticarial vasculitis

## INTRODUCTION

Vasculitis are inflammatory processes that can affect the small, medium or large-sized vessels [1]. Cutaneous vasculitis usually affect the small or medium sized vessels of the skin and subcutaneous tissue and clinically comprise a wide spectrum of diseases, ranging from skin limited lesions to systemic involvement [2].

The classification of cutaneous vasculitis and, generally, of vasculitis, has been a confusing and debate provoking topic over the last half century. Despite numerous attempts, the development of a clinically relevant and easy-to use classification system that incorporates clinical features, vessel site, histopathological and laboratory findings, and possible etiologic factors, is a goal that has not yet been fully achieved [3].

The most significant contribution derived from the consensus-based criteria specifically obtained by the combination of judgments from groups of

experts, after accurate literature reviews and developed using consensus techniques. However, few dermatologists has been involved in study aimed to better classify the cutaneous manifestation of vasculitis [4–8,9].

Herein, we review the current state of knowledge in the fields of nomenclature of cutaneous vasculitis and definition of the different clinical patterns of cutaneous vasculitis, underlining the role of dermatologist in cutaneous vasculitis diagnosis.

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## KEY POINTS

- Cutaneous vasculitis encompass a heterogeneous group of disease with different clinical, histopathological and immunofluorescence findings with various pathogenic mechanisms and clinical manifestations.
- To conclude that a patient fulfills the definition of cutaneous vasculitis, there must be no detectable involvement of systemic organs by vasculitis.
- The classification of cutaneous vasculitis has been a confusing and debate provoking topic over the last half century. The development of a classification system that incorporates clinical features, vessel site, histopathological and laboratory findings, and possible etiologic factors, is a goal that has not yet been fully achieved.
- The dermatological Addendum to the CHCC 2012 provided standardized names and definitions for many cutaneous vasculitis, representing an important basis for obtaining appropriate diagnostic criteria for cutaneous vasculitis.

## CLASSIFICATION OF VASCULITIS

In 1990, the American College of Rheumatology [4] proposed criteria for the classification of primary vasculitis based on clinical features, including seven types of vasculitis: polyarteritis nodosa (PAN), Churg–Strauss syndrome, Wegener’s granulomatosis, hypersensitivity vasculitis, Henoch–Schönlein purpura (HSP), giant cell arteritis (GCA) and Takayasu arteritis.

Although the criteria did not include microscopic polyangiitis or antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, the importance of these indications in accurate diagnosis has subsequently been recognized.

In 1994, the Chapel Hill Consensus Conference (CHCC) [5] introduced names and definitions for the major types of vasculitis using clinical and histological criteria, according to vessel size: GCA and Takayasu arteritis as large vessel vasculitis; PAN and Kawasaki disease as medium-sized vessel vasculitis; Wegener’s granulomatosis, Churg–Strauss syndrome, microscopic polyangiitis, HSP, essential cryoglobulinemic vasculitis and cutaneous leukocytoclastic angiitis (CLA) as small vessel vasculitis (SVV). These were a set of definitions, rather than classification or diagnostic criteria, even if they have been often mistakenly used as such.

As only 10 diseases of primary vasculitis were included, many diseases of vasculitis which were encountered were not actually described. In addition, the handling of eponymous disease names was controversial.

In 2006, European League against Rheumatism and Pediatric Rheumatology European Society produced consensus criteria for the classification of childhood vasculitis [6]: these criteria have underlined clinical differences of some forms of vasculitis in children in comparison with adults, such as in PAN or granulomatosis with polyangiitis (GPA); moreover, these criteria underlined that there are forms of vasculitis exclusively of children, such as Kawasaki disease, instead of GCA which is exclusively of adults.

In 2012, a new international CHCC, called CHCC 2012, modified the previous CHCC 1994 [7].

The major interests of this update was to remove definitively some traditional eponymous terms (e.g. HSP, Wegener granulomatosis) (Table 1). Moreover, the disease names were changed to objective names based on the causes or pathological conditions of the diseases.

As regards vasculitis affecting small vessels with a predominant skin involvement, two groups were included: SVV and single-organ vasculitis (SOV).

SVV was defined as ‘vasculitis predominantly affecting small vessels, defined as small intraparenchymal arteries, arterioles, capillaries and venules. Medium arteries and veins may be affected’. SVV were divided for the first time in vasculitis with paucity of vessel wall immunoglobulin deposits (ANCA-associated vasculitis) and vasculitis with predominant vessel-wall I deposits (immune complex vasculitis). This last group included types which did not involve the skin (i.e. antglomerular basement membrane disease) and many types with predominant skin involvement (i.e. cryoglobulinemia, IgA vasculitis).

SOV was defined as ‘vasculitis in arteries or veins of any size in a single organ that has no features that indicate that it is a limited expression of a systemic vasculitis’. It included CLA and cutaneous arteritis. In this category, the involved organ and the type of the affected blood vessels were included in the disease names. However, when some cases who were diagnosed with SOV subsequently develop a systemic vasculitis, the disease should be redefined into another category.

In CHCC 2012, the new group of ‘variable vessel vasculitis’, including Behçet’s disease and Cogan’s syndrome, was also identified.

Moreover, the distinction between primary and secondary forms was included, introducing for the first time etiologic criteria, with the term ‘primary’ standing for ‘non infectious vasculitides not caused by direct vessel walls invasion by pathogens’, while ‘secondary’ for vasculitis ‘caused by direct invasion of vessels wall and subsequent proliferation of pathogens with final inflammation’.

**Table 1.** 2012 International Chapel Hill Consensus Conference Nomenclature of Vasculitides

Large vessel vasculitis
Takayasu arteritis
Giant cell arteritis
Medium vessel vasculitis
Polyarteritis nodosa
Kawasaki disease
SVV
ANCA-associated vasculitis
Microscopic polyangiitis
Granulomatosis with polyangiitis (Wegener's)
Eosinophilic granulomatosis with polyangiitis (Churg–Strauss)
Immune complex SVV
Antiglomerular basement membrane disease
CV
IgA vasculitis (Henoch-Schönlein)
Hypocomplementemic urticarial vasculitis (anti-C1q vasculitis)
Variable vessel vasculitis
Behçet's disease
Cogan's syndrome
Single-organ vasculitis
Cutaneous leukocytoclastic angiitis
Cutaneous arteritis
Primary central nervous system vasculitis
Isolated aortitis
Others
Vasculitis associated with systemic disease
Lupus vasculitis
Rheumatoid vasculitis
Sarcoid vasculitis
Others
Vasculitis associated with probable cause
Hepatitis C virus–associated CV
Hepatitis B virus–associated vasculitis
Syphilis-associated aortitis
Drug-associated immune complex vasculitis
Drug-associated ANCA-associated vasculitis
Cancer-associated vasculitis
Others

ANCA, antineutrophil cytoplasmic antibody; CV, cryoglobulinemic vasculitis; SVV, small vessel vasculitis.  
Reproduced with permission [7].

Even if the 2012 revised international CHCC included SVV and SOV, it did not deal with the special features of cutaneous vasculitis and did not explicitly discuss presence of skin-limited or skin-dominant forms of vasculitis. Therefore, a consensus group composed mainly by dermatologists

was formed to propose an Addendum to CHCC 2012 that focuses on cutaneous vasculitis [9\*] (Table 2).

Accordingly, cutaneous vasculitis were divided as follows: a cutaneous component of systemic vasculitis; a skin-limited vasculitis; a skin-dominant expression or variant of a systemic vasculitis; a SOV that differs with regard to clinical, laboratory, and pathologic features from recognized systemic vasculitis.

In comparison with the CHCC 2012, the Addendum underlined some types of cutaneous vasculitis not included in the CHCC 2012.

We discuss the most relevant details added to improve the knowledges on cutaneous vasculitis.

## SMALL VESSEL VASCULITIS

The CHCC 2012 included the hypocomplementemic urticarial vasculitis (HUV) (anti-C1q vasculitis) in the group of 'immune complex SVV'. HUV was defined as 'a form of vasculitis accompanied by urticarial and hypocomplementemia affecting small vessels and associated anti-C1q antibodies'. Clinically, it is characterized by urticarial lesions, without legs predilections, which may have petechiae and postinflammatory hyperpigmentation. Glomerulonephritis, arthritis, obstructive pulmonary disease and ocular inflammation are also associated.

The Addendum distinguished HUV from the normocomplementemic urticarial vasculitis, a skin-limited vasculitis, not accompanied by systemic involvement. This form of vasculitis is associated with normocomplementemia and absence of anti-C1q antibodies.

## MEDIUM VESSELS VASCULITIS

Among medium vessels vasculitis, the Addendum focused on the so-called cutaneous PAN (cPAN) (otherwise known as cutaneous arteritis), a skin limited form of PAN, which nosographic autonomy has been longer under discussion. The CHCC 2012 included cutaneous arteritis in the SOV subgroup, underlined the potential evolution of this skin vasculitis into a systemic one, while the Addendum separated the two forms.

In fact, PAN was defined as 'a necrotizing arteritis of medium or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries or venules, not associated with ANCA', while cPAN is recognized as 'a vasculitis that affects the small arteries and arterioles in the panniculus and dermal-subcutaneous junction, without veins involvement'. Most of cPAN are confined to the legs, with skin lesions represented by livedo reticularis, macules or subcutaneous nodules, associated or not with

**Table 2.** Dermatological Addendum to the 2012 International Chapel Hill Consensus Conference Nomenclature of Vasculitides

CHCC 2012 Name	Systemic vasculitis skin component	Skin-restricted or skin-dominant variant
Large-vessel vasculitis		
Takayasu arteritis	No	No
Giant cell arteritis	Rare	No
Medium-vessel vasculitis		
Polyarteritis nodosa	Yes	Yes
Kawasaki disease	No	No
Small-vessel vasculitis		
Microscopic polyangiitis	Yes	Yes
Granulomatosis with polyangiitis	Yes	Yes
Eosinophilic granulomatosis with polyangiitis	Yes	Yes
Antiglomerular basement membrane disease	No	No
Cryoglobulinemic vasculitis	Yes	Yes
IgA vasculitis (Henoch-Schönlein)	Yes	Yes
Hypocomplementemic urticarial vasculitis (anti-C1q vasculitis)	Yes	Yes
Normocomplementemic urticarial vasculitis	Rare	Yes
Variable-vessel vasculitis		
Behçet's disease	Yes	Yes
Cogan's syndrome	Rare	No
Vasculitis associated with systemic disease		
For example, LE, rheumatoid arthritis, sarcoidosis, etc.	Yes	Yes
Vasculitis associated with probable cause		
For example, drugs, infections, sepsis, autoimmune diseases, etc.	Yes	Yes
Cutaneous single-organ vasculitis		
IgM/IgG vasculitis	No	Yes
Nodular vasculitis (erythema induratum of Bazin)	No	Yes
Erythema elevatum et diutinum	No	Yes
Hypergammaglobulinemic macular vasculitis	No	Yes

CHCC, Chapel Hill Consensus Conference; LE, lupus erythematosus.  
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ulceration. The macular form has given rise to new terms, such as macular arteritis, that have not been sufficiently defined to clearly distinguish them from PAN.

## SINGLE-ORGAN VASCULITIS

Among this subgroup, four subtypes of cutaneous vasculitis were included in the Addendum to the CHCC 2012: IgM/IgG vasculitis, nodular vasculitis (erythema induratum of Bazin), erythema elevatum et diutinum (EED) and recurrent macular vasculitis in hypergammaglobulinemia (hypergammaglobulinemic purpura of Waldenström).

### IgM/IgG vasculitis

IgM/IgG vasculitis is a vasculitis characterized by IgM and/or IgG dominant or codominant

immune deposits, affecting small vessels (predominantly postcapillary venules) in the skin. Cutaneous IgM-dominant or IgG-dominant/codominant is a leukocytoclastic vasculitis of mostly postcapillary venules, without systemic involvement (monoclonal gammopathy, systemic lupus erythematosus, rheumatoid arthritis or dermatomyositis) or cryoglobulinemia. Clinically, it is not distinguishable from IgA vasculitis; direct immunofluorescence (DIF) is necessary to make a differential diagnosis.

The evaluation of immunofluorescence pattern in the context of cutaneous vasculitis is an important challenge to characterize the diagnostic sensitivity and specificity of this technique and many studies have been carrying out to better define the characteristic of IgM/IgG vasculitis in comparison with IgA vasculitis [10<sup>■</sup>,11,12].

## Nodular vasculitis (erythema induratum of Bazin)

Nodular vasculitis is a lobular panniculitis with varying combinations of vasculitis of venules in fat lobules and/or veins or arteries of the connective tissue septa; sometimes accompanied by coagulative and caseous necrosis; and lymphocytic, neutrophilic or granulomatous inflammation.

It is called 'erythema induratum of Bazin' in presence of tuberculosis: it represents one type of hypersensitivity reaction to *Mycobacterium tuberculosis* antigens (tuberculids) [13].

The clinical picture consists of recurrent violaceous nodules with a tendency to ulceration.

Histologically, lobular panniculitis distinguishes it from cutaneous PAN and the primary localization of vasculitis in the panniculus from GPA and eosinophilic granulomatosis with polyangiitis (Churg–Strauss) (eosinophilic GPA).

## Erythema elevatum et diutinum

EED is a neutrophilic dermatosis and chronic fibrosing leukocytoclastic vasculitis, mostly of postcapillary venules, often with vascular immunoglobulin deposits. Inflammation may include eosinophils and plasma cells. Fibrosis is angiocentric and storiform.

EED is a rare disorder characterized clinically by red-brown yellowish papules, plaques or nodules distributed symmetrically at extensor surfaces of the hands and knees. Lesions are initially edematous, then become firm due to fibrosis. They appear firmer and higher at evening and after a cold temperature exposure. The disease has a chronic course with periods of waxing and waning [14].

Sometimes EED has been associated with monoclonal gammopathy, autoimmune diseases or infections.

## Recurrent macular vasculitis in hypergammaglobulinemia (hypergammaglobulinemic purpura of Waldenström)

Recurrent macular vasculitis in hypergammaglobulinemia is a cutaneous SVV with recurring macules and purpura associated with hypergammaglobulinemia (usually polyclonal, but sometimes also monoclonal) and vascular immunoglobulin deposits.

To conclude, the Addendum to CHCC 2012 improved the knowledges on cutaneous vasculitis; even if it represents a useful tool to standardize names and definitions for cutaneous vasculitis, it does not include diagnostic criteria.

Currently no satisfactory diagnostic and prognostic criteria to enable a rapid diagnosis of

cutaneous vasculitis are available. Many efforts are needed to develop guidelines useful for practising clinicians.

Moreover, some unsolved problems are still present, such as the prognostic value of perivascular depositions of immunoglobulin.

## CUTANEOUS VASCULITIS: THE DERMATOLOGICAL PERSPECTIVE

In clinical practice, the goal for a working definition of vasculitis affecting the skin should incorporate clinical relevance to management and address clinical, histologic and laboratory features, and underlying causes [15].

To date, the Kawakami algorithm [16] is still an important tool in clinical practice to diagnose primary cutaneous vasculitis, but with the new knowledges on the pathophysiology of vasculitis and newer tests in clinical use, some authors tried to introduce new diagnostic algorithm. To date, no shared and universal accepted criteria have been obtained [17].

Due to the variability of clinical symptoms and manifestations, management of vasculitis represents a special challenge requiring interdisciplinary collaboration. Dermatologists should be aware of their important role especially for making an early diagnosis, since many times the initial presentation of a vasculitis is represented by skin lesions [18]. To differentiate cutaneous vasculitis from systemic vasculitis with skin manifestations, there must be no detectable involvement of systemic organs by vasculitis [9].

A vasculitis must be suspected in the presence of skin lesions consistent with cutaneous vasculitis. The most common cutaneous lesions are represented by palpable purpura or maculopapular rash, usually localized on the legs and buttocks, due to the hydrostatic pressure and microtrauma. They are usually bilateral and symmetrical. Other common skin lesions are represented by urticarial, nonpalpable macules and patches, nodules, vesicles, bullous lesions and rarely splinter hemorrhages and ulcerations. These lesions may sometimes be combined [19].

A suspected cutaneous vasculitis must be confirmed by skin biopsy. It should be done, optimally within 24–48 h after vasculitic lesions appear. If the biopsy is poorly timed, the pathological features of vasculitis may be absent, a fact that must be considered when interpreting a negative biopsy from a patient whose clinical findings suggest vasculitis. Diagnostic yield depends on the depth of the biopsy. Generally, deep punch biopsy or excision biopsy into the subcutis is preferred; these biopsies can sample small-sized and medium-sized vessels. Shave biopsy is usually inadequate [10,20].



The most common histopathological features of cutaneous vasculitis, the so-called leukocytoclastic vasculitis [4], is characterized by perivascular infiltrate of neutrophils, nuclear dust and vessel wall, fibrinoid degeneration with endothelial edema. Rarely, eosinophils can be detected in the infiltrate. Lately, neutrophils are replaced by lympho-monocytes [10<sup>2</sup>,20,21].

A concomitant biopsy for DIF evaluation contributes to accurate diagnosis by distinguishing IgA-associated vasculitis (HSP) from IgG-/IgM-associated vasculitis, which has prognostic significance. Lesions less than 48 h old yield the most frequently positive results while older lesions may have negative DIF because of the rapid degradation of immune deposits. A diagnosis of vasculitis should not be made solely on the presence of positive DIF findings, nor should the diagnosis be excluded with a negative DIF test. The findings of DIF should be interpreted along with clinical, histologic, and other laboratory findings [18–21].

In addition to classifying a patient with vasculitis, the clinician must also attempt to identify any of the known causes for vasculitis. Based on current data, cutaneous vasculitis is associated with the following conditions: idiopathic (45–55%), infection (15–20%), inflammatory disease (15–20%), drug intake (10–15%) and malignancy (5%) [22<sup>2</sup>,23,24<sup>2</sup>].

Finally, all patients with a vasculitis, should undergo laboratory evaluation to exclude systemic involvement [2]. Goesser *et al.* [17] recently proposed an algorithm aimed to better diagnose a cutaneous vasculitis. Among laboratory examinations, complete blood count, creatinine, sedimentation rate, liver function tests and urinalysis were recommended for all the cases of suspected cutaneous vasculitis while further examinations were suggested only in case of chronic or recurrent disease with unclear underlined cause and in case of suspected systemic involvement. These examinations included: hepatitis B and C serology, Streptococcal antibodies, HIV antibody, antinuclear antibody, extractable nuclear antigen, rheumatoid factor, complement (C) levels (C3, C4, total), cryoglobulins, serum monoclonal protein study (protein electrophoresis and immunofixation), peripheral blood smear, ANCA, chest radiography and stool guaiac. Other tests based on concern for specific organ involvement, malignancy, were also reported.

## CONCLUSION

In this review, we discussed the most relevant details of newly definition criteria for cutaneous vasculitis, after accurate literature review. The Addendum to CHCC 2012 really improved the knowledges on

cutaneous vasculitis; however, many efforts are still needed to develop guidelines on diagnostic and prognostic criteria for cutaneous vasculitis.

Moreover, some unsolved problems are still present, such as the prognostic value of perivascular depositions of immunoglobulin.

We underlined the importance of dermatologist in cutaneous vasculitis diagnosis, since skin lesions are one of the main aspect both of cutaneous vasculitis and systemic vasculitis. To date, not accepted diagnostic criteria of cutaneous vasculitis have been developed.

Further research should address to provide a consensus about the nomenclature, the classification and the diagnostic criteria of cutaneous vasculitis, to improve the management of these patients.

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# Sex bias in autoimmunity

Allison C. Bill<sup>a</sup>, J. Michelle Kahlenberg<sup>b</sup>, and Johann E. Gudjonsson<sup>a</sup>

## Purpose of review

To give an overview of recently published articles addressing the mechanisms underlying sex bias in autoimmune disease.

## Recent findings

Recent studies investigating the origins of sex bias in autoimmune disease have revealed an extensive and interconnected network of genetic, hormonal, microbial, and environmental influences. Investigation of sex hormones has moved beyond profiling the effects of hormones on activity and prevalence of immune cell types to defining the specific immunity-related genes driving these changes. Deeper examination of the genetic content of the X and Y chromosomes and genetic escapees of X chromosome inactivation has revealed some key drivers of female-biased autoimmunity. Animal studies are offering further insights into the connections among microbiota, particularly that of the gut, and the immune system.

## Summary

Sex bias in autoimmune disease is the manifestation of a complex interplay of the sex chromosomes, sex hormones, the microbiota, and additional environmental and sociological factors.

## Keywords

autoimmunity, sex bias, sex-dependent gene regulation, vestigial like family member 3, X chromosome

## INTRODUCTION

The immune system functions to defend against infection. Responses must be robust and specific enough to ward off or overcome infection without causing undue harm to the organism. Autoimmune disease arises when an exaggerated or misdirected immune response damages native tissues or organs. Although individual autoimmune diseases are rare, they are collectively among the most prevalent diseases in Western society [1]. Despite intensive investigation, our understanding of the pathogenesis of autoimmune disease is incomplete. A growing body of evidence supports a model wherein environmental and lifestyle factors precipitate development of autoimmunity in genetically susceptible hosts. Cures have been elusive, and lifetime treatment is often required.

Cellular and humoral immunity are generally stronger in women; women have higher levels of circulating antibodies, more circulating CD4 T cells, more robust cytokine production in response to infection, and enhanced rejection of tumors and allografts [2]. Many autoimmune diseases show a striking female sex bias (Fig. 1) [3]. Systemic lupus erythematosus (SLE), Sjogren's syndrome, Grave's disease, and Hashimoto's thyroiditis are seven to ten times more common in women than men; multiple sclerosis (MS), rheumatoid arthritis (RA),

and scleroderma are two to three times more common [4]. Overall, it is estimated that 78% of people affected with autoimmune diseases are women [5]. For many diseases such as SLE, genome-wide association studies and meta-analyses have identified numerous risk variants, yet female sex carries a risk of autoimmunity that dwarfs that of any susceptibility locus noted to date (Fig. 2) [6,7]. The biological mechanisms underlying this bias are incompletely understood. Previous inquiry centered on the influence of sex hormones, yet female sex bias is frequently observed even in autoimmune diseases with onset in childhood, when estrogen levels do not differ between the sexes, or in postmenopausal women. More recent work suggests that the sex chromosomes themselves and sex-specific environmental factors such as sexually dimorphic microbiota are also important drivers of sex bias in autoimmunity. In this review, we discuss current

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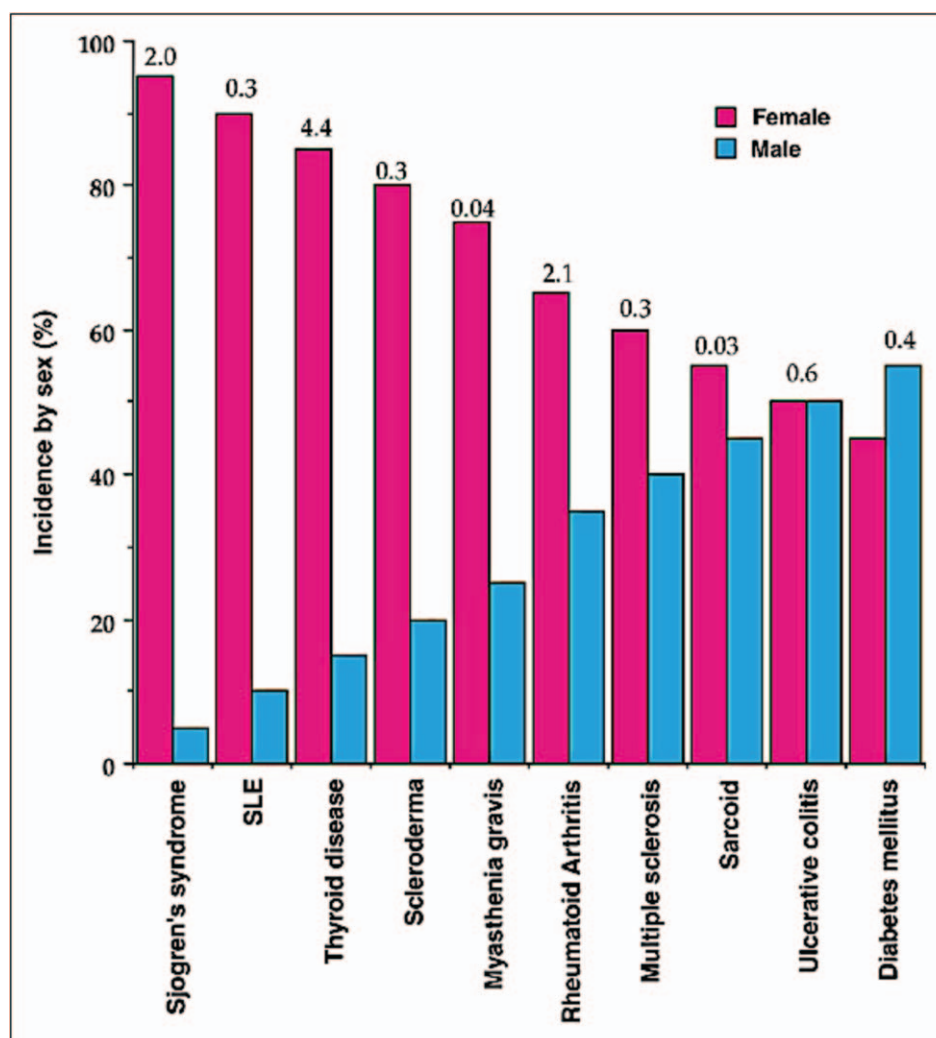
## KEY POINTS

- The mechanisms underlying sex bias in autoimmunity remain incompletely understood.
- The effects of sex hormones on autoimmune disease are mediated in part by direct regulation of key immunity factors such as the AIRE, IFN- $\gamma$ , interferon regulatory factor 5 and the intracellular TLR trafficking protein UNC93B1.
- Newly discovered nonhormonally regulated immune modulators such as VGLL3 may contribute to female-biased autoimmunity.
- The gut microbiota influences the immune response and development of autoimmunity and is itself shaped by androgens.
- These and future investigations may yield targets for more selective and therefore less toxic therapies for autoimmune disease.

and foundational studies addressing mechanisms of sex bias in autoimmunity.

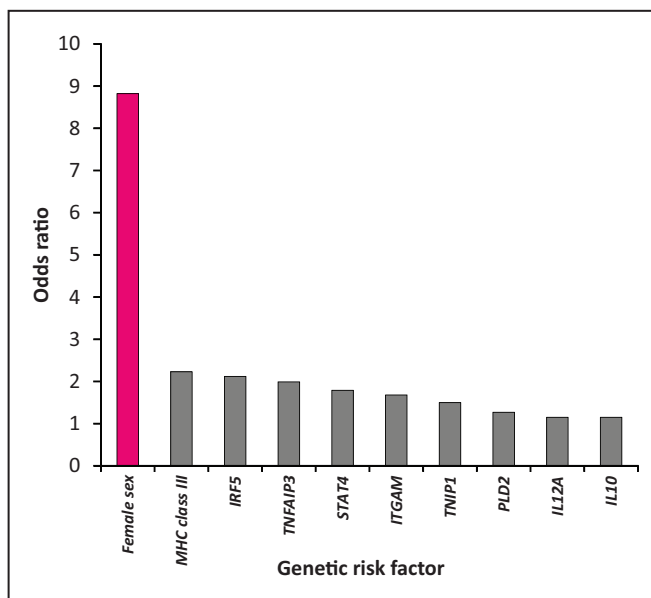
## SEX HORMONES

In the search for drivers of sex-biased autoimmunity, sex hormones represent obvious culprits. Indeed, sex hormone regulation of immunity is extensive, with interconnections to the other mechanisms discussed in this review. Sex hormones act primarily through associating with their respective intracellular receptors and binding their cognate response elements in target genes [8–10]. Sex hormone receptors are widely expressed in immune cells, and estrogen and androgen response elements are found in the promoters of several innate immunity genes [11]. Variations in autoimmune phenotype across puberty, pregnancy, and menopause demonstrate the complex regulation of immunity by sex hormones. The ease of antagonizing and



**FIGURE 1.** The sex distribution of the major autoimmune diseases. The numbers above the bars refer to the total number of disease cases ( $\times 1,000,000$ ) in the USA. Reproduced with permission [3]. SLE, systemic lupus erythematosus.





**FIGURE 2.** Female sex alone carries a risk of autoimmunity four times greater than any other known genetic risk variant for SLE. Odds ratio (OR) for female sex was calculated based on prevalence data from the Georgia Lupus Registry from 2002 [6]. ORs are shown for allelic associations at SLE susceptibility loci from a genome-wide association replication study [7].

supplementing these hormones renders them attractive as therapeutic targets and agents, but results have been inconsistent, and exposure to nonphysiologic sex hormone levels carries other intrinsic risks [12–14]. Thus, identifying and targeting the downstream immunological effectors of sex hormones may hold more therapeutic promise.

Progesterone, which is present at high levels during the luteal phase of the menstrual cycle and in pregnancy, is likely a key promoter of immune tolerance during pregnancy [15]. Progesterone is generally immune suppressive, decreasing proinflammatory mediators and inhibiting immune cell activation (reviewed in [16]). Progesterone signaling occurs primarily through progesterone receptors, which are expressed in many immune cell types including natural killer (NK) cells, macrophages, dendritic cells, and T cells [17]. At high levels, signaling may also occur through glucocorticoid receptors [18]. Progesterone decreases activation of NK cells [19], macrophages, and dendritic cells [20,21] and promotes skewing from Th1 to Th2 type T cell responses [22], which may account for the amelioration of Th1-associated autoimmune diseases such as MS and RA during pregnancy. Studies of human cord blood have shown that progesterone has strong regulatory T cell (Treg) induction activity and suppresses Th17 cell differentiation [15]. Some of the effects of progesterone may be mediated by

nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) inhibition [23].

Regulation of immunity by estrogens is more nuanced. Estrogen levels are high in pregnancy, low in menopause, and variable across the menstrual cycle. Estrogen receptor subtypes show differential expression in immune cells: estrogen receptor  $\alpha$  is highly expressed in T cells and estrogen receptor  $\beta$  is highly expressed in B cells [24]. In addition to binding estrogen response elements (EREs) in target genes, estrogen receptors also interact with ERE-independent transcription factors in immune cells [25]. Estrogens upregulate a number of key immunity factors including interferon regulatory factor 5 [26] and interferon (IFN)- $\gamma$  [27], as well as the intracellular toll-like receptor (TLR) trafficking protein UNC93B1 [28]. Estrogens also function through estrogen receptor  $\alpha$  to downregulate the autoimmune regulator (AIRE), a critical factor in central tolerance, through promoter methylation [29<sup>\*</sup>]; AIRE expression is also downregulated by progesterone and upregulated by the androgen dihydrotestosterone [29<sup>\*</sup>,30<sup>\*</sup>]. The effects of sex hormones on sex-biased DNA methylation and autoimmunity are otherwise as yet unclear [31]. In addition, estrogens regulate microRNAs (miRNAs) [32], who in turn regulate estrogen-dependent signaling [33]. Estrogens increase neutrophil numbers [34,35] but overall inhibit their activation and trafficking via multiple mechanisms, some mediated by NF- $\kappa$ B inhibition (reviewed in [36]). Estrogens enhance NK cell cytotoxicity and IFN- $\gamma$  production [37] but downregulate NK cell granzyme B secretion and cell surface activation markers [38]. Effects of estrogen on monocytes and macrophages vary by dose: production of proinflammatory cytokines is enhanced at low concentrations and suppressed at high [39]. The response of dendritic cells to estrogens is mixed, inducing production of both anti-inflammatory and Th1-type proinflammatory cytokines [40–42]. At lower concentrations, estrogens have immunostimulatory effects, promoting a Th1 response through enhancing the secretion of IFN- $\gamma$  [27,43,44]; in contrast, at high concentrations, estrogens promote a Th2 response [45–47]. In pregnant SLE patients, this Th2 shift and consequent increased production of autoantibodies often exacerbates disease [48]. Effects on Th17 cells are less clear [36]. Treg cells increase with estrogens [49,50]. Estrogens promote B cell survival, maturation, class switching, and antibody production [51–53] and interfere with peripheral negative selection of autoreactive B cells [54,55]. Oophorectomy has been reported to be protective in lupus-prone mice [56]; however, recent data suggest that this protection may not rely solely on estrogen-mediated

effects, as complete absence of estrogen receptor  $\alpha$  does not delay lupus in prone mice [57].

Androgens predominantly downregulate the immune response [58], decreasing proinflammatory mediators and inhibiting the proliferation and activation of a number of immune cell populations (reviewed in [36]). Accordingly, androgens have been shown to exert a protective effect in multiple autoimmune rodent models [30<sup>■</sup>,59,60]. This appears to be mediated in part by androgen-induced upregulation of AIRE in the male thymus: androgens increase AIRE levels by binding the androgen receptor (AR) and targeting the AIRE promoter, and the protective effects of androgens and male sex are lost in AIRE-deficient mice in a model of experimental autoimmune encephalitis [30<sup>■</sup>]. In addition, ARs are broadly expressed in neutrophil-lineage cells, with no difference in male and female expression patterns [61], and act to increase the number and trafficking of neutrophils [62]; however, androgens decrease proinflammatory responses of neutrophils [63], NK cells [64], and macrophages [65]. ARs are not expressed in peripheral lymphocytes but are found in lymphoid and nonlymphoid thymic and bone marrow cells [66], in which they limit the number of immature thymocytes and restrain active cell cycling [67]. Although androgens decrease the activation of Th1 and Th2 cells [68], Th17 cell responses are enhanced [69]. Treatment to reduce testosterone decreases Treg count [70]. Androgens have also been found to limit the number of immature type 2 innate lymphoid cells in the lung [71]. In epidermal cells, androgens were found to modulate the expression of *PRDM1*, a transcriptional repressor involved in thymic T cell apoptosis and other immune cell processes that is implicated in female-dependent autoimmune risk [72]. B cell number is negatively regulated by androgens [62].

Some studies support a role for psychosocial stress in initiation or exacerbation of autoimmune disease, although causation has been challenging to establish [73]. Response to stress occurs through the hypothalamus–pituitary–adrenal (HPA) axis, which also exhibits sexual dimorphism in cortisol response to psychosocial stressors (reviewed in [31]). Stress triggers release of glucocorticoids, which generally inhibit immune responses through decreasing production of proinflammatory cytokines and inhibiting activation and proliferation of multiple immune cell types. The HPA axis also produces prolactin, another hormone with immunological effects. The prolactin receptor is widely expressed in immune cells [74], and prolactin signaling is largely immunostimulatory [75]. In particular, prolactin may promote autoimmunity by inhibiting negative selection of autoreactive B cells, augmenting

autoantibody production [76–78]. Positive correlations of prolactin levels and disease activity have been identified in SLE patients, but the causality remains to be determined [79].

## SEX CHROMOSOMES

The presence of two X chromosomes in the female also contributes to sex bias of autoimmunity. Although canonically one of the X chromosomes is inactivated in early development, this process is imperfect, with approximately 15% of genes escaping X chromosome inactivation (XCI) [80]. A majority of the genes that escape XCI show female expression bias [81,82,83<sup>■</sup>], and there is variation among individuals in which genes escape [84]. Males with Klinefelter syndrome (karyotype XXY) have an increased risk of SLE commensurate with that of females [85], and one male patient with severe prepubertal SLE was found to have an XX karyotype due to an X–Y translocation [86], demonstrating the influence of X dosage. In contrast, females with Turner syndrome, who have complete (XO) or partial X chromosome monosomy, are at increased risk of developing autoimmune disease relative even to XX females, but the excess risk may be greater for male-predominant autoimmune conditions such as ankylosing spondylitis [87,88]. In contrast, females with Turner syndrome (karyotype XO) are at increased risk of developing autoimmune disease, but the risk is strongest for male-predominant conditions [87]. In most females, XCI is random, resulting in half of cells expressing the maternal and half the paternal X chromosome; however, some females show nonrandom silencing leading to an 80% or more predominance of one X chromosome. This skewed inactivation is more common in patients with autoimmune diseases [89,90], although it may be a consequence of autoimmunity rather than a cause [91].

Many genes with established roles in autoimmunity are located on the X chromosome. Several of these have been found to be overexpressed or hypomethylated in female but not male SLE patients [92,93], and dosage of the X-linked *TLR7* and *TLR8* genes has been shown to influence development of SLE in humans and lupus-prone mice [94,95,96<sup>■</sup>,97,98]. Recently, escape of *TLR7* inactivation in a substantial proportion of immune cells has been described in females and men with Klinefelter syndrome; this biallelic expression of *TLR7* primes for increased class switching in activated B cells and increased *TLR7* reactivity [99]. The X chromosome is also highly enriched in miRNAs [100]. miRNAs, including some located on the X chromosome, are essential for maintenance of immune

tolerance (reviewed in [101]), and a subset of X-linked miRNAs was found to be overexpressed in females, but not males, with SLE [93]. Finally, the X chromosome can become partially reactivated in lymphocytes in women, resulting in overexpression of immunity genes and possibly contributing to sex bias in SLE [102].

The Y chromosome has garnered much less attention as a driver of sex bias in autoimmunity, but evidence is accumulating. In a mouse model of autoimmune disease, Y chromosome polymorphisms, including gene copy number variation, correlate with disease susceptibility and severity [103–105], although the observed effects may also reflect impaired balancer function in mismatched X and Y chromosomes that evolved in different strains [106]. However, data from men with MS suggest the influence of the Y chromosome on autoimmunity may extend to humans [105]. Further investigation of male mice with specific Y-linked defects in immunity [107,108] and examples of human Y-linked immune variation [109] is ongoing and may shed additional light.

## GUT IMMUNOLOGY AND THE MICROBIOTA

The gut microbiota plays a critical role in maturation and modulation of innate and adaptive immunity [110] yet is itself shaped by the immune system. Both the gut immune system and microbiota exhibit sexual dimorphism. Immune tissues in the gut of male and female rodents differ in representation of immune cells, with an overall trend toward enhanced innate immunity and attenuated adaptive immunity in the male gut relative to the female [111,112], and many immune genes show sex-biased expression in mouse gut [113,114]. Although some studies have observed no differences in the diversity or composition of male and female gut microbiota, sex differences in the human gut microbiota have been extensively documented [115], raising interest in the gut microbiota as a potential driver of sex bias in autoimmune disease. In addition, microbiome aberrations have been observed in the vast majority of immune-mediated diseases, although demonstrating causation has proven a significant obstacle thus far [116], with many resorting to animal models for further investigation.

Female mice show increased microbiota diversity relative to male, and many bacterial species show sex-biased enrichment that occasionally varies with strain, age, and diet (reviewed in [115]). Sex hormones likely also play a role. In human twin studies, the microbiota of opposite-sex twins becomes more divergent after puberty relative to same-sex twins [117]. Similarly, in the nonobese diabetic (NOD)

mouse model of spontaneous autoimmune type 1 diabetes (T1D), the gut microbiota does not differ in prepubertal male and female mice; however, microbial diversity decreases in intact postpubertal males, whereas this does not occur in females and castrated males [118]. Transfer of male microbiota into germ-free female mice and female microbiota into germ-free male mice revealed that some manifestations of immunological sexual dimorphism appear to depend on sex-specific gut microbiota: regardless of the sex of the recipient, ROR $\gamma$ t<sup>+</sup>Foxp3<sup>+</sup> cells are increased in gut immune tissues of mice who received male microbiota, and T cell precursors are increased in mice who received female microbiota [113].

There is mounting evidence of a direct role for the gut microbiota in driving sex-biased autoimmunity. Female NOD mice develop spontaneous autoimmune T1D at twice the rate of male mice. Under germ-free conditions, however, incidence of T1D is equal in both sexes [119], indicating that male microbiota may confer protection. Gavage of female NOD weanlings with male NOD intestinal microbiota results in elevated serum testosterone and protects against development of T1D. This effect is abrogated in recipient female mice treated with AR antagonist, suggesting protection is conferred by a testosterone-dependent mechanism [119]. Female MRL/Mp-Fas<sup>lpr</sup> mice, who develop lupus at far higher rates than males, show significantly higher gut microbiota diversity but lower abundance of *Lactobacillales* species and increased intestinal permeability [120]. In female and castrated male Fas<sup>lpr</sup> mice, *Lactobacillales* gavage restores gut mucosal barrier function, promoting an anti-inflammatory cytokine environment in which autoantibody production decreases and renal disease improves, with increased renal Treg cells and suppression of renal Th17 cells [120]. The same benefits were not observed in intact male Fas<sup>lpr</sup> mice, suggesting that *Lactobacillus* species in the gut attenuate renal disease in lupus-prone mice in a sex hormone-dependent manner. In aggregate, the animal data suggest that sex and androgens appear to regulate gut microbiota composition and function, which reciprocally influence the immune response and development of autoimmunity.

## OTHER FACTORS

Recently, we identified the transcription factor vestigial like family member 3 (VGLL3) to be critical in orchestrating sex-biased expression of key autoimmune genes in a sex hormone-independent fashion [83<sup>¶</sup>]. VGLL3 is required for robust expression of genes implicated in autoimmunity and for mounting a full IFN-I response. In healthy skin, VGLL3

shows nuclear localization that is more prominent in women than in men. In lesional skin of patients with cutaneous lupus, however, VGLL3 shows nuclear localization in both sexes, indicating disease-dependent regulation. This suggests VGLL3 governs an autoimmunity pathway that is constitutively active in women but must be triggered by other means in men [83<sup>□</sup>]. VGLL3 is located on chromosome 3 and appears to be epigenetically regulated (unpublished observation), and its exact role in driving autoimmune diseases is being actively explored.

Sex bias is prominent in DNA methylation, affecting chromatin accessibility of immune genes [121]. The X and Y chromosomes may influence DNA methylation, as was shown for an autosomal locus in human cells [122]. Prenatal environmental exposures also show sex-specific epigenetic effects on DNA methylation (reviewed in [123]), although the underlying mechanisms remain unknown. Fetal microchimerism, wherein circulating fetal cells travel to the mother and persist for years after pregnancy [124], may predispose to development of autoimmune disease; however, studies disagree on whether autoimmune diseases are more common in women with prior pregnancies, and a definitive connection has not been established [125]. In addition, men and women differ in exposure to environmental endocrine-disrupting chemicals, with estrogenic and antiestrogenic properties that may affect genetic and epigenetic regulation of immunity (reviewed in [126,127]). Finally, sociological differences between the sexes, such as rates of smoking in men versus women, may influence the development and manifestations of autoimmune disease [128].

## CONCLUSION

In treating severe autoimmune disease, physicians often must turn to broad immunosuppressive therapies with high side effect burden and inherent risks of infection and malignancy due to decreased immune surveillance activity. The trend toward stratifying immunological research studies by sex and continuous improvements in high-throughput technologies have enabled increasingly sophisticated characterization of the differences between the male and female autoimmune phenotypes; this is evidenced by work such as the recent description of sex bias in the influence of human leukocyte antigen associations on T cell selection and expansion as revealed by T cell receptor immunosequencing of large cohorts [129]. These nuanced descriptions are helping to explain observed sex differences in infection susceptibility and

autoimmunity, but we must continue to interrogate the mechanistic underpinnings of sex-biased autoimmunity to pave the way toward development of highly specific therapeutics that spare patients the dangers of broad immunosuppression. Targeting the precise pathways that drive the female autoimmune disease burden above the baseline male prevalence will be an immense boon to human welfare, particularly if accompanied only by the relatively modest increased risk of infection and malignancy native to the male immune system.

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## Conflicts of interest

*There are no conflicts of interest.*

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# The ties that bind: skin, gut and spondyloarthritis

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## Purpose of review

This article aims to review recent literature linking epithelial barrier inflammation and arthritis in spondyloarthritis (SpA), with a critical view on how they are bound by genetic, immunological and environmental ties.

## Recent findings

The epithelia-joint axis has become an intense area of both basic and clinical SpA research. The penultimate goal is to understand the immunopathologic links between epithelial inflammation and arthritis in SpA. Inflammatory bowel disease (IBD) and psoriasis (PsO) have strong links to SpA at several levels. Clinically, there is a strong association of IBD, PsO and SpA. Genetically, there are many shared risk factors; however, there are also distinct differences in the genetics of the respective diseases. Immunologically, type 3 immunity, especially interleukin (IL)-17 and IL-23 dysregulation, has been shown to play a central role in IBD, PsO and SpA. Environmentally, a microbial dysbiosis has been noted in each of these diseases, but whether the microbial signature is similar between diseases is not clear, nor is the effect of dysbiosis on the immune response known.

## Summary

It will be crucial to determine whether the relationship between epithelia inflammation and SpA is truly causal for both the understanding of pathogenesis and for future treatment strategies.

## Keywords

gut inflammation, microbiome, spondyloarthritis

## INTRODUCTION

The epithelia-joint axis has become an intense area of both basic and clinical spondyloarthritis (SpA) research. The immediate challenge is to understand the immunopathologic link and the diagnostic utility of epithelial inflammation in relation to arthritis in SpA.

The SpA family of inflammatory joint diseases are differentiated from other forms of inflammatory arthritis based on their seronegative presentation, early age at onset (<40 years), lack of female bias and coexisting bone formation and erosion. SpA can involve both the peripheral and axial joints. Further, extra-articular manifestations are common among SpA patients, especially those involving epithelial barrier surfaces, such as psoriasis (PsO) and inflammatory bowel disease (IBD). Historically, the SpA family of diseases includes ankylosing spondylitis (AS), which by clinical definition not only involves the axial skeleton but can also feature peripheral arthritis, psoriatic arthritis (PsA), which is predominantly peripheral arthritis and by clinical definition involves PsO, reactive arthritis (ReA), which involves a prior infection with a defined set of

gastrointestinal or urinary pathogens, IBD-SpA, in which clinical IBD precedes SpA and undifferentiated SpA, if the seronegative arthritis in question does not fit the aforementioned diagnostic criteria. It is important to note that these historical forms of SpA lie on a continuum, with diagnostic criteria based on clinical observation without a defined biological basis [1–3].

Recently, classification criteria have more generally defined SpA as axial (axSpA) or peripheral (pSpA) with or without defined radiographic (x-ray) features [4]. These nuances are important

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## KEY POINTS

- Arthritic symptoms in SpA associate with skin and gut inflammation at the clinical, genetic and experimental levels.
- Whether skin/gut inflammation is a necessary precursor to arthritis is not known.
- Understanding the causal relationship between skin/gut inflammation and SpA will be crucial for predicting the success and preventing failure of novel therapeutics.

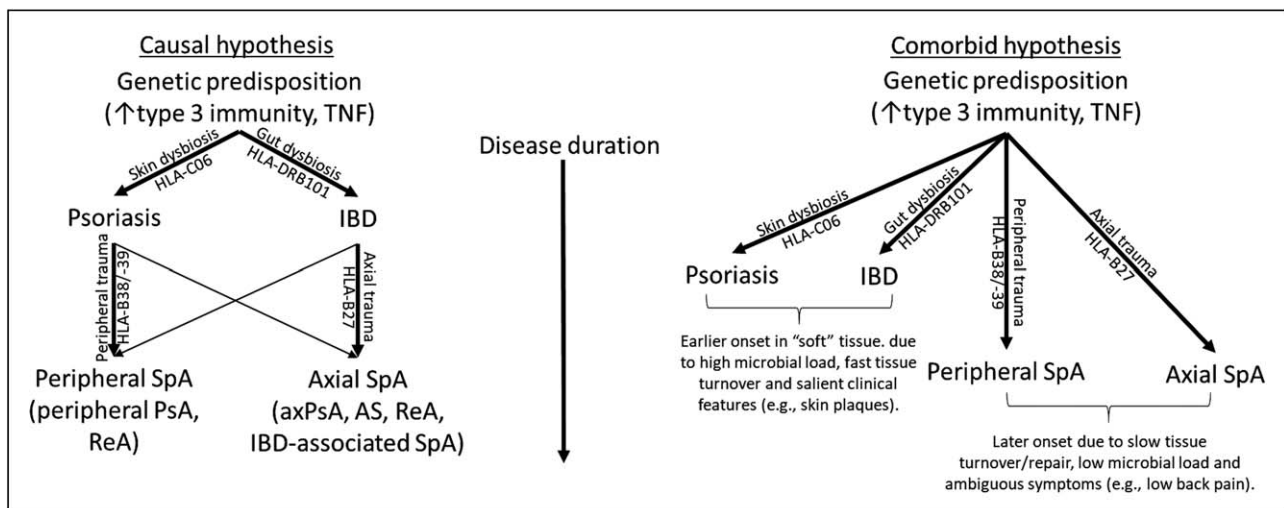
to consider when interpreting the literature, as they are not interchangeable: All AS fulfils the criteria for axSpA, but not all axSpA patients fulfil the criteria for AS. A quarter of SpA patients fulfil criteria for both PsA and AS [5], which highlights the high occurrence of axial disease in PsA. We refer to the precise diagnosis when discussing reported results.

In this article, we will broadly review the clinical, genetic, immunologic and microbial links between gut, skin and joint inflammation. Although the prevailing thought is that epithelial barrier inflammation leads to arthritis in SpA, implying a causal relationship, this dogma is not grounded in mechanistic fact. Given our current knowledge, it is equally probable that epithelial barrier inflammation coexists without causing arthritis in SpA (Fig. 1).

## CLINICAL EVIDENCE OF THE EPITHELIAL BARRIER-JOINT LINK

Epidemiologic studies have been integral in establishing the link between epithelial inflammation and SpA. PsA research is entwined with skin inflammation, largely due to the heavy weighting of PsO in the diagnostic criteria of PsA [2]. Although gut and skin inflammation is now a clinical feature for the diagnosis of axSpA, it is not essential [4]. Rheumatology-based studies have commonly reported that approximately 5–10% of AS patients have IBD, and nearly 50% of patients have subclinical gut inflammation [2,6]. Interestingly, PsO is more common in AS patients than clinical IBD, with a recent retrospective study of 4000 AS patients revealing a prevalence of nearly 5% for IBD versus 10% for PsO [7]. The susceptibility of AS patients to IBD appears to be familial: it has been reported that 35% of healthy first-degree relatives have serological markers of IBD [8], although this still remains to be confirmed. A recent study demonstrates that IBD is likely to develop *after* the diagnosis of SpA [9<sup>†</sup>].

Gastroenterology-based studies provide a complementary view to those conducted from a rheumatology perspective. In a prospective cohort of 599 IBD patients followed for 20 years, 45% of patients reported chronic back pain postdiagnosis, with 7.7% meeting diagnostic criteria for axSpA and 4.5% for AS [10]. Importantly, chronicity of IBD was significantly associated with comorbid axSpA compared with patients without back complaints. In line with



**FIGURE 1.** Conceptualizing the relationship between psoriasis, inflammatory bowel disease and spondyloarthritis. In the causal hypothesis, epithelial inflammation provides the necessary inflammatory milieu for the induction of arthritis upon the presence of a secondary trigger. In the comorbid hypothesis, extra-articular symptoms coexist with arthritis due to common genetic components. Epithelial barrier inflammation precedes arthritis in patients with comorbidity, but does not cause arthritis, HLA alleles given as representative genetic risk factors.

this observation, erosive changes in the sacroiliac joint, defined by computed tomography (CT) scans, were twice as frequent in IBD patients than controls [11<sup>¶</sup>]. A recent retrospective analysis of 626 patients with IBD reported 2.1% of patients to have AS, and 7% to have pSpA [12]. These rates are in line with a meta-analysis of 71 studies, which report 3% of IBD patients to have AS and 13% to have pSpA [13]. Importantly, this meta-analysis highlights that SpA is more commonly linked to Crohn's disease than ulcerative colitis, and that comorbidity is dependent on ethnicity. Specifically, IBD and AS are not common comorbidities in AS patients of East-Asian origin, as highlighted by the observation that only 0.4% of Han Chinese with AS have clinical IBD [14]. This ethnic evidence strongly supports comorbidity of SpA and IBD over causality.

Studies of PsO and SpA from a dermatology perspective have revealed similar observations to those from a gastroenterology view. A large study of 10 000 Danish patients with PsO reported a 13% prevalence of PsA [15], with PsO severity correlating to the development of PsA. A recent meta-analysis of almost 1 million PsO patients that reported a PsA prevalence of 20% [16], in which comorbid PsO/PsA was less common in Asian patients. Although clinical studies do not shed light on the causality between PsO and SpA, the lower rates of PsA in different ethnic groups provide further evidence of comorbidity.

### SPA GENETICS: BRIDGES AND BOUNDARIES WITH INFLAMMATORY BOWEL DISEASE /PSORIASIS

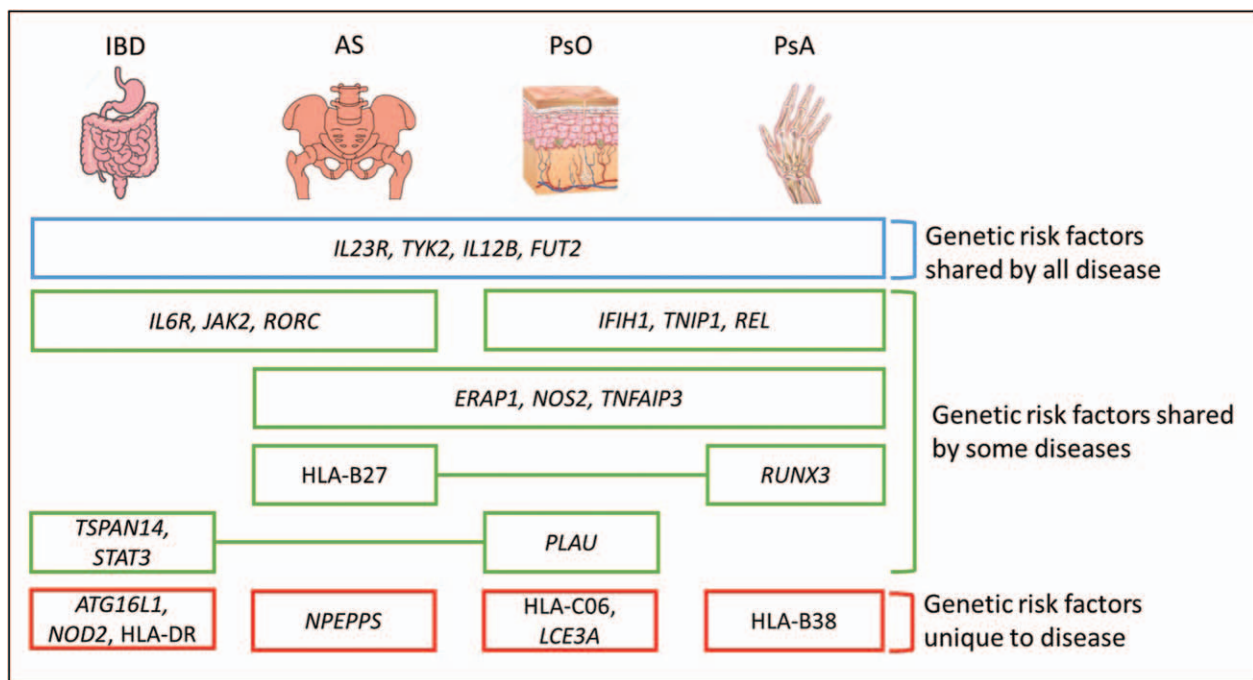
The biological link between epithelial barrier inflammation and SpA is unequivocal when viewed from a genetic perspective. Large genetic studies, namely genome-wide association studies (GWAS), have primarily been performed with the immunoCHIP platform [17], which contains a curated list of 200 000 of the estimated 5 million single nucleotide polymorphisms (SNPs) in the human genome [18]. When interpreting such studies, it is important to understand their limitations: These studies are generally powered to detect 'common' alleles, present in more than 5% of the population. These common variants generally impart only a modest risk [odds ratio (OR) < 1.5] with high risk factors, such as HLA-B27 for AS (OR = 46), having been discovered in the pre-GWAS era [19]. Further, there is considerable ethnic-bias in the occurrence of SNPs and their role in promoting autoimmune disease. For example, variants in *IL23R* are strongly associated with AS in European populations, but not East Asian [20]. The limited number of SNPs on the immunoCHIP,

and the linkage of SNPs in close proximity means that most GWAS-identified SNPs are likely markers of the primary causal SNP in the region [21]. This later observation means that GWAS must be closely examined in follow-up studies to identify causal variants and their biological effects.

The first generation of GWAS focused solely on single diseases compared with healthy controls. The discussion of PsO and IBD-specific genetic studies is outside the scope of this article; interested readers are directed to recent articles [22–25]. Initial SpA GWAS focused strictly on AS [26,27]. These GWAS confirmed the strong association of AS with HLA-B27, and revealed weaker associations with factors involved in type 3 [interleukin (IL)-17 associated] immunity (e.g. *IL23R*, *TYK2*, *IL12B*), CD8<sup>+</sup> T cell cytotoxicity (e.g. *RUNX3*, *EOMES*, *TBX21*) and antigen processing for expression on class I major histocompatibility complex (MHC) (e.g. *NPEPPS*, *ERAP1*, *ERAP2*, *LNPEP*).

GWAS of PsA have been confounded by its close relationship to PsO. Studies contrasting PsA/PsO have confirmed the close genetic overlap, yet have revealed striking differences [28]: Non-MHC associations have revealed PsO-specific genes, such as the costimulatory molecule *TNFRSF9* (4-1BB), and *LCE3C*, an epithelial keratinization gene. On the contrary, PsA, but not PsO, is associated with *TNFAIP3* (A20). Even more striking differences are seen in the MHC loci: HLA-C06 (HLA-Cw6) is a strong risk factor for PsO, while HLA-B08, -B27, -B38 and -B39 are risk factors for PsA [29]. The MHC loci correlations with specific disease have been replicated in clinical studies [5,30<sup>¶¶</sup>], with HLA-B27 being linked to severity of enthesitis in PsA patients [31] and HLA-C06 being linked to psoriasis severity [32]. In striking contrast, fine mapping of the MHC locus in IBD has revealed strongly linked to HLA-DRB101, a weak link to HLA-C12 and no link to HLA-B [33].

Accumulating GWAS data have allowed for posthoc multidisease meta-analyses. It is important to highlight that single disease studies do not take into account comorbidities, so these studies must be approached with caution, especially when interpreting PsO data in light of comorbid SpA. Farh *et al.* [21] demonstrated that AS and IBD are very closely linked at the genetic level, and reasonably linked to psoriasis. In contrast, autoimmune disease such as systemic lupus erythematosus, rheumatoid arthritis and celiac disease are poorly correlated to IBD/PsO/SpA at the genetic level. A more recent meta-analysis that combined AS, IBD, PsO and primary sclerosing cholangitis expanded on the known genetic risk factors, and supported the close association of epithelial barrier disease to AS [34].



**FIGURE 2.** Shared and unique genetic risk factors associated with epithelial barrier and joint inflammation. Selected GWAS-associated genes used to illustrate similarities and differences in the genetics of the respective diseases.

GWAS and their meta-analyses build a picture of shared and distinct loci (Fig. 2). When viewed as a whole, these risk factors explain shared biological underpinnings, especially in type 3 immunity, but highlight distinct pathways that dictate tissue localization, such as MHC usage and tissue-specific genes. Perhaps the best illustration of this is the complex association of SpA and *NOD2*, one of the strongest risk factors for IBD. SpA patients with microscopic, Crohn's disease like gut inflammation do have a similar burden of the IBD *NOD2*-risk allele compared with SpA patients with noninflamed gut and healthy individuals [35]. On the contrary, although *NOD2* variants are associated with an earlier age at onset of IBD in SpA patients, they do not affect the age of onset for arthritic symptoms [36]. These genetic distinctions suggest that SpA can be comorbid, but is not strictly dependent on clinical or subclinical IBD and psoriasis.

### TYPE 3 IMMUNITY AT THE EPITHELIAL BARRIER AND JOINT

As highlighted by the genetics of IBD, PsO and SpA, perturbations to type 3 immunity are central to the pathogenesis of these inflammatory diseases. Type 3 immune responses are defined as the effector arm of immunity that produces IL-17, or related cytokines such as IL-22 [37]. These effector cytokines can be induced by IL-23; however, other cytokines, such as

IL-7, have been implicated [38]. Cells producing these cytokines generally express the transcription factor ROR $\gamma$ t, and include group 3 innate lymphoid cells (ILC3), conventional CD8 $^{+}$  T (T<sub>C</sub>17) cells and CD4 $^{+}$  T (T<sub>H</sub>17) cells. IL-17 producing innate-like T cells such as  $\gamma\delta$  T cells, mucosal-associated invariant T (MAIT) cells and natural killer T (NKT) cells, as well as certain IL-17 producing myeloid cells such as neutrophils and mast cells, can also be considered type 3 immune cells.

Members of type 3 immunity exhibit prominent roles in epithelial barrier surfaces. Th17 cells and ILC3 are featured heavily in IBD literature [39], while in psoriasis Tc17 cells appear pathogenic [40,41]. The key function of type 3 immunity at these surfaces is to maintain barrier integrity [42,43] through production of IL-22 and IL-17, which promote epithelial proliferation [44] and tight junction formation [45], respectively.

Type 3 immune cells have been broadly implicated in the pathogenesis of SpA [46]. Animal studies support the involvement of Th17 and Tc17 cells in the SKG mouse [47,48] and HLA-B27 transgenic rat [48], and  $\gamma\delta$  T cells in the IL-23 minicircle model [49 $^{**}$ ]. Although the SKG mouse model supports the notion that systemic autoreactive T cells mediate tissue inflammation by trafficking to joint tissue [50], the IL-23 minicircle model is centred around the activation of tissue resident  $\gamma\delta$  T cells [49 $^{**}$ ]. These phenomena are not mutually exclusive:



recruited Th17 cells activate joint resident ILC and stromal cells to mediate arthritis in the SKG mouse [51<sup>■</sup>]. In humans, a range of type 3 immune cells are elevated in the blood of SpA patients, including ILC3, Th17 cells and  $\gamma\delta$  T cells [46,52]. In joint tissue, immunopathology-based studies of AS patients found IL-17 to be present in myeloid cells of the axial skeleton in humans [53]. Further to this, ligaments from the axial skeleton contain populations of tissue resident ILC3 that produced IL-17 in response to IL-23 [54<sup>■</sup>]. Studies of synovial fluid from peripheral joints of SpA patients have found an enrichment of MAIT cells [38], Tc17 [55] and ILC3 [56,57]. Given the extensive list of CD8<sup>+</sup> T cell related genes linked to AS and PsA, it is likely that future research will reveal a pathogenic role for Tc17 cells, or tissue resident memory (Trm) in SpA.

The relationship between type 3 immune cells in anatomically distinct sites is not clear: do patients with IBD/PsO/SpA have a shared systemic elevation of type 3 immunity, in which local trauma such as epithelia barrier damage or mechanical stress induces tissue-specific immune activation? According to such a view, antecedent epithelial inflammation can be explained by the sensitivity of this tissue to environmental changes, and rapid turnover rate when compared with joint tissue. Alternatively, can type 3 immune cells activated in the skin or gut traffick to joints to induce arthritis? During an immune response, cells are 'educated' to go to certain tissues through the induced expression of tissue specific trafficking molecules such as integrins and chemokine receptors. Examples of this include  $\alpha 4\beta 7$ , which is essential for T cell recruitment to the gut, and  $\alpha E\beta 7$ , which is important for cell trafficking to the skin [58]. Studies of rheumatoid arthritis synovial tissue have shown that the receptors for these integrins are also present in the joint [59], suggestive of shared trafficking mechanisms between the epithelial barrier and joint. Further support comes from epithelial barrier trafficking molecule enrichment on synovial fluid-derived T cell lines [60] and type 3 immune cells in the blood and synovial fluid of AS patients [57,61]. Systematic studies of trafficking molecule expression in SpA patients is required to strengthen the barrier-joint correlation in humans.

The link between type 3 immunity, IBD/PsO and SpA has provided the rationale for its targeting. Biologics targeting the  $\alpha 4\beta 7$  integrin are approved for therapeutic use in IBD; however, they may worsen or even induce arthritis [62,63]. Anti-IL-17 mAbs have proven to be very successful in psoriasis [64], which is in stark contrast to their failure in IBD [65], demonstrating tissue-dependent roles for these cytokines. Anti-IL-17 therapy has been successful in both PsA [66] and AS [67] without preexisting

IBD. Likewise, anti-IL-23 trials have yielded conflicting results: although it is successful in treating IBD [68], PsO [69] and PsA [70<sup>■</sup>], it failed to meet primary endpoints in AS patients [71<sup>■</sup>]. These success and failures trials highlight the divisions between epithelial barrier inflammation and arthritis in SpA.

Animal studies have yet to directly address the underlying mechanism of extra-articular manifestations in SpA, which precede arthritis in SpA animal models precede arthritis [72]. Nevertheless, immune cells in the gut have been shown to traffic to the eye, triggering uveitis and to the kidney, leading to glomerulonephritis [73,74]. It is also possible that microbes, or their products (such as LPS) traffick from the epithelial barrier to the joint, as has been long shown for ReA [3]. Such an observation warrants closer examination of the microbiome in barrier surfaces as potential modulators of systemic immunity.

## MICROBIAL DYSBIOSIS: ACTION OR REACTION?

The term microbiome defines the collection of microorganisms and their genomes that inhabit different anatomical locations both in and on humans. Dysbiosis ensues when the microbiome composition deviates from normal, and has been observed in inflammatory and autoimmune diseases, including PsO [75<sup>■</sup>] and IBD [76]. The clinical association of these conditions with SpA and their link to skin and gut dysbiosis raise questions regarding a possible link between the microbiome and SpA pathogenesis. Does dysbiosis *stem* from inflammatory changes, or could it be implicated in *mediating* epithelial involvement in the pathogenesis of SpA?

IBD, PsO and SpA are all characterized by gut dysbiosis. In SpA, decreased gut bacterial diversity has been reported in patients with PsA [77], axSpA [78<sup>■</sup>] and AS [79], but not ReA [80]. Although describing the microbiome characteristics of these conditions in detail is beyond the scope of this review, we note that the gut microbiota in stool from PsA patients resembles IBD dysbiosis, both are characterized by a decrease in diversity [81] and in the genera *Akkermansia*, *Ruminococcus* and *Alistipes*. Interestingly, *Akkermansia muciniphila*, reported absent in one PsA cohort [77] and decreased in IBD compared with controls [82], is a mucus-degrading gut symbiont that converts mucin into short-chain fatty acids (SCFAs), highlighted below for their anti-inflammatory effects. Nevertheless, despite earlier reports of reduced microbial diversity in PsO [77], a recent study reports a significantly *higher* gut microbial diversity in PsO patients than healthy controls [83<sup>■</sup>]. Furthermore, the latter study reports increased abundances of genera



*Akkermansia* and *Ruminococcus*, which the authors suggest may be due to higher PsO severity in their cohort.

In contrast to the gut microbiome, the skin microbiome is less studied, with no SpA-specific studies. The skin is the body's first line of defense against toxic substances and pathogens, serving as a physical barrier and as an arsenal of immune cells and antimicrobial mediators [84,85]. Although to a lesser degree than the gut, it must tolerate the wide range of microorganisms harboured within its 1.8 m<sup>2</sup> diverse ecosystem and is credited with educating our immune system to tolerate resident microorganisms while being able to mount an effective immune response against pathogens [48]. Changes in the skin microbiome have been described in PsO [86–88], most recently demonstrating a relative enrichment of *Staphylococcus aureus* (in both lesional and nonlesional skin) and an underrepresentation of *S. epidermidis* and *Propionibacterium acnes* relative to healthy skin [89<sup>¶</sup>]. Furthermore, *S. aureus* affects T-cell differentiation, demonstrated by a strong Th17 polarization following colonization of newborn mice, while mice colonized with *S. epidermidis* and uncolonized controls showed no such response [89<sup>¶</sup>]. This suggests that *S. aureus* can specifically trigger the Th17 response, which may contribute to IL-17 driven inflammation in psoriasis (and, perhaps, PsA).

Dysbiosis in the skin and gut are not necessarily mutually exclusive. Although this field is still nascent, early studies support a role for the gut microbiome on skin physiology, both directly through microbial metabolites and indirectly through the immune system [90]. It has even been hypothesized that the trifecta of the gut, skin and joint form a unique axis of inflammation in PsA [91]. In such an axis, it is even possible that bacteria may cross-colonize the respective tissues: bacterial DNA has been detected in the blood of psoriasis [83<sup>¶</sup>,92] and PsA patients [93], though their microbiome of origin remains elusive.

Mechanistically, the link between dysbiosis and arthritis in SpA is not clear. Evidence in support of microbial-mediated induction of SpA comes from the observation that HLA-B27 transgenic rats raised in a germfree environment do not develop inflammatory intestinal or peripheral joint disease and display a milder axial arthritis than their conventionally raised counterparts [94]. Interestingly, HLA-B27 alters the repertoire of the gut microbiome – transgenic animals have a different microbiota compared with their wild-type counterparts, even in the absence of gut inflammation [95] – which may, in part, mediate susceptibility SpA in HLA-B27 positive individuals. Mechanistic studies outside of the SpA

field have revealed that commensal microorganisms ferment dietary fibres to produce SCFAs, which exert anti-inflammatory effects on gut cells by decreasing the expression of pro-inflammatory cytokines [96] and promoting regulatory T (Treg) cell responses [97,98]. The result is protection against inflammatory disorders, including arthritis and colitis. Furthermore, the gut microbiome helps in maintaining a balance between immune tolerance of harmless antigens and protecting the host against pathogens [99]. On the contrary, certain bacteria have pro-inflammatory effects. For example, segmented filamentous bacteria have been associated with various Th17-mediated diseases, including arthritis [100], raising the possibility that an altered gut flora may favour the production of Th17 over Tregs.

Despite extensive evidence that there are dysbioses in the skin and gut of SpA patients, the evidence on hand does not provide a mechanistic answer as to whether the dysbiosis is a cause or effect of systemic inflammation. Given the aforementioned evidence of the immunoregulatory effects of microbes, it is quite likely that they have an effect on arthritis from their niches in the skin or gut. It is of the utmost importance to determine whether this is a triggering or exacerbating effect.

## CONCLUSION

We need to examine our assumptions in light of the facts. There is clear correlation between epithelial barrier inflammation and SpA, driven by shared genetic and environmental risk factors. But how do these ties bind together inflammation in anatomically distinct locations? At this stage, it is tempting to speculate that epithelial barrier inflammation leads to arthritis through the transmission of immune factors or cells (Fig. 1). The data on hand, however, equally support the theory that these features are bound through a shared immunologic and genetic background, yet are not temporally dependent on one another. This relationship between gut, skin and joint inflammation is not only important to understand both in terms of understanding pathogenesis but also for treatment strategies, as has been highlighted by arthritic flares in IBD patients treated with integrin-blocking biologics [62].

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## Conflicts of interest

There are no conflicts of interest.

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# Osteoarthritis following meniscus and ligament injury: insights from translational studies and animal models

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## Purpose of review

The interaction between joint injuries and posttraumatic osteoarthritis (PTOA) is generally thought to be mechanical in nature, however, surgical intervention has little effect on the development of PTOA. Little is known about the biological underpinning of how meniscus and anterior cruciate ligament (ACL) tears lead to cartilage degeneration. This review summarizes the latest findings regarding biological factors that influence how the knee responds to meniscus and ligament injuries, how meniscus and/or ACL tears turn the joint in the direction of PTOA and whether patient risk for PTOA after meniscus/ACL injury can be predicted.

## Recent findings

Literature indicates that numerous intrinsic and extrinsic factors are associated with the biological response of the knee to injuries associated with PTOA. Gene/protein biomarkers provide insight into the biologic response of the knee to meniscus/ACL tears and the relationship to osteoarthritis in at-risk patients. Animal studies detail the time-course of disease pathogenesis and inform about the molecules that potentially alter the course of disease.

## Summary

The molecular metabolic state of the meniscus/ACL after injury is associated with several biological factors. The limited studies to date provide initial evidence on the early molecular manifestations of injury, suggesting possible mechanisms for further study.

## Keywords

biomarkers, gene expression, molecular markers, risk factors, therapy

## INTRODUCTION

Osteoarthritis is a disease of the entire joint resulting from the complex disruption of normal interactions between different tissues in the knee. It is a chronic debilitating disease, a major cause of morbidity in the aging population, causes significant pain and suffering, and has only a limited selection of medical treatments. If unabated, osteoarthritis progression leads to total joint failure and surgical replacement, a costly procedure associated with significant risks. Thus, it would be advantageous to delay or halt disease progression before reaching end-stage joint failure and arthroplasty. The optimal time for therapeutic intervention has not been determined. Osteoarthritis develops over decades, beginning long before clinical symptoms become apparent. Once radiographic changes are detectable, the process is irreversible. Despite advances in preclinical studies, the pathways controlling early cartilage degeneration in osteoarthritis remain undefined. One of the challenges to identifying osteoarthritis pathogenesis is the relative lack of information on early steps in the disease

process. Posttraumatic osteoarthritis (PTOA), however, is defined by onset after a known joint trauma. Anterior cruciate ligament (ACL) and meniscus tears are leading causes of joint trauma. Altered biomechanics in ACL-deficient knees likely lead to cartilage damage [1] and PTOA in about 50% of people [2,3]. Nearly 50% of the individuals with meniscus injuries also develop PTOA over time [2,4]. Although both meniscus and ACL tears are known risk-factors for osteoarthritis, little is known about how meniscus

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## KEY POINTS

- The metabolic state of meniscus and ACL tears relates to numerous patient-specific intrinsic and extrinsic factors, which may advance our understanding of how this injury turns the knee in the direction of osteoarthritis.
- Gene and protein biomarkers provide insight into the biologic response of the knee to meniscus and ACL tears, which may predict the risk for developing osteoarthritis.
- Animal studies detail the time-course of osteoarthritis disease pathogenesis and identify molecules that potentially alter the course of disease.
- Efforts are directed towards identification of patients at elevated risk for developing PTOA after meniscus and ACL injury.
- Given the long lag between meniscus and ligament injuries and the clinical manifestations of PTOA, early intervention may lead to meaningful changes in if, when and how rapidly PTOA develops.

and ACL injuries affect the biology of the joint. Although only 12% of the overall osteoarthritis burden results from joint trauma [5], these injuries affect predominantly young, physically active patients, with potentially significant long-term implications.

As the nature and time of initial injury can be defined, PTOA is a model to study disease pathogenesis, which could be informative for primary osteoarthritis. Here, we will discuss three distinct and yet related aspects to address this knowledge gap: biological factors that influence how the knee responds to meniscus and ligament injuries, how meniscus and/or ACL tears turn the joint in the direction of PTOA and whether patient's risk for PTOA after meniscus/ACL injury can be predicted. We expect that this information will summarize current knowledge and identify opportunities to study biologic and clinical interventions for early treatment to delay or prevent PTOA in patients with meniscus and ligament injuries.

## RISK FACTORS ASSOCIATED WITH MENISCUS AND LIGAMENT INJURIES AND POSTTRAUMATIC OSTEOARTHRITIS

Presently, there is no consensus on the risk-factors associated with PTOA following meniscus and ACL tears. However, numerous intrinsic (age, obesity, sex, genetics, etc.) and extrinsic (activity level, prior injury or surgery, tear pattern, etc.) factors have some evidence suggesting a possible link with PTOA (Table 1).

## MENISCUS TEARS: FROM TRANSCRIPTOME TO THE CLINIC

### Clinical perspectives

Following partial meniscectomy, there is an elevated risk for functional deterioration of cartilage, often resulting in osteoarthritis. Irreparable damage in osteoarthritis joints include meniscus tears, maceration, and fragmentation, but little is known about the change in the histological structure. Some histological changes in degenerative meniscus tears without knee osteoarthritis include increased Safranin-O-staining intensity, decreased cell density, and some loss of collagen fiber organization [6]. There is also some evidence for pathological calcification of the cartilage matrix in osteoarthritic meniscus [7].

### Protein biomarkers

Biomarkers of cartilage and joint diseases provide insight into the pathophysiology of PTOA [8]. Limited available data demonstrate increased levels of pro-inflammatory cytokines (IL-6, IL-8, and TNF- $\alpha$ ), which could hamper cartilage regeneration [9]. Although aggrecan, COMP, MMP-13 and TIMP-1 do not appear to predict outcome of meniscus and ACL injuries [10], matrix metalloproteinase activity and prostaglandin E2 are elevated in the synovial fluid of meniscus tear patients [11]. This study showed that serum MMP-3 could be a potential biomarker for early knee osteoarthritis. The strong correlation between serum biomarkers and microstructural changes to cartilage after joint trauma demonstrates potential use of these biomarkers for precisely assessing joint degeneration (PTOA progression) after meniscus tears.

### Transcriptomic signatures

Gene expression profiling of meniscus tissues/cells is emerging as a powerful tool to understand the molecular biology of the meniscus. Genes associated with inflammation and cytokine production are elevated in the meniscus from knees with osteoarthritis whereas those associated with DNA repair are repressed. Elevation of genes enriched for inflammation pathways has been reported in older patients whereas genes associated with matrix synthesis were elevated in younger patients [12]. Obesity-related differences in transcript expression show the highest number of differentially expressed gene transcripts between obese and overweight patients, suggesting that there may be a weight-threshold above which the injured meniscus responds differently [13]. Gene expression in torn meniscus is associated overall with early degenerative changes in the knee, but only a limited number of specific

**Table 1.** Risk factors that influence meniscus and ligament tears and posttraumatic osteoarthritis after meniscus and ligament tears

Factor	Functional attributes		
	Meniscus	ACL	PTOA
Age	Increasing age is associated with meniscus injury (Jones, 2012, 22488232).	Younger age is associated with higher ACL injury rate (Roos, 1995, 7740937).	Sustaining ACL injury early in adulthood leads to greater lifetime risk and earlier onset of knee osteoarthritis and TKA (Suter, 2017, 27214559). Age is a significant factor predictive of osteoarthritis after ACL tear (Shelbourne, 2017, 28806096).
Sex	Male sex is associated with meniscus injury (Jones, 2012, 22488232).	Female sex is a risk factor for ACL tear (Smith, 2012, 23016083).	Prevalence of osteoarthritis is higher in females than males (Cross, 2014, 24553908).
Obesity	Obesity is associated with an increased risk of first hospitalization because of meniscus lesions (Kontio, 2017, 29237499). Obesity is associated with decreased knee flexion after arthroscopic partial meniscectomy (Kluczynski, 2017, 28969948). Higher BMI is associated with medial meniscus body extrusion (Zhang, 2017, 27939623).	There is a consistent association between overweight status and developing arthritis among patients having an ACL reconstruction (DiSilvestro, 2017, 29189335).	Elevated BMI is associated with knee osteoarthritis in elite football players. Future research should investigate ways to minimize the risk of osteoarthritis after knee surgery in these athletes (Smith, 2017, 27940573).
Genetics	There is some evidence for genetic predisposition for meniscus damage (Englund, 2016, 26318660).	Familial disposition is a risk factor for ACL tear (Smith, 2012, 23016083). Currently, there is insufficient evidence to conclude that genetics plays a role in ACL tears.	Genetic factors play a key role in all forms of arthritis including PTOA (Sandell, 2012, 22231237).
Tear pattern	Root tears are associated with greater pain than meniscal tears or maceration (McFarlane, 2017, 28043939). There is no relationship between meniscal damage and meniscal symptoms (McFarlane, 2017, 28043939). Young people with ACL injuries have a very high associated incidence of meniscal pathology (Reid, 2017, 26165552). Ipsilateral meniscus tear is associated with medial meniscus body extrusion (Zhang, 2017, 27939623).	Longitudinal and bucket handle tears are highly associated with ACL injuries (Jarraya, 2017, 28057326).	Combined ACL and meniscus tear increased the risk for osteoarthritis and TKA (Suter, 2017, 27214559). Medial meniscectomy is a significant factor predictive of PTOA after ACL tear (Shelbourne, 2017, 28806096). Horizontal tear of lateral discoid meniscus is a significant risk factor for radiographic progression to high grade osteoarthritis after arthroscopic partial meniscectomy (Ahn, 2017, 28987527). There is a lack of data on the relevance of different morphologic types of meniscal tears to the natural history of knee osteoarthritis (Jarraya, 2017, 28057326). Concomitant meniscal disease in patients undergoing ACL reconstruction leads to a greater risk of future osteoarthritis (Wang, 2017, 26506844).
Prior surgery	Young people with ACL injuries have a very high associated incidence of meniscal disease at the time of surgery (Reid, 2017, 26165552).	Surgical ACL reconstruction constitutes a second trauma to the acutely injured joint resulting in a prolonged elevation of already high synovial fluid levels of inflammatory cytokines (Larsson, 2017, 28522220). Lesser biomechanical loading in the ACL reconstructed limb is related to deleterious joint tissue metabolism (Pietrosimone, 2017, 28150869). Severe osteoarthritis changes were found in the patients who underwent partial meniscus resection (Järvälä, 2017, 28661696).	Previous ACL reconstruction and partial meniscectomy is associated with knee osteoarthritis in elite football players (Smith, 2017, 27940573). Patients treated nonoperatively after isolated ACL tears are at a significantly higher risk of osteoarthritis compared with age and sex-matched subjects without ACL tears (Sanders, 2017, 27221641).
Activity level	Service in Army or Marine Corps is associated with meniscus injury (Jones, 2012, 22488232). Ballgames, gymnastics and jogging predicted the highest risk of meniscus lesions (Kontio, 2017, 29237499). A gradual increase in activity (e.g. frequency, duration, intensity) may be warranted prior to returning to activities that involve running (Cattano, 2017, 27035929).	Participation in high-impact physical activities, including military service and athletics, places younger patients at higher risk of developing PTOA (Showery, 2016, 27181491).	The incidence of PTOA as an indication for arthroplasty is significantly higher than among civilians (Murtha, 2017, 27177309).
Prior injury	The ACL rupture is associated with a decreased risk of meniscectomy (Jiang, 2017, 29207986).	Prior injury increases the likelihood of ACL tear (Smith, 2012, 23016083).	Meniscectomy constitutes a risk factor for the progression of osteoarthritis after ACL reconstruction (Nakamae, 2018, 29589491). A history of prior injury is associated with new knee injuries with and without osteoarthritis (Driban, 2015, 26034152).
Chronicity of injury	Duration of complaint is associated with high risk of meniscectomy (Jiang, 2017, 29207986).	Severe osteoarthritis changes were found in the patients who had the longest delay from the primary injury to ACL reconstruction (Järvälä, 2017, 28661696).	Prolonged symptom duration is a significant risk factor for radiographic progression to high grade osteoarthritis (Ahn, 2017, 28987527).

In parentheses are last name of first author, year of publication and PubMed ID number; ACL, anterior cruciate ligament; PTOA, posttraumatic osteoarthritis; TKA, total knee arthroplasty.

genes demonstrate this relationship [14<sup>¶</sup>]. A molecular link between the gene expression pattern in meniscus tears and the degree of cartilage chondrosis in the same knee has also been reported [12]. Traumatic meniscus tears exhibit a higher inflammatory/catabolic response than degenerative tears, suggesting a (molecular) biological distinction between traumatic and degenerative tears [15<sup>¶</sup>]. Available meniscus transcriptome studies provide baseline information but more study is needed to understand how the biology of meniscus tears relates to PTOA.

## ANTERIOR CRUCIATE LIGAMENT TEARS: FROM TRANSCRIPTOME TO THE CLINIC

### Clinical perspectives

ACL-reconstruction does not prevent or reduce the risk for knee osteoarthritis, in fact, patients may be at higher risk for osteoarthritis [16].

### Protein biomarkers

Synovial fluid levels of inflammatory markers in the ACL-deficient knees likely correlate with the extent of cartilage damage [17]. The synovial fluid may exhibit a repair response immediately postinjury. This could contribute to the early cascade of joint degeneration by exposing joint tissues to an inflammatory milieu [18<sup>¶¶</sup>] and may trigger the release of inflammatory mediators that lead to long-term cartilage damage [19,20]. Limited data on protein biomarkers are available in subjects undergoing ACL-reconstruction [21,22] or subjects with meniscus/ligament injuries [23<sup>¶</sup>]. Findings from these studies suggest that changes in biomarker concentrations after ACL injuries reflect an alteration in cartilage turnover and joint metabolism [24,25]. It is plausible that there is a robust inflammatory reaction in the injured joint and a coordinated anabolic response, in an attempt to promote tissue healing and restore joint homeostasis and function [21,26]. Furthermore, surgical ACL reconstruction constitutes a second trauma resulting in a prolonged elevation of already high levels of inflammatory cytokines for example, IL-6, IL-8, IL-10, TNF and IFN $\gamma$  [27]. Increased synovial fluid concentrations of aggrecan and COMP are related to knee injury, but acute and chronic synovial fluid concentrations of aggrecan, COMP, MMP-3 and TIMP-1 have not been shown to predict PTOA after ACL injury [10<sup>¶¶</sup>].

### Transcriptomic signatures

Although there is clinical evidence that the risk for meniscal and chondral lesions in the knee increases

with time from ACL injury [28], little is known about how the biology of the injured ACL varies over time and contributes to osteoarthritis. It is plausible, and even likely, that the biology of the injured ACL has significant implications for the healing potential of the ligament. Some studies have characterized the biology of human ACL remnants [29,30] or have isolated mesenchymal stem cells [31] whereas others have utilized animal models of ACL injury [32]. The healing potential of the ACL in the knee is not clear. Although there is little evidence that the ACL has the ability to heal spontaneously, some surgeons advocate repair of the injured ligament, usually with an acute injury and rarely for chronic ACL tears. It has been reported the injured ACL loses extracellular matrix (ECM) over time [29,31–33]. Periostin (POSTN) expression is significantly higher in acute ACL tears (< 3 months from injury) compared with chronic tears (> 12 months). Changes in POSTN expression are accompanied by reduced expression of several ECM collagen genes, suggesting a possible link between POSTN and ECM synthesis. At the same time, our recent data indicate a crosstalk between ACL and chondrocytes plausibly via POSTN [34<sup>¶¶</sup>], which is also known to exacerbate cartilage catabolism via up-regulation of MMP-13 [35]. A recent study identified differentially expressed microRNAs in ACL from osteoarthritis patients [36]. Genes (e.g. *ADAMTS-1*, *BMP-2*, *RUNX-2*, *COL1A1* and *COL1A2*, *IL-6* and *TGF- $\beta$* ) involved in cartilage development and remodeling, collagen biosynthesis and degradation, inflammatory response and extracellular matrix homeostasis were predicted as potential targets of the dysregulated miRNAs.

## IDENTIFICATION OF PATIENTS AT RISK FOR DEVELOPING POSTTRAUMATIC OSTEOARTHRITIS

Meniscal injury is a definite risk-factor for osteoarthritis, with 50% of patients showing radiographic evidence for osteoarthritis within 10 years of injury [2,4]. Currently, it is not possible to identify the 50% who will develop osteoarthritis. Recent evidence using whole genome expression analysis of the cartilage from meniscus tear patients suggests that they can be subcategorized based on their expression of osteoarthritis ‘risk’ alleles and osteoarthritis gene expression [37]. In these preliminary experiments, 50% of patients clustered expressing ‘osteoarthritis risk’ alleles and 30% of patients expressed osteoarthritis-characteristic transcripts in the macroscopically normal cartilage. These patients may be at the highest risk for genesis and progression to knee osteoarthritis. The identification of patients at very

high risk for osteoarthritis after meniscus and ACL tears will facilitate the development and evaluation of interventions to delay or prevent osteoarthritis. In order to make a more evidence-based selection of patients who are at higher risk for developing PTOA after meniscus and ligament tears, knowledge of prognostic factors is essential. If the disease could be detected early or predicted by molecular parameters, treatments could be devised to intervene early and delay or prevent osteoarthritis.

If we can identify patients that will progress to osteoarthritis, treatment of the patients will change dramatically. Those patients that are not at risk avoid further evaluation and treatment. Patients who are at risk can be counseled to make lifestyle changes and are candidates for drugs that modify disease progression or reverse specific damage. Many such treatments are under investigation worldwide that could dampen or prevent the effects of injury if administered before changes become irreversible.

### **CAN WE MOVE OSTEOARTHRITIS DETECTION FROM END-STAGE DISEASE TO EARLY MOLECULAR MARKERS?**

The question is, do patients with meniscus tear exhibit evidence for early osteoarthritis in gene expression signatures of the cartilage? Currently, osteoarthritis is diagnosed at the terminal disease stage at which time little can be done to prevent total knee arthroplasty. Our goal is to move osteoarthritis from terminal disease to early detection by molecular markers (gene transcripts). Developing simple tests that allow early osteoarthritis detection is one of the top priorities in osteoarthritis research. Cutting-edge molecular markers based on stem cells, proteins, mRNAs, and epigenetic markers can now be identified. Thus, predicting the likelihood of progression to osteoarthritis is a critical factor that would facilitate early treatment to delay or prevent progression.

### **LESSONS LEARNED FROM ANIMAL MODELS**

Studies of human osteoarthritis are limited by difficulty in defining disease onset and progression, as well as its phenotypic heterogeneity. Basic mechanistic studies are often performed with tissue obtained at the time of joint replacement, which represents end-stage disease. These studies have focused largely on changes present in the cartilage; however, osteoarthritis is a condition that affects the entire joint as an organ. Various animal models of osteoarthritis have been developed in order to

study the disease process under more controlled conditions where disease onset and stages of progression can be better defined and evaluated. Animal models offer critical tools to evaluate the detrimental effects of specific meniscus or ligament injury in the initiation and progression of PTOA; help identify novel therapeutic approaches; and assess the effects of specific gene knockouts on the susceptibility to PTOA.

Small animals offer advantages as preclinical models to study PTOA, particularly genetically modified strains. In such models, meniscus and ACL damage can be achieved by surgical transection or by noninvasive mechanical means. Among all the meniscus and ACL injury models, surgical destabilization of medial meniscus (DMM) [38,39] and non-invasive ACL tear [40–42] are the most widely used and characterized models. It is obvious that meniscus and ligament tears differentially alter normal mechanics, but whether the underlying biology that drives PTOA is different or not remains unknown. Nevertheless, animal models have been used for target validation studies. Gene knockout mice have been shown to demonstrate significant protection against osteoarthritis progression (Table 2).

Salient features of PTOA after DMM generally include cartilage matrix loss, cartilage fibrillation and destruction, mild synovitis, subchondral bone thickening and osteophyte formation. The major pathways identified are the extracellular matrix–receptor interaction and the focal adhesion pathways along with the Wnt, Hedgehog and TGF- $\beta$  signaling and inflammatory pathways [43,44,45] and pain-related signaling [46]. ACL tear models also shed some light on understanding the early molecular biology after ACL rupture. The consistent phenotypes include chondrocyte apoptosis, loss of cartilage proteoglycan, synovitis and some ectopic bone formation. There is also fragmentation and cellular uptake of aggrecan. COMP expression also changes autophagy. New data reports an up-regulation of genes encoding acute pro-inflammatory markers, inducible nitric oxide synthase, IL-6 and IL-17, and the matrix degrading enzymes, ADAMTS-4 and MMP-3 in femoral cartilage (where the cartilage injury occurs), concomitant with extensive cartilage damage and bone remodeling [46]. This knowledge may guide appropriate interventions to delay or arrest the inflammatory arm of PTOA.

### **STATUS OF TREATMENT OPTIONS FOR MENISCUS AND ANTERIOR CRUCIATE LIGAMENT TEARS**

Regenerative medicine seeks to harness the potential of cell biology for tissue replacement therapies,



**Table 2.** Factors that protect or exacerbate destabilization of medial meniscus-induced osteoarthritis in mouse gene knockout models

Gene	Description	Function	Effect(s) <i>in vivo</i>	First author, year, PubMed ID
<i>Pai1</i>	Plasminogen activator inhibitor 1	A serine protease inhibitor that primarily inhibits tissue-type and urokinase-type plasminogen activators; acts as an inhibitor of fibrinolysis	Accelerated subchondral osteopenia	Moritake, 2017, 28893232
<i>Redd1</i>	Regulated in development and DNA damage response 1	Inhibits mammalian target of rapamycin to arrest cell growth and proliferation; enhances stress resistance and cell survival	Exacerbated the severity of injury-induced osteoarthritis	Alvarez-Garcia, 2017, 28334504
<i>Cdkn1a</i>	Cyclin-dependent kinase inhibitor 1 (p21)	A potent inhibitor of cell cycle progression	Increased susceptibility to osteoarthritis; more inflammation	Kihara, 2017, 28128866
<i>Ucma</i>	Upper zone of growth plate and cartilage matrix-associated protein	Involved in the negative control of osteogenic differentiation of osteochondrogenic precursor cells	Increased structural damage, proteoglycan loss, cell apoptosis	Stock, 2017, 28086000
<i>Ccl2/Ccr2</i>	C-C motif chemokine ligand 2/C-C motif chemokine receptor 2	Modulate monocyte/macrophage recruitment in multiple inflammatory diseases	Protected from osteoarthritis; reduced synovial macrophages and inflammation	Raghu, 2017, 27965260
<i>Fbxo32</i>	F-box protein 32 (Atrogin-1)	Mediates the ubiquitination and subsequent proteasomal degradation of target proteins	Failed to affect posttraumatic osteoarthritis cartilage destruction	Kim, 2017, 27480933
<i>Lcn2</i>	Lipocalin 2	Mediates metabolic homeostasis, apoptosis, and immune responses	No alteration in osteoarthritis-associated cartilage destruction	Choi, 2017, 27477830
<i>Wnt16</i>	Wnt family member 16	Nuclear translocation of $\beta$ -catenin and transcriptional activation of target genes	Increased osteoarthritis and cell apoptosis; reduced lubricin expression	Nalesso, 2017, 27147711
<i>Dp1</i>	D prostanoid receptor 1	Plays protective role in osteoarthritis, inhibits cytokine-induced catabolism	Exacerbated cartilage degeneration, increased proteoglycan degradation	Ouhaddi, 2017, 28544596

which will restore lost tissue functionality. We have recently reviewed different intra-articular delivery systems for joint diseases [47] whereas others have reviewed biological therapies for meniscus [48,49] and ligament tears [50]. From these reviews, we can conclude that recent intra-articular approaches concentrate on platforms that are safe, tunable and highly translatable, meniscus tissue engineering is on the horizon and there is a proliferation of potential

biological augmentation approaches for ACL repair and reconstruction. Long-term studies with larger cohorts and technique validation are limited in humans. Regardless, there is an increasing interest in the use of biological products in patients (Table 3). In animal models, various molecules (mostly biochemical agents) have been tested. Taking advantage of these studies, here, we provide a comprehensive list of current potential biological therapies (Table 4).

**Table 3.** Potential therapies for human meniscus and anterior cruciate ligament lesions

Treatment type	Procedure	Condition	Study patients	Maximum follow-up	Effect	First author, year, PubMed ID
Mesenchymal stem cells/collagen-scaffold implant	Arthroscopy	Isolated medial meniscus tear	5 (4 males, 1 female)	2 years	Safe; augment avascular meniscal repair	Whitehouse, 2017, 28186682
Allogenic mesenchymal precursor cells + hyaluronan	Intra-articular injection	ACL reconstruction	17 (12 males, 5 females)	2 years	Safe; well tolerated; improved symptoms and structural outcomes	Wang, 2017, 28768528
Poly-lactide bioabsorbable implant	Arthroscopy	Partial meniscus loss	16 (9 males, 7 females)	6 years	No adverse effects; significant improvement in functional and activity level	Filardo, 2017, 27395355
Actifit polyurethane meniscal scaffold	Arthroscopy	ACL reconstruction	15 (8 males, 7 females)	6 years	Safe; improved pain and activity scores	Leroy, 2017, 28373139
Grafts soaked with platelet-rich plasma	Arthroscopy	ACL reconstruction	42 (not known)	1 year	No complications; improved pain and activity scores	Ji, 2017, 29798604 <sup>a</sup>
Platelet-rich plasma	Arthroscopy	Meniscus tear	37 (30 males, 7 females)	3.5 years	Improved healing; improved functional outcomes	Kaminski, 2018, 29713647
Platelet-rich plasma with platelet-rich fibrin	Arthroscopy	Meniscus repair	17 (9 males, 8 females)	6 months	Improved activity and pain scores	Kemmochi, 2018, 29881226
Allografts with platelet-rich plasma	Intra-articular injection	Meniscus repair	31 (14 males, 17 females)	2 years	Improved all functions and pain outcomes	Zhang, 2018, 29699841
Platelet-rich plasma	Arthroscopy	ACL reconstruction	50 (22 males, 28 females)	2 years	Improved activity and pain scores	Walters, 2018, 29741923

<sup>a</sup>Article in Chinese (abstract was used for above information).

**Table 4.** Therapeutic opportunities in animal models of meniscus and anterior cruciate ligament injuries

Molecule	Description	Action	Type	Species	Route	Effect(s) <i>in vivo</i>	Model	First author, year, PubMed ID
cFos/AP-1 inhibitor	cFos/activator protein (AP)-1 inhibitor, T-5224	Controls expression by binding to the AP-1 site on the promoters of inflammatory cytokine and MMP	T-5224 dissolved in polyvinylpyrrolidone solution	Mouse	p.o.	Prevented cartilage destruction and osteophyte formation	DMM	Motomura, 2018, 29476740
Trehalose	D(+)-Trehalose dehydrate	An autophagy activator through mTOR independent pathway	Biochemical compound	Mouse	p.o.	Exerted antiapoptotic effects through the suppression of oxidative stress-induced mitochondrial injury and ER stress	DMM	Tang, 2017, 28981117
$\alpha$ -MG	Alpha Mangostin	Anti-inflammatory; antioxidant	Biochemical agent	Rat	i.p.	Ameliorated cartilage damage	DMM	Tianlong, 2017, 28858724 Pan, 2017, 28915439
Exosomes (ESC-MSCs)	Exosome from embryonic stem cell-induced mesenchymal stem cells	Immunomodulatory; anabolic	Purified exosomes	Mouse	i.a.	Impeded cartilage degeneration	DMM	Wang, 2017, 28807034
Paeonol	Phenolic compound	Antioxidant; anxiolytic-like; anti-inflammatory	Biochemical agent	Rat	i.a.	Alleviated DMM-induced articular cartilage degeneration	DMM	Liu, 2017, 28910961
Fisetin	Polyphenol and flavonoid	Antioxidant; anti-inflammatory; antiangiogenic; antitumor activities	Biochemical agent	Mouse	p.o.	Alleviated the progression of osteoarthritis	DMM	Zheng, 2017, 28213268
Plumbagin	A plant extract	Anti-inflammatory	Biochemical agent	Mouse	p.o.	Prevented cartilage degeneration, less synovitis	DMM	Zheng, 2017, 28168658
Proteoglycan 4	Lubricin, a mucinous glycoprotein	Joint lubricant	Purified recombinant protein	Yucatan minipig	i.a.	Lowered osteoarthritic biomarkers; reduced cartilage damage; suppressed joint inflammation	DMM	Waller, 2017, 28129516
MMP-13/ADAMTS-4	Matrix metalloproteinase 13/a disintegrin and metalloproteinase with thrombospondin motifs 4	Matrix degrading enzymes	Chemically modified siRNA	Mouse	i.a.	Less cartilage degeneration, improvement in histological score	DMM	Hoshi, 2017, 28120109
Ginsenoside-Rg5	Steroid glycosides and triterpene saponins	Anticancer; anti-inflammatory; antiangiogenic	Biochemical agent	Rat	i.g.	Reduced inflammatory cytokines; prevented cartilage degradation; inhibited apoptosis	ACLT+MMT	Zhnag, 2017, 28112382
Bindarit	Synthetic molecule	Inhibits expression of the CCL2, CCL8 and CCL7; attenuates inflammation	Biochemical agent	Mouse	p.o.	Abrogate CCL2 secretion	DMM	Raghu, 2017, 27965260
Gremlin 1	A diffusible protein that binds to ligands of the TGF- $\beta$ family	A BMP-2 inhibitor	Recombinant protein	Rat	i.a.	Attenuated exercise-induced osteoarthritis	DMM	Iijima, 2017, 27965139
Butein	A polyphenolic compound	Antioxidant; antifibrogenic; anti-inflammatory	Biochemical agent	Mouse	i.p.	Decreased cartilage erosion; lowered osteoarthritis	DMM	Zheng, 2017, 27863298
RS-504393	Selective antagonist	Specific CCR2 antagonist	Synthetic chemical	Mouse	p.o.	Ameliorated osteoarthritis	DMM	Longobardi, 2017, 27856294
rhGDF5	Recombinant human growth and differentiation factor	Stimulate anabolic (repair) response	Purified recombinant protein	Rat	i.a.	Reduced cartilage lesions	MMT	Parrish, 2017, 27851984
NAPA	A N-acetyl phenylalanine derivative	Anti-inflammatory	Biochemical agent	Mouse	i.a.	Improved cartilage thickness; reduced osteoarthritis; suppressed catabolic enzymes	DMM	Veronesi, 2017, 27836674
Sulf1	Sulfatase 1	Edits the sulfation pattern of heparin sulfate proteoglycan	Recombined protein	Mouse	i.a.	Suppressed glycosaminoglycan loss; reduced expression of matrix degrading enzyme(s)	DMM	Otsuki, 2017, 27808352
MR16-1	Anti-IL-6 receptor neutralizing antibody	Neutralizes IL-6 catabolic effects	Monoclonal antibody	Mouse	i.p.	Alleviate DMM-induced osteoarthritis	DMM	Latourte, 2017, 27789465
AM/UC	Amniotic membrane and umbilical cord tissues	Potent anti-inflammatory; support wound healing	Particulate	Rat	i.a.	Lessen overall joint destruction	MMT	Raines, 2017, 27707109
Atrogin-1	Encoded by <i>Fbxo32</i> gene	Blocks ubiquitination and proteasomal degradation pathway and transcriptional activity of NF- $\kappa$ B	Adenovirus	Mouse	i.a.	No effect on cartilage destruction	DMM	Kim, 2017, 27480933

**Table 4 (Continued)**

Molecule	Description	Action	Type	Species	Route	Effect(s) in vivo	Model	First author, year, PubMed ID
LCN2	Lipocalin 2	Mediates metabolic homeostasis, apoptosis, and immune responses	Adenovirus	Mouse	i.a.	No effect on cartilage degeneration	DMM	Choi, 2017, 27477830
Protandim	A potent nutraceutical	Antioxidant, anticatabolic	Not known	Mouse	i.a.	Reduced osteoarthritis, lower cartilage loss	DMM	Abusarah, 2017, 27463229
Tanezumab	Nerve growth factor inhibitor	Plays a role in pain and physical function	Monoclonal antibody	Rat	s.c.	Prevented gait deficiency; resulted in increased cartilage damage	MMT	LaBranche, 2017, 27381034
PTH 1-34	Parathyroid hormone (teriparatide)	Inhibits cartilage degeneration and terminal differentiation of chondrocytes, anabolic	Hormone	Rat	s.c.	Upregulated Wnt/ $\beta$ catenin signaling pathway, downregulated Runx2	ACLT+MMx	Ma, 2017, 29039525
Isoliquiritigenin	A natural flavonoid extracted from licorice	Vasorelaxant, antiviral, antioxidant, antiplatelet aggregation, anti-inflammatory	Biochemical agent	Mouse	i.p.	Inhibited NF- $\kappa$ B p65 expression, increased cartilage thickness and production of proteoglycan, chondroprotective	ACLT	Ji, 2017, 29039445
XG-ADSCs	Xanthan gum (a polysaccharide) and adipose-derived stem cells	Gum is cartilage protective; cells possess tissue regeneration potential	A vehicle glue and self-renewal stem cell product	Rat	i.a.	Attenuated osteoarthritis	ACLT	Mei, 2017, 29030067
Halofuginone	A coccidiostat (halogenated derivative of febafugine)	Inhibits development of T helper 17 cells, has potential to treat autoimmune diseases	Synthetic compound	Mouse		Reduced the expression of matrix degrading enzymes; lowered osteoarthritis; prevented cartilage loss	ACLT	Mu, 2017, 28944864
Tanshinone IIA	Phenanthrenequinone constituent of Chinese medicinal herb Danshen	Anti-inflammatory. Antioxidant	Biochemical agent	Rat	p.o.	Reduced levels of inflammatory cytokines	ACLT+MMx	Jia, 2017, 28849083
Chitosan+HA	Chitosan (polysaccharide) and hyaluronic acid	Chondroprotective, chondroproliferative	Hybrid hydrogel formulation	Rabbit	i.a.	Enhanced subchondral bone loss	ACLT	Rieger, 2017, 28810851
Hylan G-F 20	High-molecular-weight crosslinked hyaluronan composed of hylan A mixed with hylan B,	Viscosupplementation, a hyaluronan derivative	Fluid	Rabbit	i.a.	Prevented macroscopic lesions	ACLT	Levillain, 2017, 28554494
Pterostilbene	A phytoalexin and an analog of resveratrol, natural component of blueberry	Antioxidant, antiproliferative, apoptosis inducer, antihyperglycemic, antidiabetic	Biochemical agent	Rats	p.o.	Prevented cartilage degeneration and promoted nuclear translocation of Nrf2 in cartilage	ACLT+MMx	Xue, 2017, 28410217
Lenalidomide	A thalidomide derivative	An inhibitor of tumor necrosis factor alpha, immunomodulatory properties	Chemical agent	Mouse	i.p.	Suppressed the expression of TNF- $\alpha$ and I $\kappa$ B1 and reduced the number of CD31 positive cells in the subchondral bone	ACLT	Wang, 2017, 28358372
Ginsenoside Rg1	Major active ingredients of ginseng	Inhibits inflammatory responses	Biochemical agent	Rat	p.o.	Attenuated cartilage degeneration; reduced type II collagen loss and MMP-13 levels	ACLT	Cheng, 2017, 28287423
Ginsenoside-Rg5	Major active ingredients of ginseng	Inhibits inflammatory responses	Biochemical agent	Rat	p.o.	Prevented cartilage degradation; inhibited cartilage apoptosis	ACLT+MMx	Zhang, 2017, 28112382
A2M	Alpha-2-macroglobulin (a blood product)	Bio-inhibitor for catabolic enzymes	Biological product	Rat	i.a.	Chondroprotective, attenuated cartilage degeneration	ACLT	Zhang, 2017, 28743292
Resveratrol	3,4',5-trihydroxystilbene	Anti-inflammatory	Biochemical agent	Mouse	DMM	Delayed articular cartilage degeneration; promoted chondrocyte autophagy	DMM	Qin, 2017, 28669597

ACLT, anterior cruciate ligament transection; BMP, bone morphogenetic protein; CCL2, C-C motif ligand 2; CCL7, C-C motif ligand 7; CCL8, C-C motif ligand 8; CCR2, C-C chemokine receptor type 2; CD31, cluster of differentiation 31; DMM, destabilization of medial meniscus; ER, endoplasmic reticulum; i.a., intra-articular; i.g., intra-gastric; i.p., intraperitoneal; IL-6, interleukin 6; I $\kappa$ B1, leucine-rich alpha2-glycoprotein 1; MMP, matrix metalloproteinase; MMT, medial meniscus transection (tear); MMx (partial) medial meniscectomy; NF $\kappa$ B, nuclear factor kappa B; Nrf2, nuclear factor 2; p.o., per os (oral); p65, transcription factor p65 (NF $\kappa$ B subunit); Runx2, Runx-related transcription factor 2; s.c. subcutaneous; siRNA, small interfering ribonucleic acid; TGF- $\beta$ , transforming growth factor beta; TNF- $\alpha$ , tumor necrosis factor alpha.

## CONCLUSION

Current review of literature suggests an emerging paradigm in the injury-inflicted osteoarthritis. Clearly, the metabolic state of meniscus and ligament tears relates to several patient-specific factors, which may advance our understanding of how this injury turns the knee in the direction of osteoarthritis. No doubt, the meniscus and ACL are key tissues related to the development of osteoarthritis in the knee. Future investigations will evaluate biological risk factors in patients after meniscus and ligament injuries and follow cohorts to identify information that truly predicts osteoarthritis. The goal is to identify mechanistic pathways that could be targets for interventions to delay or prevent this development. This information could lead to more precise prediction of osteoarthritis risk following meniscectomy or ACL-reconstruction that in turn can guide the development of tailored interventions to decrease that risk. Given the long lag between meniscus and ligament injuries and the clinical manifestations of PTOA, early intervention may lead to meaningful changes in if, when and how rapidly PTOA develops. This would shift the current treatment paradigm for patients with meniscus or ACL injury from management of symptoms to altering the natural history of disease.

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## Conflicts of interest

*There are no conflicts of interest.*

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# Molecular taxonomy of osteoarthritis for patient stratification, disease management and drug development: biochemical markers associated with emerging clinical phenotypes and molecular endotypes

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## Purpose of review

This review focuses on the molecular taxonomy of osteoarthritis from the perspective of molecular biomarkers. We discuss how wet biochemical markers may be used to understand disease pathogenesis and progression and define molecular endotypes of osteoarthritis and how these correspond to clinical phenotypes.

## Recent findings

Emerging evidence suggests that osteoarthritis is a heterogeneous and multifaceted disease with multiple causes, molecular endotypes and corresponding clinical phenotypes. Biomarkers may be employed as tools for patient stratification in clinical trials, enhanced disease management in the primary care centres of the future and for directing more rational and targeted osteoarthritis drug development. Proximal molecular biomarkers (e.g. synovial fluid) are more likely to distinguish between molecular endotypes because there is less interference from systemic sources of biomarker noise, including comorbidities.

## Summary

In this review, we have focused on the molecular biomarkers of four distinct osteoarthritis subtypes including inflammatory, subchondral bone remodelling, metabolic syndrome and senescent age-related endotypes, which have corresponding phenotypes. Progress in the field of osteoarthritis endotype and phenotype research requires a better understanding of molecular biomarkers that may be used in conjunction with imaging, pain and functional assessments for the design of more effective, stratified and individualized osteoarthritis treatments.

## Keywords

biochemical marker, biomarker, clinical phenotype, drug development, molecular endotype, molecular taxonomy, osteoarthritis, patient stratification

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## KEY POINTS

- Osteoarthritis is the most common form of joint disease.
- The molecular taxonomy of rheumatoid arthritis is becoming well established but more research is required to unravel the molecular taxonomy of osteoarthritis.
- Molecular biomarkers are important tools for patient stratification, disease management and drug development.
- Biomarkers are associated with the emerging clinical phenotypes of osteoarthritis and may be used to define the underlying molecular endotypes.
- Proximal molecular biomarkers in synovial fluid are more likely to distinguish between molecular endotypes than those in systemic circulation highlighting the role of synovial fluid biobanks in future osteoarthritis research.

## INTRODUCTION

The 20th century witnessed an unprecedented increase in average human lifespan [1]. The same trend continues in the 21st century as the world's human population continues to expand and age, presenting significant challenges for governments and healthcare systems working to ensure that people live longer and healthier lives without suffering severe disability [2]. According to the World Health Organization (WHO) (<http://www.who.int>) between 2015 and 2050, the proportion of the world's population over 60 years will increase from 12% to 22% (<http://www.who.int/news-room/fact-sheets/detail/ageing-and-health>). By 2020, the number of people aged 60 years and older will outnumber children younger than 5 years. Enhanced longevity combined with the worldwide obesity crisis mean that an ever-growing number of people are susceptible to age-related diseases, particularly musculoskeletal diseases [3]. Osteoarthritis is the most common form of joint disease and a major cause of pain and disability, affecting the welfare of 240 million people across developed as well as developing countries [4]. Consequently, the impact of osteoarthritis on society is substantial, grossly under-estimated and rising at an alarming rate [5].

Osteoarthritis is a modern disease in terms of human evolution. Osteoarthritis is closely linked to the beginning of the 'Anthropocene,' an epoch dating from the commencement of significant human impact on the Earth's geology and ecosystems. It is defined by the global domination of the human species and the rapid climate and

environmental change brought about by irreversible impacts of anthropogenic activity. Although our ancestors have been around for more than six million years, they had relatively little impact on the planet. In contrast, modern humans (*Homo sapiens*) have had the most destructive global impact in recent geological time [6]. Although civilization as we know it is only about 6000–8000 years old and industrialization started in the 1800s, humans had the most significant impact on the planet over the last 200 years. So, what is the relevance of this global change to joint disease, and especially to osteoarthritis?

It has been suggested that osteoarthritis is an 'evolutionary mismatch disease' in which modern-day factors are crucially important [7]. There is compelling evidence that the industrial revolution caused a sharp rise in the incidence of inflammatory diseases such as cancer, obesity and arthritis. Independent risk factors for osteoarthritis have, therefore, either arisen or have become amplified in the postindustrial era [8].

The increased dependency of humans on mechanised vehicles, the significantly reduced levels of physical activity associated with urbanization, and the consumption of obesogenic processed foods (i.e. foods high in sugar and saturated fat and low in fibre) have been proposed to be key components of the modern environment that is believed to drive the development of obesity [9] and the sedentary activities and behaviour changes associated with becoming overweight and obese. These behavioural changes are essential prerequisites for the pathogenesis and progression of osteoarthritis [10]. However, it could also be argued that these are not the only reasons for an increase in osteoarthritis in modern humans. There is data from archaeological digs of Native American settlements that suggests that a combination of major alterations in diet (i.e. relying more on carbohydrates derived from agriculture and less on hunting and gathering) and increased workload predisposed to the development of arthritis. Therefore, it would seem plausible to suggest that a sedentary lifestyle predisposes to the development of obesity, which leads to osteoarthritis, whereas a poor diet coupled with heavy workloads leads to osteoarthritis through alternative mechanisms.

Although osteoarthritis poses a large and growing burden to society, results of clinical trials of potential disease-modifying osteoarthritis drugs (DMOADs) and other disease-modifying interventions have been disappointing. One potential explanation is that we are still using blunt and ineffective tools for classifying osteoarthritis and monitoring disease progression. Conventional radiography still remains the endpoint in clinical trials of potential

disease-modifying interventions because of regulatory agency requirements. Major radiographic hallmarks of primary osteoarthritis are progressive loss of articular cartilage together with osteophyte formation, subchondral sclerosis and subchondral cysts [11]. However, radiography has some major limitations. First, it is an indirect and relatively insensitive measure of the limited and/or focal articular cartilage loss that is typical of early-stage osteoarthritis [12]. Second, radiography provides a snapshot of structural joint changes at a single point in time but fails to measure dynamic changes during the process of osteoarthritis pathogenesis and informs about the quality of joint tissues to a limited extent. Third, radiography is limited by reproducibility problems and a poor correlation between radiographic findings and symptoms. Fourth, radiography is good for assessing bone attrition and osteophytes, but is not able to show synovitis and bone marrow lesions [13].

Another potential reason for the disappointing results so far of clinical trials of disease-modifying interventions might be that osteoarthritis is a heterogeneous disease. Studies suggest that knee osteoarthritis may be a heterogeneous collection of overlapping subtypes with different clinical phenotypes and different molecular endotypes [14]. For example, osteoarthritis is hypothesized to have inflammatory and metabolic components in addition to biomechanical features [15]. Therefore, there is an urgent need to develop more sensitive tools (biomarkers) for osteoarthritis classification and monitoring. It is envisaged that such tools will help diagnose osteoarthritis at an earlier stage, even during the asymptomatic and molecular phase. Ideally, these biomarkers would also improve our ability to enrich clinical trials with osteoarthritis progressors and help develop patient stratification strategies to identify the right patient for the right treatment. Altogether, this would reduce the required duration and size of clinical trials and improve the ability to demonstrate effectiveness of potential disease-modifying interventions.

The aim of this focused review is to discuss existing and emerging biochemical markers that may be used to supplement our understanding of the molecular taxonomy of osteoarthritis and define endotypes corresponding to clinical phenotypes. This article does not attempt to provide exhaustively detailed definitions of osteoarthritis and osteoarthritis biomarkers. We provide brief and concise definitions and frameworks and refer readers to other recent reviews that provide background information about clinical osteoarthritis phenotypes and molecular endotypes and recent progress on biochemical markers.

## MOLECULAR ALTERATIONS IN OSTEOARTHRITIS

Once symptoms and structural changes from osteoarthritis become clinically apparent, the disease process very likely is already in the final common pathway that follows from numerous initiating and inciting events. Although the precise sequence of molecular events involved in the pathogenesis of osteoarthritis is not clear and may vary between individuals, there are biomechanical [16], inflammatory [15] and metabolic [17<sup>••</sup>] factors that have been shown to play key roles in the initiation and progression of the disease. We now know that chondrocytes are not simply passive participants and bystanders in disease progression. Chondrocytes become progressively inflammatory and activated in osteoarthritis. The increased pro-catabolic and pro-inflammatory factors in osteoarthritis reduce anabolic activity, alter cellular metabolism and disturb the delicate balance between extracellular matrix (ECM) synthesis and degradation [18]. Other joint tissues can contribute to the loss of homeostasis and metabolic regulation in the joint as well, as osteoarthritis also involves the synovial membrane [19,20], subchondral bone [21] and peri-articular soft tissues [22].

Synovitis appears to be a very common feature in both the early and late phases of osteoarthritis [23], with infiltrating macrophages, T cells and mast cells [24]. Synovitis and the innate inflammatory network [25] expectedly play a key role in osteoarthritis; pro-inflammatory cytokines were most frequently found in the inflamed synovium [24]. Catabolic and pro-inflammatory mediators such as cytokines, reactive oxygen species (ROS), nitric oxide (NO), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and neuropeptides from the inflamed synovium, all affect chondrocyte metabolism and matrix turnover in the cartilage [26]. Synovitis leads to excess production of proteolytic enzymes responsible for cartilage breakdown [27]. Cartilage matrix catabolism releases molecules that perpetuate synovial inflammation, creating a vicious and self-perpetuating cycle [27]. Inflammatory mediators from chondrocytes and synoviocytes also drive oxidative stress and inflict joint damage by releasing ROS [28]. Once activated by stress because of pro-inflammatory cytokines, prostaglandins and ROS, the normally quiescent articular chondrocytes become activated and undergo a phenotypic shift described as 'chondro-senescence' with the development of a senescence-associated secretory phenotype (SASP) [29] and further disruption of homeostasis and metabolism in cartilage [30].

## BIOMARKERS IN OSTEOARTHRITIS

Biomarkers in osteoarthritis have been divided into 'dry' and 'wet' markers [31,32]. 'Dry' biomarkers, for



example, include parameters derived from imaging or questionnaires. ‘Wet’ biomarkers are biochemical markers that can be measured in body fluids, cells and/or extracts of cells and tissues, and include proteins, protein fragments, bioactive lipids [33], metabolites [34] and/or extracellular genomic material [35]. Existing osteoarthritis biomarkers are classically categorized according to the targeted osteoarthritis process, as either markers of cartilage degradation/synthesis, bone degradation/synthesis or synovial tissue inflammation and fibrosis [36–38].

The BIPED system first introduced by Bauer *et al.* [39] later introduced for biochemical markers for osteoarthritis by van Spil *et al.* [36] and finally extended by Kraus *et al.* [40] classified biochemical markers into six categories corresponding to burden of disease, investigational, prognostic, efficacy of intervention, diagnostic biomarkers and safety biomarkers (BIPEDs).

Moreover, in 2011, the OARSI/FDA osteoarthritis Biomarkers Working Group classified biomarkers into four categories according to their current level of qualification for drug development (i.e., exploration, demonstration, characterization and surrogacy levels) [40]. More recently, regulatory authorities such as the Food and Drug Administration (FDA) (<https://www.fda.gov>) have published draft guidelines to help define the usage of biomarkers for drug development and clinical usage, including the BEST criteria (<https://www.ncbi.nlm.nih.gov/books/NBK326791/>). The BEST glossary aims to capture distinctions between biomarkers and clinical assessments and to describe their distinct roles in biomedical research, clinical practice and medical product development. The BEST criteria may be most suited to formulating, testing and validating a hypothesis for a biomarker. BEST provides a more detailed level of distinction concerning the clinical usage of the biomarker in question, which is needed when approval, qualification or labelling are needed or desired for that biomarker.

Metabolic changes are common to cartilage, bone and synovium and these joint tissues should all be considered as important sources of circulating biomarkers. These tissues are all affected by external and internal drivers of disease progression, such as inflammation, injury or biomechanical alterations, metabolic reprogramming and immunomodulation. Synovial tissues are not the only source of biochemical markers, but also, they dominate the published literature and are themselves targets of the biomarkers that have inflammatory bioactivity. In other words, ECM breakdown products that serve as wet biomarkers of joint damage can function also as pro-inflammatory and catabolic signalling molecules.

## DEFINITION OF OSTEOARHRITIS PHENOTYPES AND ENDOTYPES

A clear definition of clinical phenotypes and molecular endotypes is crucial for a mechanistic understanding of any disease and we are only beginning to recognize this in osteoarthritis research and clinical practice. Soluble biochemical markers are particularly useful for the molecular endotyping of patients and for generating molecular profiles that are related to distinct clinical phenotypes. However, osteoarthritis patients may have overlapping clinical phenotypes, complicating the task of identifying distinct molecular endotypes and their corresponding clinical profiles. Moreover, comorbidities might correspond to the presence and extent of endotypes and phenotypes.

Research into molecular endotypes and clinical phenotypes in other areas, such as asthma, suggest that the term ‘clinical phenotype’ should relate to the presentation of a disease, whereas ‘molecular endotype’ should pertain to the molecular pathogenesis of a disease and ignore its clinical presentation [41<sup>¶</sup>]. Unfortunately, these definitions are not yet uniformly used in osteoarthritis literature [42]. Efforts to standardize definitions and approaches are underway.

Current evidence suggests that osteoarthritis patients can fall into multiple endotypic subgroups defined on the basis of the main driver of disease. The primary concept that we intend to focus on will be the molecular markers of disease endotypes, the main driver of disease, rather than clinical phenotypes. Thus far, a number of mechanistic osteoarthritis subgroups have been proposed:

- (1) Inflammatory phenotype (local and systemic)
- (2) Metabolic syndrome phenotype
- (3) Senescent ageing-related phenotype
- (4) Endocrine phenotype (oestrogen deficiency)
- (5) Sarcopenic muscle phenotype (beyond the scope of this review)

On the basis of a systematic review, six main clinical phenotypes were proposed by Dell’Isola *et al.* [43], including inflammatory, metabolic syndrome and bone and cartilage metabolism phenotypes. Other groups including ours have proposed slightly different phenotype descriptions based on pathophysiological characteristics [17<sup>¶¶</sup>]. Although different articles have used different and sometimes confusing terminologies, especially confusing the terms endotype and phenotype, efforts are currently underway to develop a unified framework for defining osteoarthritis phenotypes and conducting and reporting research based on distinct phenotypes. Therefore, the aim of this review is to focus on

biomarkers of the molecular endotypes as the main drivers of osteoarthritis and how they relate to the emerging phenotypes (Fig. 1) (Fig. 2).

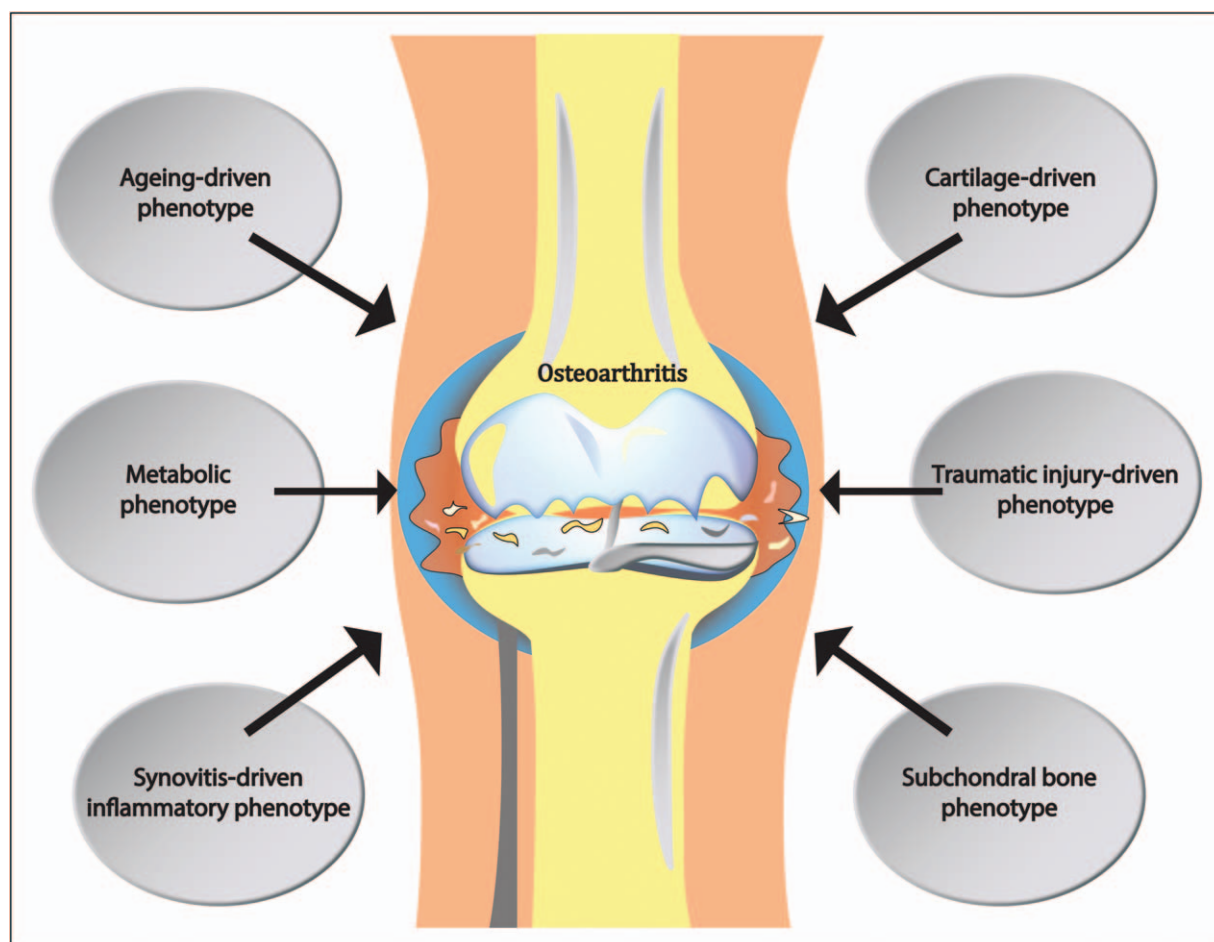
### BIOMARKERS OF THE INFLAMMATORY ENDOTYPE

Recent research suggests that osteoarthritis has substantial inflammatory components in addition to mechanical components [15]. The inflammatory endotype of osteoarthritis could be characterized by high levels of local and systemic inflammatory biomarkers [44]. There is evidence for involvement of systemic inflammation in osteoarthritis, with evidence of increased circulating c-reactive protein (CRP, a protein produced by the liver in response to inflammation) [45], c-reactive protein M (CRPM, an inflammatory derivative of CRP), tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) [46], interleukin 6 (IL-6, an inflammatory cytokine largely derived from the liver), interleukin 17 (IL-17, from T cells), chemokine

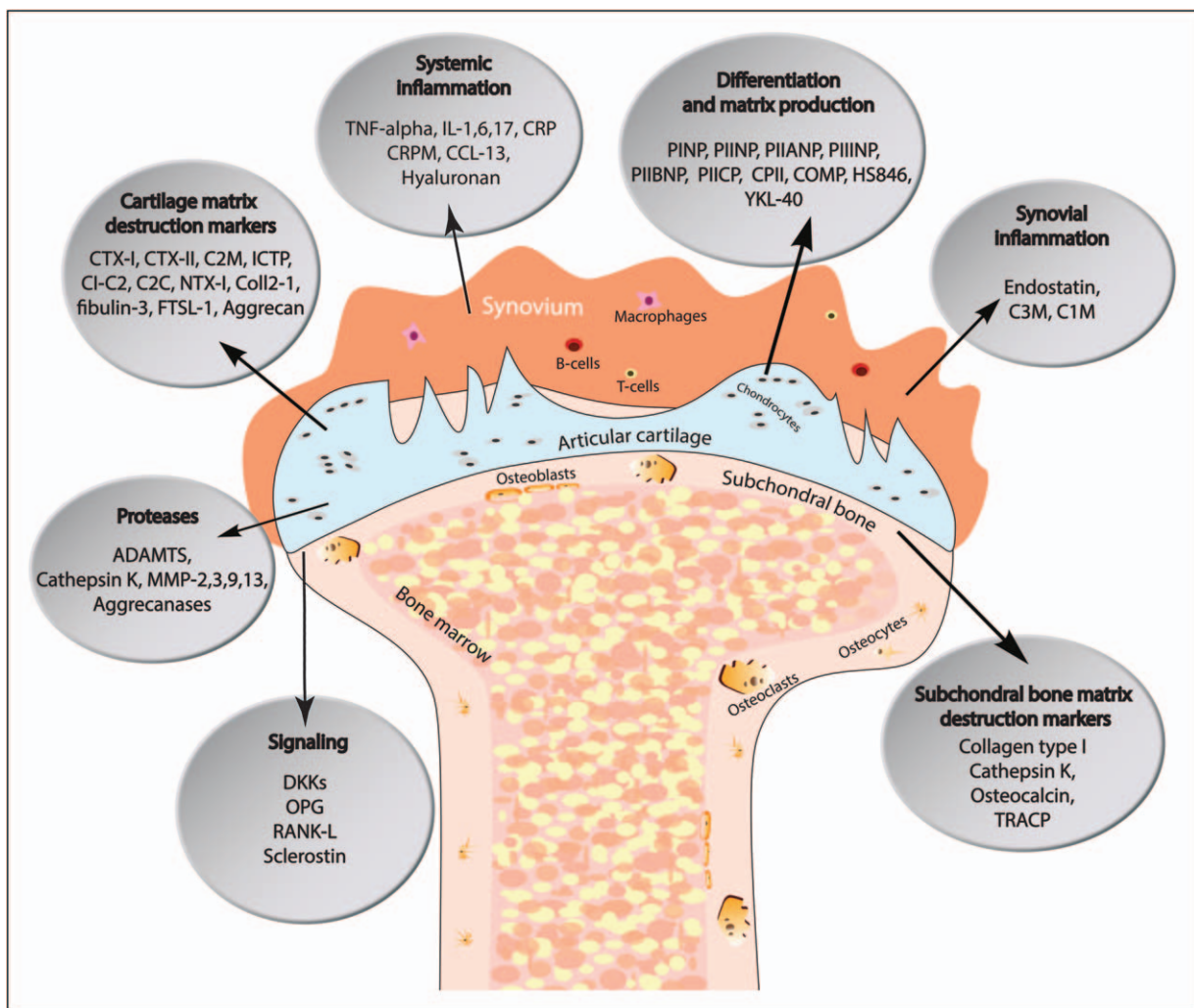
(C-C motif) 13 (CCL13), other chemokines and serum hyaluronan. In addition, the inflammatory endotype is associated with markers of 'local' or 'synovial' inflammation, including C3M, C1M (a marker of tissue collagen turnover) [47], synovial fluid-derived hyaluronan and endostatin, which is an angiogenic marker implicated in Sjögren's syndrome [48], systemic sclerosis [49], collagen-induced arthritis [50] and adjuvant-induced arthritis [51]. Endostatin is an angiogenesis inhibitor, which interferes with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF) [38].

### BIOMARKERS OF THE BONE ENDOTYPE (BONE REMODELLING AND OSTEOPHYTES)

The relationship between bone turnover and osteoarthritis has been established in the osteoarthritis



**FIGURE 1.** Emerging phenotypes of osteoarthritis. Patients with osteoarthritis may potentially be classified into distinct phenotypic subgroups defined on the basis of the main driver of disease. Cartilage, bone and synovium are all affected by external and internal drivers of disease such as inflammation, traumatic joint injury or biomechanical alterations, metabolic reprogramming and immunomodulation.



**FIGURE 2.** Biomarkers of osteoarthritis. Cartilage matrix destruction markers: C-terminal telopeptide of collagen I and II (CTX-I and CTX-II), matrix metalloproteinases (MMP)-derived collagen degradation neo-epitopes of collagen II – C2M, C-telopeptide of type I collagen (ICTP), peptides C1–C2, collagenase-generated protein (C2C), N-telopeptide of type I collagen (NTX-I), collagen type II peptide – Coll2-1, fibulin-3, follistatin-related protein 1 (FTSL-1), Aggrecan. Systemic inflammation markers: TNF-alpha, interleukin 1,6,17 (IL-1,6,17), C-reactive protein (CRP), MMP-dependent degradation product of C-reactive protein (CRPM), chemokine C-C, ligand 13 (CCL-13), hyaluronan. Differentiation and matrix production markers: N-terminal propeptides of type I, II, III collagens (PINP, PIINP, PIIINP); N-terminal propeptide of procollagens IIA (PIIANP) and IIB (PIIBNP), C-terminal propeptide of type II collagen PIICP, type II synthesis C-propeptide (CPII), cartilage oligomeric matrix protein (COMP), heat-shock protein 846 (HS846), human cartilage glycoprotein-39 (YKL-40). Synovial inflammation markers: endostatin, neoepitope biomarkers: MMP-derived collagen degradation neoepitopes of collagen I, III (C1M, C3M). Subchondral bone matrix destruction markers: collagen type I, Cathepsin K, osteocalcin, tartrate-resistant acid phosphatase (TRACP). Signalling markers: Dickkopf proteins (DKKs), osteoprotegerin (OPG), nuclear factor  $\kappa$ B ligand (RANKL), sclerostin. Proteases: cleaving proteases A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), Cathepsin L, matrix metalloproteinases 2,3,9,13 (MMP-2,3,9,13), aggrecanases.

field, and has received substantial interest in recent years, particularly in the context of biochemical markers [38,52,53]. This relationship is dependent on stage and origin of disease. The bone effects are isolated to changes in the subchondral compartment of the joint. Furthermore, bone–cartilage cross-talk involves biomarkers, biochemical and

biomechanical signalling between these tissue compartments and is crucially important in osteoarthritis disease progression [54]. The development of osteophytes has also been shown to be important for the development of pain in later disease stages [55]. Osteophytes themselves were not painful but they are markers for other sources of pain and as



they are a hallmark of osteoarthritis development, they could be an important target for pain management and retarding disease progression [56]. Bone remodelling is a dynamic process where the process of bone resorption by osteoclasts is balanced by adequate amounts of new bone formation by the bone-forming osteoblasts [57]. Structural and molecular changes in the subchondral bone region are associated with increased vascularization, bone marrow lesions and increased microfractures, all leading to substantial increases in bone remodelling and tissue turnover [58].

Several biomarkers exist for the measurement of bone matrix tissue turnover [59]. C-terminal telopeptide of collagen I (CTX-I) measures the degradation of collagen type I by the cysteine protease cathepsin K secreted by osteoclasts during bone resorption [60]. CTX-I, also exists in an alpha isomerized form associated with turnover of young bone [61]. In a longitudinal study of patients with symptomatic and radiographic osteoarthritis, alpha CTX was associated with increased subchondral bone turnover measured by bone scintigraphy and was associated with osteoarthritis progression based on osteophyte score and JSN [61]. It has been proposed that novel pharmacological and biological treatment for osteoarthritis should include targeting of the bone–cartilage interface and biochemical markers that can measure dynamic changes at this interface will be useful for osteoarthritis drug development [58] (Fig. 1).

## BIOMARKERS OF THE METABOLIC SYNDROME ENDOTYPE

Osteoarthritis has been shown to be associated with an increased prevalence of metabolic syndrome (MetS) [62,63]. Metabolic syndrome associated osteoarthritis (MetS-OA) has been suggested as a subtype of osteoarthritis owing to the association between osteoarthritis and metabolic syndrome [64]. The metabolic syndrome phenotype has been defined in different ways, also because of differences between available datasets. Francis Berenbaum has defined MetSOA as: 'A generalised definition of MetS-OA is a patient aged between 45–65 years with generalised osteoarthritis, with a minimum of one component of metabolic syndrome, that is overweight or obese.' Although this is a useful and important definition, there is no generalized consensus about this definition. Others have defined MetS-OA as a knee osteoarthritis patient with diabetes mellitus and obesity, irrespective of hypertension [65]. Another Dutch study on patients with knee osteoarthritis has identified five osteoarthritis subtypes including 'obese phenotype,' which corresponds to MetS-OA [66]. Almost every component

of the metabolic syndrome has been associated with incidence and/or progression of osteoarthritis, although results are sometimes contradictory between studies [67,68]. Moreover, the relevance of individual metabolic syndrome components may differ between joints [69].

Various metabolic factors including lipid toxicity, insulin resistance, adipokines and systemic low-grade inflammation are likely to contribute to osteoarthritis pathogenesis. Metabolic triggered inflammation, also known as 'metaflammation,' is a subclass of inflammation, which may involve many similar molecules and signalling pathways involved in classical inflammation and which is initiated by factors involved in metabolic diseases, potentially as a result of conditions associated with metabolic surplus [70]. Metaflammation induced by lipids, cytokines, adipokines and vitamin D has been implicated in osteoarthritis pathogenesis [71]. Increased adipose tissue in overweight and obese patients with osteoarthritis results in increased levels of circulating adipokines, which may have a direct role in joint degradation [72,73]. The adipokines leptin, resistin, visfatin and adiponectin have been found to be at higher concentrations in either plasma or synovial fluid in osteoarthritis patients compared with control counterparts [74]. Hyperglycaemic-associated advanced glycation end products (AGEs) are known to accumulate in muscle and connective tissues and cross-link with target proteins [3<sup>o</sup>]. Nonenzymatic glycation of collagen results in pathologic stiffening of cartilage and ECM. The advanced glycation end (AGE) product, pentosidine, has been found to be present in synovial fluid, serum and cartilage from patients with osteoarthritis [75].

A different approach to the proposed metabolic syndrome phenotype might include innovative omics technologies such as metabolomics. One study identified three metabolically distinct subcategories, endotypes of knee osteoarthritis that differed in synovial fluid levels of acylcarnitines, glycerophospholipids, sphingolipids and a biogenic amine [76]. However, the subtypes identified in this study did not coincide with classical risk factors for osteoarthritis (e.g. sex, age) or components of the metabolic syndrome (e.g. hypertension, diabetes mellitus). Also, the potential clinical consequences of these findings remain to be established.

## BIOMARKERS OF THE SENESCENT AGEING-RELATED ENDOTYPE

A well known inverse relationship exists between mammalian longevity and the aging rate of collagen, expressed as a progressive increase in the stiffness of collagen-rich tissues like arteries, lungs and



articular cartilage. During senescence, extracellular matrix is characterized by decreased solubility and proteolytic digestibility. An age-related accumulation of pentosidine in human ECM has been demonstrated [77]. In a recent study, age-related osteoarthritis severity in guinea pigs was associated with markedly increased levels of glycated, oxidized, and nitrated amino acids [78<sup>\*\*\*</sup>]. In addition, glucosepane and dityrosine increased progressively with age and the advance of disease in both osteoarthritis animal models and osteoarthritis patient samples [78<sup>\*\*\*</sup>]. Glucosepane was described as a major cross-linker of the senescent ECM, accounting for collagen modification in diabetes, and potentially, in age-related hormonal alterations [79]. Furthermore, the altered secretion of cytokines by senescent cells impacts the immune system and its response to joint tissue trauma, as well as the interactions between those cells and the local tissue environment [80]. These data also emphasize the potential intersection of the senescent osteoarthritis endotype with other endotypes, including the metabolic and inflammatory endotypes discussed earlier.

## CONCLUSION

Osteoarthritis is a heterogeneous disease with multiple causes and corresponding molecular endotypes and clinical phenotypes. To help the osteoarthritis field move forward, we need to be clear about the meaning of molecular endotypes and clinical phenotypes and avoid confusing them in the published literature. We also need to develop new biomarker tools that will enable patient stratification as these are likely to be important for paving the way for the development of effective prevention and treatment strategies. Combining clinical data, imaging results and carefully selected panels of biochemical markers could help in advancing patient stratification and lead to better-designed clinical trials and more personalized and effective treatments for osteoarthritis patients.

A systematic review published by van Spil *et al.* [36], 8 years ago, concluded that 'None of the current biochemical markers are sufficiently discriminating to aid diagnosis and prognosis of osteoarthritis in individuals or limited numbers of patients, or performs so consistently that they could function as an outcome in clinical trials.' There still is a high demand for biochemical markers in osteoarthritis to enable drug development and facilitate translational research [81<sup>\*</sup>].

The main focus of recent research has been on testing whether available biochemical markers of tissue turnover and/or inflammation can differentiate between subtypes of osteoarthritis patients and

healthy controls, whether they relate to signs and symptoms of osteoarthritis on a group level, and/or whether they relate to disease progression. However, to advance the field, a number of steps ahead are required. First, the field should move to studying osteoarthritis at the molecular and preradiographic or early-radiographic stage, because the ability to distinguish between molecular endotypes and the corresponding clinical phenotypes might be easier at earlier disease stages, before all phenotypes coalesce in a final common pathway. Second, biochemical markers should actually be tested for their ability to differentiate between subtypes of osteoarthritis patients and not just for associations with disease parameters at a group level or during comparison with healthy controls. Third, novel techniques (e.g. -omics techniques) might be particularly useful for osteoarthritis phenotype research as they assess a panel of markers rather than just one or a few. Fourth, it might be hypothesized that local biochemical markers (e.g. synovial fluid) might better distinguish between molecular endotypes because there is less interference from systemic sources of noise, including comorbidities.

In summary, progress in the field of osteoarthritis endotype and phenotype research is considered important to the design of effective, stratified osteoarthritis treatment and to counter the burden that osteoarthritis poses to individuals and societies worldwide.

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### Conflicts of interest

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- of outstanding interest

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