Editorial introductions

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

EDITOR IN CHIEF

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.

SECTION EDITORS

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 the American College of Rheumatology and the recipient of the 2012 EULAR award for Meritorious Service in Rheumatology.

Yusuf Yazici

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

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

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

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

Jose U. Scher

Jose U. Scher, MD is associate professor in the Department of Medicine, at New York University Langone Medical Center, USA. He serves as Director of the NYU Psoriatic Arthritis Center, USA and the Microbiome Center for Rheumatology and Autoimmunity at NYU School of Medicine, USA, mostly clinical/translational research-oriented facilities to study the early environmental and immunologic events in RA and psoriatic diseases. As the Director of the NYU Psoriatic Arthritis Center, he oversees patient care, education and translational research initiatives in psoriasis and psoriatic arthritis. His main area of research is related to the role of the human microbiome in autoimmune and rheumatic diseases and has published seminal work on the field.

Dr Scher is founding member of Psoriasis and Psoriatic Arthritis Clinics Advancement Multicenter 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.

Mukundan Attur

Mukundan Attur, PhD, is Associate Professor of Medicine in the Department of Medicine, Division of Rheumatology, of NYU School of Medicine, NYU Langone Health, New York, USA. He received his PhD degree from Madurai Kamaraj University, India, and joined the NYU Division of Rheumatology. Dr Attur's interests are in the expression and role of inflammatory mediators in osteoarthritic cartilage, with the aim to elucidate the autocrine and paracrine mechanisms of action of inflammatory cytokines and lipids (eicosanoids) in chondrocytes using genomics and proteomics approaches. Dr Attur is privileged to work under the supervision of Dr Steven B. Abramson, with whom he has developed transcriptome- and protein-based biomarkers to identify subjects at risk for development of severe knee osteoarthritis. Currently, as Director of the Rheumatology Research Laboratory at NYU Langone Orthopedic Hospital, Dr Attur has established and maintained arthritis Biobank. His current research focuses on the pathophysiology, diagnosis and treatment of osteoarthritis, with special interest in extracellular non-collagenous proteins expressed in bone and cartilage. Dr Attur through proteogenomics- system biology approach has identified several genes and proteins that are examined as biomarkers to predict knee osteoarthritis development and progression. He is the author of over 77 publication and numerous invited reviews.

Cryoglobulinemic vasculitis: pathophysiological mechanisms and diagnosis

Marie N. Kolopp-Sarda[a,b] *and Pierre Miossec*[a,c]

Purpose of review
Cryoglobulins (CG) are immunoglobulins that precipitate in the cold, and dissolve at 37°C. *In vivo*, in cold exposed tissues and organs, they can induce vasculitis and occlusive vasculopathy after deposition on vascular endothelium under low temperature and high concentration conditions. Clinical manifestations are cutaneous (purpura, ulcers, vasomotor symptoms, and livedo reticularis), rheumatological (arthralgia and arthritis), and peripheral neuropathy (paresthesia and pain in the lower limbs). In profound organs such as the kidneys, CG deposition is less temperature-dependent, favored by local protein and anion concentrations, and can lead to glomerulonephritis. This review will focus on cryoglobulinemic vasculitis and vascular lesion, and their diagnosis.

Recent findings
The mechanisms of vascular lesions of pathogenic CG in function of CG type and their characteristics are better defined. Optimal conditions for CG detection are critical. The importance of looking for underlying diseases, especially hepatitis C virus status in mixed CG, is reminded.

Summary
A decision diagram for CG vasculitis diagnosis based on clinical and biological parameters is proposed.

Keywords
complement, cryoglobulinemia, HCV, rheumatoid factor, vasculitis

INTRODUCTION

Cryoglobulins (CG) are immunoglobulins (Ig) that precipitate in the cold and dissolve on rewarming. They were first reported by Wintrobe and Buell in 1933 in a patient with multiple myeloma, whose serum formed a cryogel at +4°C [1]. Then Meltzer *et al.* described the first series with a more precise characterization of CG and description of clinical manifestations. From that study, Meltzer's triad with fever, asthenia, and arthralgia describes the clinical signs of cryoglobulinemic vasculitis [2,3].

The reasons for cold insolubility of Ig depend on many intrinsic and extrinsic factors, when compared to normal Ig [4–9]. Structural anomalies (amino acid mutations or insertions/deletions, atypical glycosylation with reduced sialylation) resulting in modified tertiary structure may explain Ig solubility decrease. Formation of monoclonal Ig aggregates and immune complexes by noncovalent bonds such as hydrogen bonds or Fab-Fc interactions in rheumatoid factor positive CG are involved in such cold precipitation. *In vitro* and *in vivo*, these phenomena depend on environmental physicochemical conditions such as temperature, pH, and ionic strength [9,10].

The formation of aggregates or immune complexes induced by exposure to cold temperature is the key to these pathogenic mechanisms. CG cause symptoms of vascular defects on exposure to cold, most commonly involving extremities. They are responsible for vasculitis of small and medium-size vessels, involving frequently skin, joints, nerves, and kidneys. However, it is difficult to predict the pathogenicity of CG only on structure and type.

This review will focus on cryoglobulinemic vasculitis, its pathophysiological mechanisms, and diagnosis in the context of different underlying diseases and types of CG. A focus will be upon the best way of detection and characterization of CG.

[a]Immunogenomics and inflammation research Unit EA 4130, University of Lyon, [b]Immunology Laboratory and [c]Department of Immunology and Rheumatology, Clinical Immunology Unit, Hospices Civils de Lyon, Lyon, France

Correspondence to Pierre Miossec, Clinical Immunology Unit, Department of Immunology and Rheumatology, Hospital Edouard Herriot, 69437 Lyon Cedex 03, France. E-mail: pierre.miossec@univ-lyon1.fr

Curr Opin Rheumatol 2021, 33:1–7
DOI:10.1097/BOR.0000000000000757

KEY POINTS

- Cryoglobulins are immunoglobulins precipitating in the cold, *in vivo* and *in vitro*.
- Optimal conditions of temperature and sampling are essential to the detection of CG.
- Precipitation of immune complexes or aggregates of CG in small and medium vessels is responsible for cryoglobulinemic vasculitis.

CRYOGLOBULIN CLASSIFICATION ACCORDING TO BROUET *ET al.*

The CGs are classified into three types according to their immunochemical composition and the association to underlying pathologies [11▪▪]. Type I CG are composed of monoclonal Ig, more frequently IgM than IgG [12▪], rarely IgA [11▪▪,13–17]. They account for 10% of all CG [12▪]. They are commonly secondary to B-cell lymphoproliferative disorders.

Mixed CG, type II and type III CG, are composed of monoclonal and/or polyclonal Ig of all isotypes [18], they account for 90% of all CG [12▪]. Type II CG associate monoclonal and polyclonal Ig and represent 44% of all CG, and type III CG are only composed of polyclonal Ig and account for 47% [12▪]. Mixed CG are secondary to viral, bacterial, and parasitic infections, autoimmune diseases or are essential (no underlying disease) [19▪].

DETECTION AND CHARACTERIZATION OF CRYOGLOBULINS

Precipitation temperature of CG is variable according to their immunochemical characteristics and could be as high as 36°C [20]. For detection, the laboratory must receive blood samples collected under the best conditions, to avoid a false negative detection because the CG has already precipitated in the tube. Blood sample should be collected in 3–5 ml tubes with red top and clot activator and immediately deposited at 37°C until taken over to the laboratory. If these sampling conditions cannot be fulfilled, it is better to send the patient to the laboratory.

Cryoprecipitates are detected by visual observation of the serum placed for 7 days at +4°C. If no precipitate is observed, this negative detection must be confirmed on at least two other samples, especially in a suggestive clinical context, because 10% samples with a first negative detection are positive on another sample [12▪]. Characterization of CG consists in their typing (Ig isotype and monoclonal and/or polyclonal composition) for their classification [21▪]. CG quantification must be specific Ig measurement in the cryoprecipitate [21▪]. Cryocrit measurement (% volume of cryoprecipitate/total serum) is not a sensitive and specific technique and must be abandoned [22–24]. The final CG concentration is adjusted to the initial volume of serum and expressed as milligrams per liter of serum.

Hypocomplementemia is often associated with CG. Exploration of the complement system (C3 and C4 fractions, CH50 functional activity) is a useful tool for CG diagnosis. Decrease C4 (<0.10 g/L) associated or not with decreased C3 (<0.80 g/L) and CH50 was found in 24% of CG-positive serum [12▪]. Consumption of complement fractions C3 and C4 associated with the presence of CG is characteristic of complement classical pathway activation, involved in vessel inflammation and pathogenicity [25].

Rheumatoid factor activity (RF) in mixed CG is part of their definition [11▪▪,18]. The presence of RF in the CG contributes to the formation of immune complexes that deposit in vessels, with complement activation and increased inflammation [12▪] (Fig. 1). These mechanisms are involved in cryoglobulinemic vasculitis, especially in hepatitis C virus (HCV) infection [26,27]. RF measurement in cryoprecipitate is an important element for CG characterization and cryoglobulinemic vasculitis diagnosis. RF measurement in serum is not specific enough [12▪,27].

CG detection is essential even in the absence of vasculitis manifestations because of interferences of CG with the determination of other biological parameters (total blood cell count, serum total proteins and Ig measurements, and so on). The presence of a CG must be known by any laboratory to treat samples with strict preanalytic conditions before analysis.

CONSEQUENCES OF THE DETECTION OF A CRYOGLOBULIN

Detecting a CG is important for the diagnosis of cryoglobulinemic vasculitis and its treatment, and for the diagnosis of an underlying disease as described in the definition.

Type I cryoglobulins

Detection of type I CG, even without associated clinical manifestations, should lead to the search for a lymphoproliferative disease and this could allow monitoring for the appearance of a malignant disease. Type I CG is associated for 63–86% with monoclonal gammopathies of undetermined significance or of clinical significance when associated to CG manifestations [28▪▪], with Waldenström macroglobulinemia accounting for 18–33%, with multiple myeloma for 11–20%, and with other lymphoproliferative diseases such as chronic lymphocytic leukemia and some

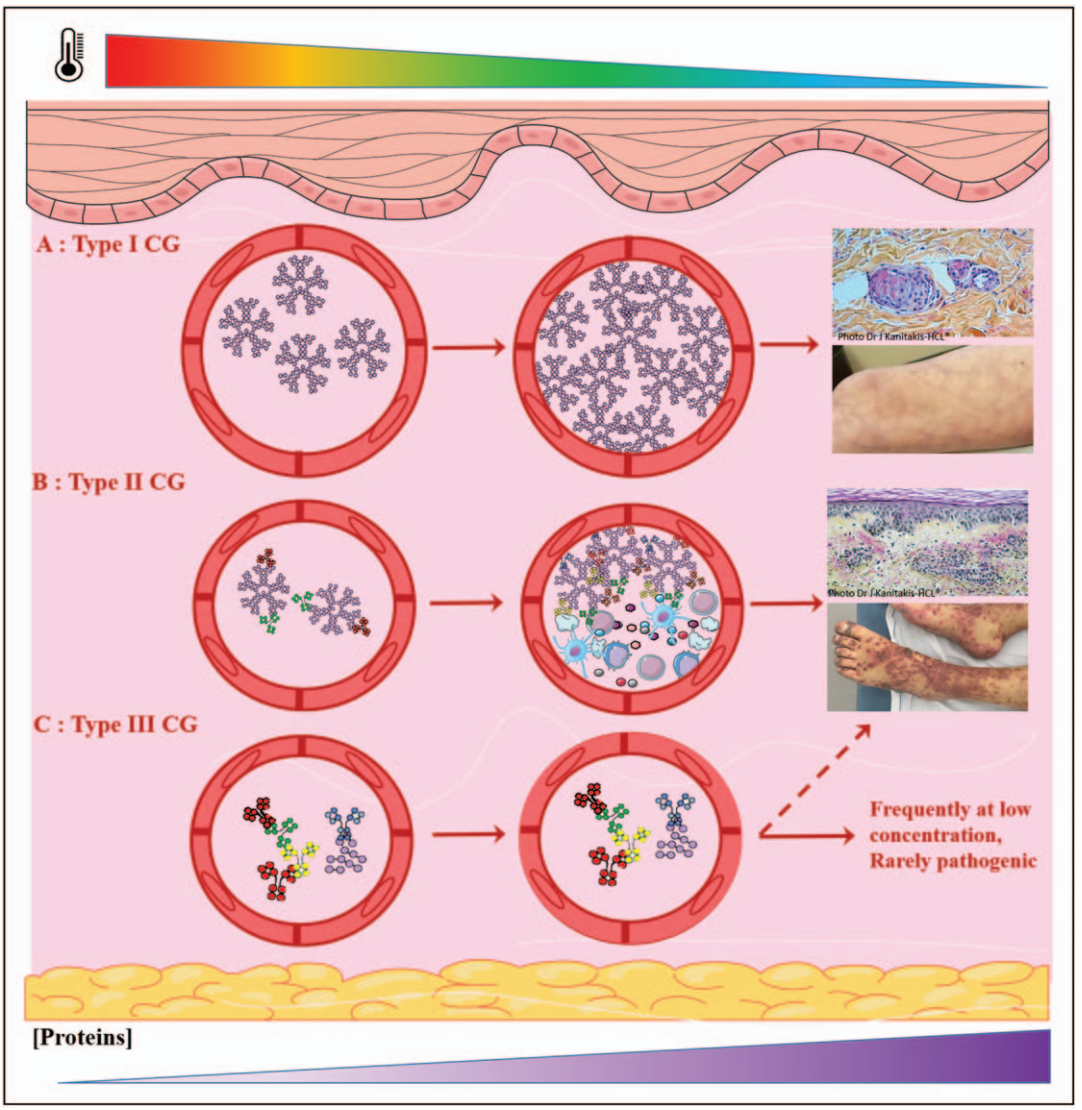

FIGURE 1. Pathophysiological mechanisms of cryoglobulinemic vasculitis in skin, depending on temperature and protein concentration. (a) Mechanism of vessel obstruction by type I CG. Occlusive vasculopathy is more frequently associated with type I CG, that are highly concentrated compared to mixed CG. This is a mechanical obstruction of venules and arterioles by large aggregates of type I CG, more frequently with type I IgM CG than with type I IgG. Monoclonal CG form aggregates that are larger when protein concentration increases and temperature decreases. Skin biopsy shows obstructive vasculitis (top picture). Clinical manifestation in the skin is *livedo reticularis* (bottom picture). (b) Mechanism of cryoglobulinemic vasculitis by type II CG. Circulating type II RF positive CG in small vessel form large immune complexes when temperature decreases, with more IgG fixed on RF IgM [7]. This phenomenon is amplified with high concentrated CG. These immune complexes contain complement fractions that favor their fixation on endothelium and local inflammation, with the recruitment of inflammatory cells and cytokine synthesis, resulting in vasculitis. Skin biopsy shows leucocytoclastic vasculitis (top picture). The most frequent clinical manifestation in the skin is purpura, more often in the lower limbs, that could evaluate in necrotic purpura. This mechanism is also involved in type I CG vasculitis, after the formation of larger aggregates at low temperature, that could deposit on vascular endothelium [37]. (c) Circulating type III CG. Type III CG are less concentrated and have more rarely RF activity. Even in the case of RF positive type III CG, immune complexes are small, RF IgG or polyclonal IgM bind less IgG and are less prone to deposit. Generally, no inflammation and no tissue injury are associated with type III CG. Alternatively, mechanisms like type II CG vasculitis are described (see b).

lymphomas, especially marginal zone lymphoma and mantle zone lymphoma, for 11–20% [29–32].

The measurement of M-spike on serum electrophoresis could be underestimated if serum sample containing type I monoclonal CG is conserved at 4°C before analysis. Patient monitoring must be done knowing the presence of a CG to warm up the serum sample before electrophoresis.

Mixed cryoglobulins

Mixed CG are found secondary to viral infections, especially chronic HCV (50%), HBV, or HIV (for <5%) [12▪]. They could be transient following acute bacterial or parasitic infections. They are also associated with auto-immune diseases, especially systemic lupus erythematosus, Sjögren's syndrome, and rheumatoid arthritis [12▪]. In the special case of CG associated with Waldenström macroglobulinemia, the monoclonal IgM (most often IgM kappa) could have an RF activity (IgM anti-IgG) and the CG will be classified as a type II CG associating monoclonal IgM and polyclonal IgG, and not as a type I CG.

Chronic HCV infection is responsible for 50% of mixed CG and 35% of HCV-infected patients have a CG [12▪]. Detection of CG in HCV infected patients is useful for the diagnosis of extrahepatic clinical manifestations and their treatment, and especially to look for B lymphoma secondary to chronic activation of B cells [33–35]. Because of this important association of HCV and CG, it is essential to look for HCV infection in CG positive patients and conversely, to look for CG in all HCV patients. A recent study reported that these detections are not sufficiently carried out; only 38% of CG positive patients had a biological diagnosis of HCV infection, and 30% of HCV positive patients had a detection of CG [36▪].

PATHOPHYSIOLOGICAL MECHANISMS OF CRYOGLOBULINEMIC VASCULITIS

The pathogenicity of CG depends first on their type, isotype, antigenic specificity, and concentration, second, on local conditions of temperature, protein, and anion concentrations, and complement activation.

In tissues exposed to cold, such as skin (where temperature is about 28°C), extremities, joints, and peripheral nerves, CG can deposit in small and medium vessels, and cause vasculitis and obstruction. The formation of precipitating aggregates and immune complexes is not immediate in these organs, explaining why clinical signs appear after prolonged exposure at low temperature [8,37].

The clinical manifestations of type I CG result from the aggregation of monoclonal Ig, the largest aggregates are formed with pentameric IgM compared to monomeric IgG, and are favored by both low temperature and high CG concentration [38–40]. Complement fractions are bound in type I CG aggregates and increase inflammation when deposited on small vessel endothelium. Large aggregates of high concentrated monoclonal CG can also mechanically obstruct vessels and lead to occlusive vasculopathy (Fig. 1a).

The clinical manifestations of mixed CG, more frequently type II CG, are linked to the formation of immune complexes in cold exposed tissues and their deposition on vascular endothelium (Fig. 1b). Type II CG most commonly consist of monoclonal IgM with RF activity associated with polyclonal IgG [12▪]. Multivalency of IgM and low temperature contribute to large size immune complexes [7,10,41,42]. RF activity promotes immune complex formation, with the binding of complement fractions, in particular C1q, C4, and C3 [41]. In cold exposed tissues, these large immune complexes containing complement fractions are not eliminated as normal immune complexes (fixation on erythrocytes via complement receptors). They are deposited on endothelium where they activate complement classical pathway *in situ* and cause local inflammation [42]. Type III CG are less pathogenic than type II CG [26,43] because their concentration is lower and RF activity of polyclonal IgM or IgG is less frequent [12▪], and consequently immune complexes are smaller (Fig. 1c). Thus, the two main conditions for cryoprecipitation and tissue injury, high concentration and RF, are not fulfilled (Fig. 1c).

Kidneys are also target organs of CG, although the physiological temperature is 37°C and does not decrease even in extreme conditions. The pathophysiological mechanisms of less-temperature dependent precipitation of CG result more from local protein and anion concentrations [8,44,45]. Type I membranoproliferative glomerulonephritis (MPGN) is the most frequent kidney disorder associated with type I and type II CG, characterized by mesangial cell proliferation and structural changes in glomerular capillary walls. In glomeruli, an increase of protein concentration and variation of anion concentration could lead to CG aggregation, and to their deposition on glomerular membrane and formation of thrombi in glomeruli capillaries [44].

In conclusion of these mechanisms, typing and quantifying CG and determining their RF activity and complement levels are essential to define their potential pathogenic capacity. However, there is still a high heterogeneity between CG characteristics and clinical manifestations.

DIAGNOSIS PROCESS OF CRYOGLOBULINEMIC VASCULITIS

Definition and classification

CG can induce vasculitis of small vessels, predominantly capillaries, venules, or arterioles, but medium arteries and veins can also be affected. Mixed CG secondary to viral infections are included in 'vasculitis with probable etiology' in the 2012 Chapel Hill consensus conference on the nomenclature of vasculitis [46].

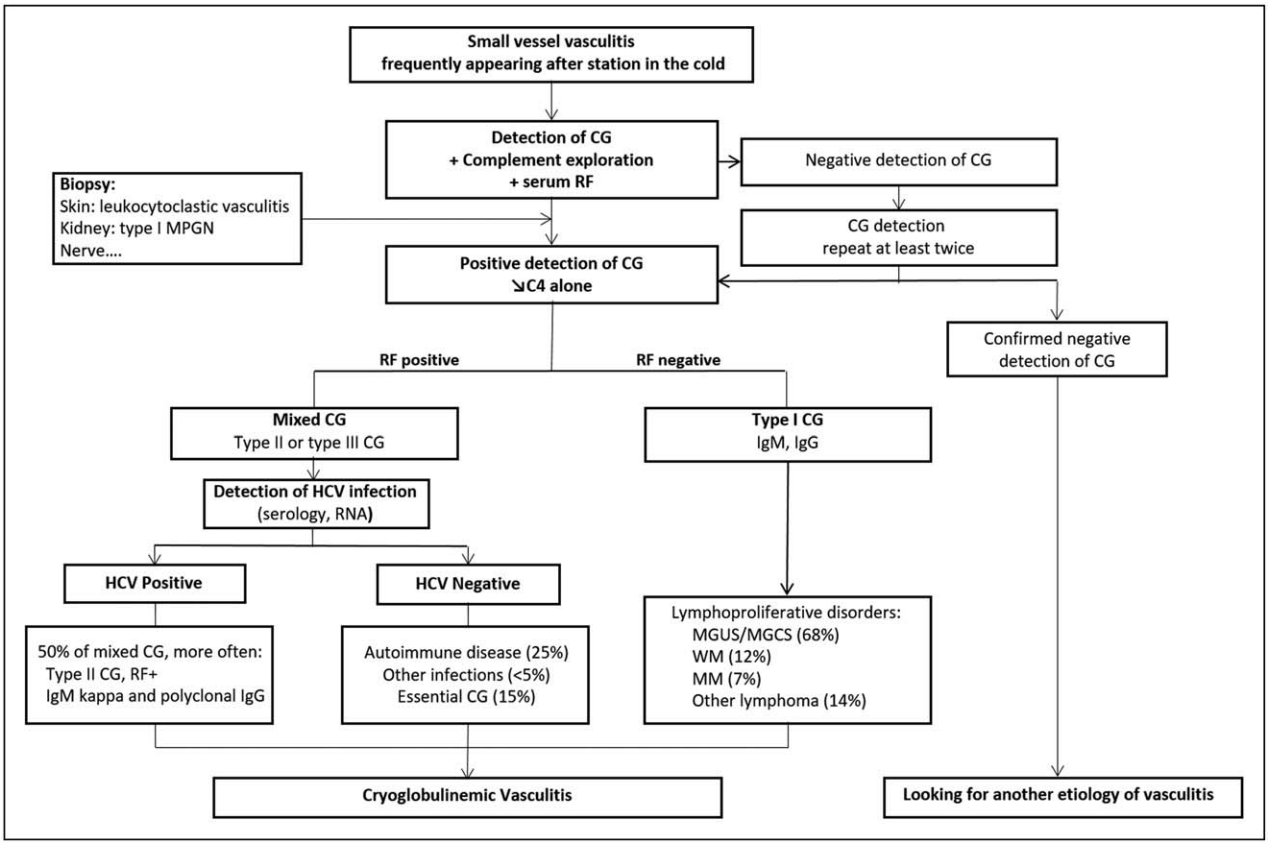

FIGURE 2. Decision diagram for cryoglobulinemic vasculitis diagnosis in a suggestive clinical context. Detection and characterization of CG (typing, quantification) associated with serum complement exploration and RF measurement, provide additional diagnostic elements for cryoglobulinemic vasculitis. In the case of the detection of mixed CG, a diagnosis of HCV infection must be done because it is the most frequent associated disease.

Classification criteria for cryoglobulinemic vasculitis were proposed and validated in prospective studies [47–49]. This classification takes into account the detection of CG in serum in at least two determinations at ≥12-week interval, clinical questionnaire (three simple questions about clinical manifestations), clinical items (general symptoms, articular, cutaneous, and/or neurologic involvements), and laboratory items (decrease C4, positive serum RF, positive monoclonal component). These classification criteria are important for epidemiologic studies, because of their high specificity, but cannot be used for diagnosis. Diagnostic criteria for cryoglobulinemic syndrome, based on clinical and biological criteria, are less validated [50,51] and must be adjusted by the clinician, even in patients without all the above classification criteria, especially for the 12-week interval between two CG detections.

Diagnosis of cryoglobulinemic vasculitis

Figure 2 proposes a decision diagram for cryoglobulinemic vasculitis diagnosis, based on simple clinical and immunological features. The two main elements leading to the diagnosis of vasculitis related to CG are first, suggestive clinical manifestations (cutaneous, rheumatological, neurological manifestations, which may be associated with renal manifestations) with specific location (lower limbs) and, second, biological changes. In such context, the detection of CG is essential, associated simultaneously with serum complement exploration and RF measurement, in cryoprecipitate and in serum. If the first CG detection is negative, it is important to take at least two other samples to confirm the absence of CG [12▪].

A biopsy of inflammatory tissue (skin, nerve) or renal biopsy can provide additional diagnostic elements, although they are not very specific. Leukocytoclastic vasculitis is commonly found in skin biopsy. In renal biopsy, type I membranoproliferative glomerulonephritis is the most frequent and specific histological description of renal disease [25,52].

In a mixed CG with C4 decrease and RF positive, a diagnosis of HCV infection must be done because it is the most frequent associated disease, with a specific and effective treatment. In case of negative

HCV detection, autoimmune disease is the next option, frequently with a type III CG [12▪]. More rarely, mixed CG could be detected after other infections such as HBV or HIV, bacterial and parasitic infections. An RF negative type I CG with C4 decrease and any CG with a monoclonal component should lead to search for a lymphoproliferative disease.

CONCLUSION

Cryoglobulinemic vasculitis is a rare but severe manifestation of CG, for which diagnosis combines clinical signs characteristic of this vasculitis and biological parameters, with CG detection, with optimal conditions of sampling, and characterization (typing, quantification, RF activity), and serum complement exploration. A complete characterization is essential to assess CG pathogenicity and the associated inflammatory mechanisms. Diagnosis of cryoglobulinemic vasculitis will influence treatment, depending on the underlying disease, commonly HCV or autoimmunity. Treatment of cryoglobulinemic vasculitis depends on the essential or secondary nature of CG.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Wintrobe M, Buell M. Hyperproteinemia associated with multiple myeloma. Bull Johns Hopkins Hosp 1933; 52:156–165.
2. Meltzer M, Franklin EC, Elias K, *et al.* Cryoglobulinemia–a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. Am J Med 1966; 40:837–856.
3. Meltzer M, Franklin EC. Cryoglobulinemia–a study of twenty-nine patients. I. IgG and IgM cryoglobulins and factors affecting cryoprecipitability. Am J Med 1966; 40:828–836.
4. Middaugh CR, Gerber-Jenson B, Hurvitz A, *et al.* Physicochemical characterization of six monoclonal cryoimmunoglobulins: possible basis for cold-dependent insolubility. Proc Natl Acad Sci U S A 1978; 75:3440–3444.
5. Gerber-Jenson B, Kazin A, Kehoe JM, *et al.* Molecular basis for the temperature-dependent insolubility of cryoglobulins. X. The amino acid sequence of the heavy chain variable region of McE. J Immunol 1981; 126:1212–1216.
6. Erickson BW, Gerber-Jenson B, Wang AC, Litman GW. Molecular basis for the temperature-dependent insolubility of cryoglobulins–XI. Sequence comparison of the heavy-chain variable regions of the human cryoimmunoglobulins McE and Hil by metric analysis. Mol Immunol 1982; 19:357–365.
7. Brandau DT, Trautman PA, Steadman BL, *et al.* The temperature-dependent stoichiometry of mixed cryoimmunoglobulins. J Biol Chem 1986; 261: 16385–16391.
8. Lawson EQ, Brandau DT, Trautman PA, *et al.* Kinetics of the precipitation of cryoimmunoglobulins. Mol Immunol 1987; 24:897–905.
9. Lawson EQ, Brandau DT, Trautman PA, Middaugh CR. Electrostatic properties of cryoimmunoglobulins. J Immunol 1988; 140:1218–1222.
10. Brandau DT, Lawson EQ, Trautman PA, Middaugh CR. Thermodynamics of monoclonal and mixed cryoimmunoglobin solubilization. Immunol Invest 1987; 16:21–32.
11. ▪▪ Brouet J-C, Clauvel J-P, Danon F, *et al.* Biologic and clinical significance of cryoglobulins: a report of 86 cases. Am J Med 1974; 57:775–788.
This paper is the basis for the current classification of CG.
12. ▪ Kolopp-Sarda MN, Nombel A, Miossec P. Cryoglobulins today: detection and immunological characteristics of 1675 positive samples out of 13439 patients over 6 years. Arthritis & Rheumatology 2019; 71:1904–1912.
this epidemiological study on a very large cohort of patients recruited in all medical units reports recent data on repartition, characterization of CG, and associated biological parameters and diseases.
13. Auscher C, Guinand S. [Investigation of a cryo-precipitable bêtea2A-globulin]. Clin Chim Acta 1964; 9:40–48.
14. Pruzanski W, Jancelewicz Z, Underdown B. Immunological and physicochemical studies of IgA1(γ) cryogelglobulinaemia. Clin Exp Immunol 1973; 15:181–191.
15. Slavin RG, Suriano JR. Dreesman G: Studies on cryogelglobulinemia associated with IgA myeloma proteins. Int Arch Allergy Appl Immunol 1971; 40:739–748.
16. Yamaguchi N, Kawai K, Kagami K, *et al.* [A case of idiopathic monoclonal IgA cryoglobulinemia (author's transl)]. Nippon Naika Gakkai Zasshi 1977; 66:414–421.
17. Lalezari P, Kumar M, Kumar KM, Lawrence C. Inhibition of cold insolubility of an IgA cryoglobulin by decanedicarboxylic acid and related compounds. Am J Hematol 1983; 15:279–288.
18. Meltzer M, Franklin EC. Cryoglobulins, rheumatoid factors and connective tissue disorders. Arthritis & Rheumatism 1967; 10:489–492.
19. ▪ Roccatello D, Saadoun D, Ramos-Casals M, *et al.* Cryoglobulinaemia. Nature Reviews Disease Primers 2018; 4:1–16.
this recent review focuses on clinical manifestations of CG and discusses treatment options of secondary CG
20. Nishimura Y, Nakamura H. Human monoclonal cryoimmunoglobulins. I. Molecular properties of IgG3 kappa (Jir protein) and the cryo-coprecipitability of its molecular fragments by papain. J Biochem 1984; 95:255–265.
21. ▪ Kolopp-Sarda M-N, Miossec P. Cryoglobulins: An update on detection, mechanisms and clinical contribution. Autoimmunity Reviews 2018; 17:457–464.
Detection of CG in good conditions of sampling is critical. In this review, details on conditions of sampling and characterization are reported.
22. Shihabi ZK. Cryoglobulins: an important but neglected clinical test. Ann Clin Lab Sci 2006; 36:395–408.
23. Sargur R, White P, Egner W. Cryoglobulin evaluation: best practice? Annals of Clinical Biochemistry 2009; 47:8–16.
24. Vermeersch P, Gijbels K, Marien G, *et al.* A critical appraisal of current practice in the detection, analysis, and reporting of cryoglobulins. Clinical Chemistry 2008; 54:39–43.
25. Alchi B, Jayne D. Membranoproliferative glomerulonephritis. Pediatric Nephrology 2010; 25:1409–1418.
26. De Rosa FG, Agnello V. Observations on cryoglobulin testing: I. the association of cryoglobulins containing Rheumatoid factors with manifestation of cryoglobulinemic vasculitis. J Rheumatol 2009; 36:1953–1955.
27. Gorevic P. Rheumatoid factor, complement, and mixed cryoglobulinemia. Clin Dev Immunol 2012; 2012:1–6.
28. ▪▪ Fermand J-P, Bridoux F, Dispenzieri A, *et al.* Monoclonal gammopathy of clinical significance: a novel concept with therapeutic implications. Blood 2018; 132:1478–1485.
the concept of monoclonal gammopathy of clinical significance is very important for type I CG definition and management. Monoclonal CG are rarely associated with multiple myeloma, but they are responsible for specific clinical manifestations, and must be considered as hematological diseases for their treatment.
29. Terrier B, Karras A, Kahn J-E, *et al.* The spectrum of type I cryoglobulinemia vasculitis: new insights based on 64 cases. Medicine (Baltimore) 2013; 92:61–68.
30. Néel A, Perrin F, Decaux O, *et al.* Long-term outcome of monoclonal (type 1) cryoglobulinemia. Am J Hematol 2014; 89:156–161.
31. Harel S, Mohr M, Jahn I, *et al.* Clinico-biological characteristics and treatment of type I monoclonal cryoglobulinaemia: a study of 64 cases. Br J Haematol 2015; 168:671–678.
32. Sidana S, Rajkumar SV, Dispenzieri A, *et al.* Clinical presentation and outcomes of patients with type 1 monoclonal cryoglobulinemia. Am J Hematol 2017; 92:668–673.
33. Cacoub P, Poynard T, Ghillani P, *et al.* Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. Arthritis Rheum 1999; 42:2204–2212.
34. Ferri C, La Civita L, Longombardo G, *et al.* Mixed cryoglobulinaemia: a crossroad between autoimmune and lymphoproliferative disorders. Lupus 1998; 7:275–279.
35. Ferri C, Sebastiani M, Giuggioli D, *et al.* Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. Semin Arthritis Rheum 2004; 33:355–374.

36. Kolopp-Sarda MN, Miossec P. Contribution of Hepatitis C Infection to a Large ■ Cohort of Cryoglobulin-Positive Patients: Detection and Characteristics. Front Immunol 2020; 11:1183.
CG secondary to HCV infection are compared to non-HCV CG, for their immunological characteristics and RF activity. The results support the importance of looking for the HCV status of patients with mixed CG, and conversely to search for CG in patients with HCV infection.
37. Wang Y, Lomakin A, Hideshima T, *et al.* Pathological crystallization of human immunoglobulins. Proc Natl Acad Sci U S A 2012; 109:13359–13361.
38. Kochwa S, Smith E, Brownell M, Wasserman LR. Aggregation of IgG globulin in vivo. II. Physicochemical properties of the isolated protein. Biochemistry 1966; 5:277–285.
39. Hall CG, Abraham GN. Reversible self-association of a human myeloma protein. Thermodynamics and relevance to viscosity effects and solubility. Biochemistry 1984; 23:5123–5129.
40. Vallas V, Farrugia W, Raison RL, *et al.* Dissimilar aggregation processes govern precipitation and gelation of human IgM cryoglobulins. J Mol Recognit 2007; 20:90–96.
41. Ng YC, Schifferli JA. Clearance of cryoglobulins in man. Springer Semin Immunopathol 1988; 10:75–89.
42. Sansonno D, Tucci FA, Ghebrehiwet B, *et al.* Role of the Receptor for the Globular Domain of C1q Protein in the Pathogenesis of Hepatitis C Virus-Related Cryoglobulin Vascular Damage. J Immunol 2009; 183:6013–6020.
43. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 1992; 327:1490–1495.
44. Di Stasio E, Bizzarri P, Casato M, *et al.* Cl- regulates cryoglobulin structure: a new hypothesis for the physiopathological mechanism of temperature non-dependent cryoprecipitation. Clin Chem Lab Med 2004; 42:614–620.
45. Di Stasio E, Bizzarri P, Bove M, *et al.* Analysis of the dynamics of cryoaggregation by light-scattering spectrometry. Clin Chem Lab Med 2003; 41:152–158.
46. Jennette JC, Falk RJ, Bacon PA, *et al.* 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitide**s**. Arthritis & Rheumatism 2013; 65:1–11.
47. De Vita S, Soldano F, Isola M, *et al.* Preliminary classification criteria for the cryoglobulinaemic vasculitis. Ann Rheum Dis 2011; 70:1183–1190.
48. Quartuccio L, Isola M, Corazza L, *et al.* Performance of the preliminary classification criteria for cryoglobulinaemic vasculitis and clinical manifestations in hepatitis C virus-unrelated cryoglobulinaemic vasculitis. Clin Exp Rheumatol 2012; 30:S48–52.
49. Quartuccio L, Isola M, Corazza L, *et al.* Validation of the classification criteria for cryoglobulinaemic vasculitis. Rheumatology (Oxford) 2014; 53:2209–2213.
50. Invernizzi F, Pietrogrande M, Sagramoso B. Classification of the cryoglobulinemic syndrome. Clin Exp Rheumatol 1995; 13:S123–S128.
51. Damoiseaux J. The diagnosis and classification of the cryoglobulinemic syndrome. Autoimmun Rev 2014; 13:359–362.
52. Coliche V, Sarda M-N, Laville M, *et al.* Predictive factors of renal involvement in cryoglobulinaemia: a retrospective study of 153 patients. Clin Kidney J 2018; 12:365–372.

Management of primary vasculitides with biologic and novel small molecule medications

Naomi Serling-Boyd[a] *and Zachary S. Wallace*[a,b,c]

Purpose of review
Vasculitides can affect small, medium and/or large vessels, leading to end-organ damage, decreased quality of life and death. Glucocorticoids remain the backbone of treatment for systemic vasculitis but are associated with numerous toxicities. In recent years, the efficacy of glucocorticoid-sparing biologic and novel small molecule therapies has been demonstrated.

Recent findings
In giant cell arteritis, tocilizumab was superior to glucocorticoid monotherapy in maintenance remission and cumulative glucocorticoid exposure and is now approved for the treatment of giant cell arteritis. In addition to the previously demonstrated efficacy of rituximab for remission induction in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, recent trials have also demonstrated its superiority for remission maintenance compared to alternative approaches. Mepolizumab is superior to standard of care alone with regard to remission rates and glucocorticoid-sparing effect in refractory eosinophilic granulomatosis with polyangiitis. Avacopan has shown significant promise in ANCA-associated vasculitis as part of a glucocorticoid-free induction regimen in a recently completed phase 3 trial. Use of biologics in rarer vasculitides remains guided by reports from small case series.

Summary
Biologics and other novel therapies have an increasingly important role in the management of systemic vasculitis. Additional studies are needed to define their optimal use and to guide their use in more rare forms of vasculitis.

Keywords
biologic, glucocorticoid, vasculitis

INTRODUCTION

Vasculitis refers to inflammation involving the vessel wall and is often categorized according to the size of the affected vessels (i.e. small, medium or large). These conditions may be idiopathic in cause (i.e. primary) or may develop in the context of another underlying disease (e.g. infection and malignancy) or exposure to medication (e.g. hydralazine) or environmental toxin (e.g. levamisole). Glucocorticoids are a cornerstone of therapy for many of the primary vasculitides. However, glucocorticoids are associated with numerous toxicities such that while taking them, 90% of patients experience at least one adverse event, such as hypertension, diabetes, cataracts, glaucoma, osteoporosis and serious infections, among others (Fig. 1) [1,2]. The association between adverse events and glucocorticoid exposure is dose dependent; for each 1000 mg increase in cumulative glucocorticoid exposure, the risk of an adverse event increases by up to 5% [3]. Thus, for chronic conditions like many types of vasculitis, glucocorticoid-sparing therapies are critical for improving patient outcomes. Although conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) are often used, there is a rapidly expanding role for biologic medications and other oral, small molecule, targeted therapies in a variety of systemic vasculitides. For the purpose of this review, we define a biologic medication as one that is produced from or contains components of a living organism. In this review, we will focus on recent

[a]Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, [b]Harvard Medical School and [c]Department of Medicine, Clinical Epidemiology Program, Mongan Institute, Massachusetts General Hospital, Boston, Massachusetts, USA

Correspondence to Zachary S. Wallace, MD, MSc, Division of Rheumatology, Allergy and Immunology, Clinical Epidemiology Program, Massachusetts General Hospital, 100 Cambridge Street, 16th Floor, Boston, MA 02114, USA. Tel: +1 617 724 2507; e-mail: zswallace@partners.org

Curr Opin Rheumatol 2021, 33:8–14
DOI:10.1097/BOR.0000000000000756

KEY POINTS

- Because of the chronicity of many primary vasculitides and the risk of long-term glucocorticoid toxicity, glucocorticoid-sparing medications play a key role in the management.
- Several biologics, including tocilizumab for GCA, rituximab for ANCA-associated vasculitis and mepolizumab for eosinophilic GPA, have received regulatory approval for treatment based on randomized controlled trials, though data supporting the use of these and other biologics in other vasculitides remain limited to case series and other small studies.
- Ongoing clinical trials evaluating the efficacy and glucocorticoid-sparing effects of biologics and novel small molecules are expected to further inform the management of systemic vasculitis moving forward.

updates in the management of primary vasculitides, with a focus on recent advances in biologic and small molecule targeted therapy.

GIANT CELL ARTERITIS

Giant cell arteritis (GCA) is one of the large-vessel vasculitides and is the most common form of primary vasculitis. It has an annual incidence of 17 per 100,000 people over 50 years old in North America [4] and most often affects women of northern European descent who are over 50 years of age. Until recently, glucocorticoid monotherapy (at a dose of 1 mg/kg/day and tapered gradually over at least 1 year) was the standard of care, though some studies suggested a potential glucocorticoid-sparing effect of methotrexate [5].

The management of GCA shifted with the recent regulatory approval of tocilizumab, an interleukin (IL)-6 receptor antagonist, as a glucocorticoid-sparing treatment for GCA (Table 1). An initial small (N = 20) randomized controlled trial assessed the efficacy of tocilizumab and found higher relapse-free survival rates in the tocilizumab group than the placebo group, which received glucocorticoid monotherapy (85 vs. 20% in the placebo group; $P = 0.001$) [6]. This was followed by the Giant Cell Arteritis Actemra (GiACTA) trial (N = 251) in which patients were randomized to one of four arms; the proportion achieving sustained glucocorticoid-free remission (primary outcome) at 52 weeks was 56% in the weekly tocilizumab group, 53% in the every other week tocilizumab group, 18% in the 52-week prednisone only group and 14% in the 25-week prednisone only group [7]. Serious adverse events were reported more often in the prednisone groups (22–25% in the prednisone groups vs. 14–15% in the tocilizumab groups). The prednisone groups also had greater cumulative glucocorticoid doses over

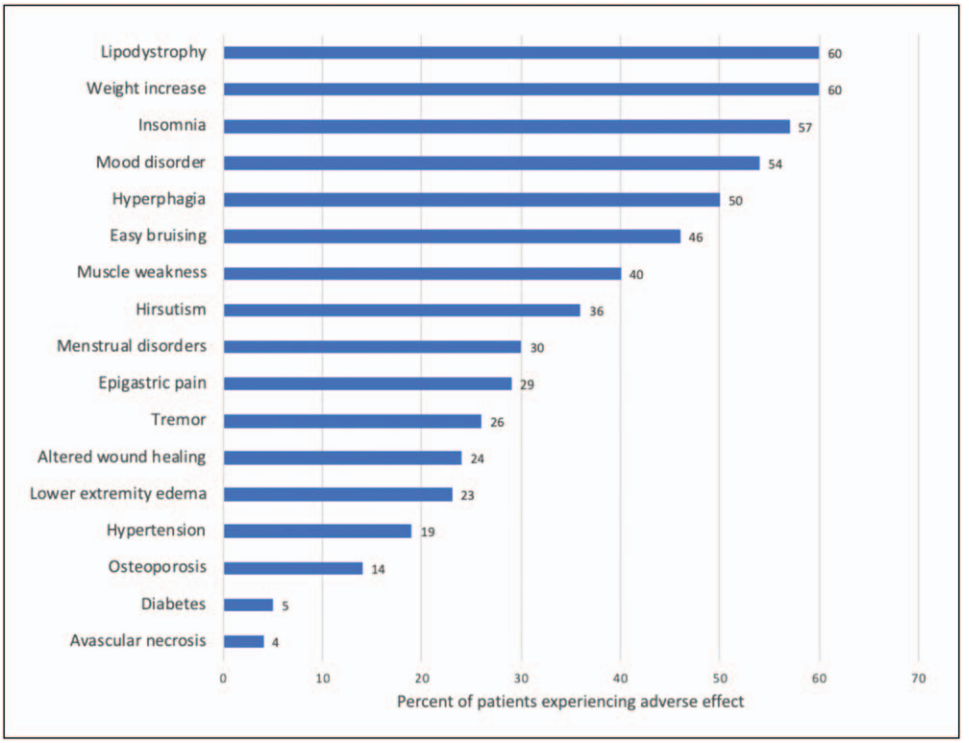

FIGURE 1. Percentage of patients experiencing various glucocorticoid-related adverse effects after 6 months of glucocorticoid treatment by self-reported questionnaire. Data source: [2].

Table 1. Biologic and small molecule, targeted treatments for rheumatic diseases

Rheumatic disease	Treatments currently in use	Under evaluation in clinical trials
Giant cell arteritis	Tocilizumab[a]	Abatacept Anakinra Upadacitinib
ANCA-associated vasculitis	Rituximab[a] (for remission induction and maintenance)	Avacopan Belimumab
Takayasu arteritis	TNF inhibitors Tocilizumab[a]	
Cryoglobulinemic vasculitis	Rituximab	
Primary angiitis of the central nervous system	Rituximab	
Behcet's disease	TNF inhibitors (especially for neuro-Behcet's)	Ustekinumab Anakinra
Polyarteritis nodosa	TNF inhibitors Rituximab Tocilizumab	

TNF, tumor necrosis factor.
[a]Supported by the data from randomized controlled trials; otherwise, data limited to cohort studies, case series or case reports.

52 weeks (3296–3818 mg in the prednisone groups vs. 1862 mg in each of the tocilizumab groups) [7].

Other biologics have been studied in GCA in smaller studies and yielded less dramatic results. Abatacept, a cytotoxic T-lymphocyte-associated protein 4 immunoglobulin, was studied in a randomized controlled withdrawal trial in which all patients received abatacept up-front with glucocorticoids; subsequently, those achieving remission (N = 41) were randomized to continue or discontinue abatacept (both received a total of 28 weeks of glucocorticoids). Relapse-free remission was observed in 48% in the abatacept continuation group compared with 31% in the glucocorticoid monotherapy group ($P = 0.049$). There was no difference in the frequency or severity of adverse events between the treatment arms. Although promising, additional studies are needed to further evaluate the efficacy of abatacept for GCA [8]. Ustekinumab, an IL-12/23 inhibitor, was evaluated for GCA in an open-label single-arm study (N = 25) that suggested that it may lead to less glucocorticoid exposure and reduce the risk of relapse [9]. However, a subsequent single-arm, open-label study (N = 13) evaluating ustekinumab in combination with a 6-month prednisone taper was terminated early because of 70% of the initially enrolled patients experiencing disease flares [10▪].

The precise role of tocilizumab in the approach to GCA management remains controversial and undefined [11]. Additional studies (e.g. trials, cohort studies and cost-effectiveness studies) are needed to evaluate the optimal use of tocilizumab for the treatment of GCA (e.g. timing of initiation and duration of treatment) and its long-term ability to prevent large vessel and other complications. Current guidelines for the management of GCA reflect this uncertainty, recommending initial treatment with high-dose glucocorticoids and the use of tocilizumab in the setting of refractory or relapsing disease or for patients at increased risk of glucocorticoid-related complications [12▪▪].

The success of the GiACTA trial has prompted tremendous interest in programs evaluating novel approaches to GCA management. Ongoing clinical trials are studying tocilizumab in combination with a short 2-month prednisone taper (ClinicalTrials.gov; NCT03726749), an IL-6 receptor inhibitor (sarilumab) (ClinicalTrials.gov; NCT03600805), and novel targets, such as Janus kinase with upadacitinib (ClinicalTrials.gov; NCT03725202), and IL-1with anakinra (ClinicalTrials.gov; NCT02902731).

TAKAYASU ARTERITIS

Takayasu arteritis is a form of large-vessel vasculitis involving the aorta and its primary branches that tends to affect women under the age of 50 years. Its incidence varies across the globe, with an estimated incidence of 2.6 cases per million in the United States and up to 60 cases per million in Japan [13]. Glucocorticoids in combination with DMARDs, especially conventional synthetic DMARDs (e.g. methotrexate and leflunomide), have traditionally been the standard of care for treatment because of difficulty tapering glucocorticoids to reasonably low doses [12▪▪]. Recently, biologic DMARDs, such as tumor necrosis factor (TNF) inhibitors and tocilizumab, have been studied and increasingly been used as first-line therapy, though there is a paucity of data to guide these practices.

Data from both retrospective and prospective open-label series suggest that TNF inhibitors can reduce disease activity and glucocorticoid exposure in Takayasu arteritis, though results should be interpreted with caution as there have not been randomized controlled trials evaluating the efficacy of these medications [12▪▪]. A recent small (N = 36) randomized controlled trial evaluated the efficacy of tocilizumab in Takayasu arteritis to prevent relapse after remission was achieved with glucocorticoids. In that trial, tocilizumab decreased the time to relapse of disease in the per-protocol analysis [hazard ratio 0.3, 95% confidence interval (CI): 0.11–1.00, $P = 0.03$], though this difference was not significant in the intention to treat analysis (hazard ratio 0.4, 95% CI: 0.15–1.10; $P = 0.06$) [13]. Additional trials with large cohorts are need to further assess the efficacy of tocilizumab and TNF inhibitors for the treatment of Takayasu arteritis. The decision to use either a TNF inhibitor or tocilizumab for Takayasu arteritis should be based on the patient's comorbidities and the presence of any relevant contraindications to either medication [12▪▪].

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a category of diseases that includes granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (eGPA), all of which tend to affect small vessels and are often characterized by the presence of antibodies to either myeloperoxidase or proteinase 3. For moderate to severe disease, remission induction therapy typically involved the combination of cyclophosphamide and glucocorticoids until the RAVE trial (N = 197) demonstrated the noninferiority of rituximab (anti-CD20 monoclonal antibody) when compared to cyclophosphamide for remission induction (64 vs. 53%, respectively; $P < 0.001$ for noninferiority) [14]. Today, providers have a number of treatment options for the management of AAV, including rituximab, cyclophosphamide, mepolizumab, azathioprine, methotrexate and mycophenolate mofetil, guided by disease type and severity, treatment phase, patient preference and comorbidities [15]. Recently, a number of additional trials have further evaluated rituximab, as well as mepolizumab, in AAV.

MAINRITSAN1 was a randomized controlled trial (N = 115) that demonstrated the superiority of rituximab over azathioprine for remission maintenance (major relapse rates of 5 vs. 29%; $P = 0.002$) and comparable safety profiles of both drugs in patients with GPA or MPA [16]. These results have been confirmed in those with relapsing AAV in the recently completed, but not yet published, RITAZAREM randomized controlled trial [17▪]. Notably, rituximab was associated with improved survival in long-term follow-up of MAINRITSAN1 participants [18].

Following demonstration of rituximab's efficacy for remission maintenance, additional trials were conducted to better define its use in AAV. The MAINRITSAN2 trial (N = 162) randomized patients with GPA or MPA to fixed retreatment (every 6 months) or a tailored-approach to retreatment (when $CD19^+$ B cells or ANCA reappeared) for remission maintenance over 18 months, with the primary endpoints of number of relapses or worsening disease activity measured at month 28. Those randomized to tailored dosing required fewer rituximab infusions (3 vs. 5) but did not experience a significantly higher risk of relapse than those randomized to fixed dosing (10 vs. 17%; $P = 0.2$) [19]. Although the results from MAINRITSAN 2 are compelling, there is a concern that there was a numerical difference in the proportion flaring with tailored therapy that may have been statistically significant if the trial cohort has been larger.

Most recently, the MAINRITSAN3 trial randomized 97 patients with GPA or MPA who had completed MAINRITSAN2 to an additional 18 months of rituximab or placebo. In this trial, those randomized to continue rituximab had superior relapse-free survival compared to those randomized to discontinue rituximab (96 vs. 74%, respectively; $P = 0.008$) [20▪▪]. Rates of hypogammaglobulinemia, a concern with prolonged rituximab exposure, were similar in the rituximab and placebo-treated patients. The rates of serious infection were numerically greater in the group randomized to continued rituximab compared to placebo (12 vs. 9%) [20▪▪]. Additional studies, including cost-effectiveness studies, are needed to define the optimal role for different maintenance strategies in AAV, especially those using varying approaches to frequency and duration of rituximab use.

Although belimumab, a monoclonal antibody targeting B-lymphocyte stimulator (BLyS), also targets B cells, it was not found to have benefit with regard to risk of relapse when added to azathioprine for remission maintenance in patients with GPA or MPA [21▪]. Although there is not a clear role for belimumab in AAV management at this time, it was observed in the trial that none of the patients who received rituximab for induction and were subsequently treated with belimumab had a relapse, raising the question of whether belimumab may have a role in patients who receive rituximab for remission induction [21▪].

Mepolizumab, a monoclonal antibody against IL-5, prevents interaction between IL-5 and the surface of eosinophils and was recently evaluated in a randomized clinical trial (N = 136) for the treatment of eGPA, a condition characterized by eosinophilic infiltration in affected tissue. The addition of mepolizumab to standard immunosuppressive therapy in patients with relapsing or refractory eGPA unable to discontinue glucocorticoids led to higher rates of remission (accrued remission of at least 24 weeks in 28 vs. 3%; $P < 0.001$) and less glucocorticoid exposure over 1 year (mean prednisone dose of 9.2 vs. 13.5 mg) when compared with placebo, prompting regulatory approval [22]. Mepolizumab may be particularly helpful for eGPA cases with asthma and or sino-nasal disease requiring chronic glucocorticoids. Notably, this trial excluded patients with severe eGPA (e.g. glomerulonephritis and cardiomyopathy).

In addition to recent developments using B-cell and eosinophil-targeted therapy, other biologics have been studied for AAV in small trials. In an open-label trial (N = 20) of patients with nonsevere GPA, 90% of abatacept-treated patients had disease improvement, 80% achieved remission and 73% discontinued prednisone. These compelling findings require additional study in a randomized controlled trial [23].

Following the results of two promising phase 2 clinical trials [24,25], a steroid-free regimen with avacopan, a nonbiologic oral antagonist of the human C5a receptor, was recently found to be noninferior to standard glucocorticoid regimens for remission induction in GPA or MPA when combined with rituximab or cyclophosphamide in a large randomized controlled trial (N = 331). Preliminary results indicate that avacopan led to higher rates of sustained remission (65.7 vs. 54.9%, respectively; $P = 0.007$ for superiority of avacopan), reduced glucocorticoid toxicity and higher quality of life scores when compared to standard glucocorticoid-containing regimens [26].

Ongoing clinical trials in AAV are evaluating a low-dose glucocorticoid regimen beginning at 0.5 mg/kg/day (ClinicalTrials.gov; NCT02198248), benralizumab versus mepolizumab for eGPA (ClinicalTrials.gov; NCT04157348) and the combination of rituximab and belimumab (ClinicalTrials.gov; NCT03967925). There are also other trials evaluating other complement inhibitors, like IFX-1, which binds to C5a (ClinicalTrials.gov; NCT03712345).

CRYOGLOBULINEMIC VASCULITIS

Cryoglobulinemic vasculitis is a small-vessel vasculitis caused by cryoglobulinemic immune complex deposition. The most common form of cryoglobulinemic vasculitis is due to hepatitis C infection. The treatment approach is multipronged and includes treatment of the underlying cause, which is especially important in cases due to viral illnesses or malignancy, targeting circulating B cells with rituximab to decrease the production of cryoglobulins in severe disease, and the addition of plasmapheresis in severe cases (e.g. glomerulonephritis, diffuse alveolar hemorrhage and vasculitic neuropathy) [27]. Rituximab has been found to be efficacious and steroid sparing in both viral and nonviral-associated cryoglobulinemic vasculitis with estimated efficacy of 67% for peripheral neuropathy, 77% for weakness, 79% for arthralgia and 85% for cutaneous ulcers [28].

PRIMARY ANGIITIS OF THE CENTRAL NERVOUS SYSTEM

Primary angiitis of the central nervous system (PACNS), or CNS vasculitis, has traditionally been treated with glucocorticoids with or without cyclophosphamide. The relapsing nature of this disease and toxicity of prolonged cyclophosphamide exposure has led to interest in trying biologics for the treatment of PACNS. Rituximab, in particular, has been reported to have potential efficacy in PACNS in several case reports or series [29,30]. Indeed, the inflammatory infiltrate in PACNS is often lymphocytic, with a frequent predominance of B lymphocytes, supporting the use of a B-cell depleting agent [29,30].

BEHCET'S DISEASE

Behcet's disease is characterized by recurrent oral and/or genital ulcers and features of a variable vessel vasculitis (small, medium and large vessels may be affected). Although medications, such as colchicine, azathioprine and apremilast, are effective for mucocutaneous ulcers, the vascular manifestations of Behcet's disease often require additional therapy [31▪,32]. TNF inhibitors are recommended in refractory cases of Behcet's disease and as first-line therapy in cases with neurologic involvement. Infliximab has been the most studied TNF inhibitor for the treatment of Behcet's, followed by adalimumab [33]. In a case series of patients (N = 27) with Behcet's disease manifesting as refractory vasculitis, 80% experienced complete clinical remission within 3 months of initiating a TNF inhibitor [34]. There have also been case series reporting the successful use of ustekinumab and anakinra for refractory Behcet's disease, though additional studies are needed [33,35].

POLYARTERITIS NODOSA

Polyarteritis nodosa (PAN) is a form of medium-vessel vasculitis, most often affecting the skin, nerves, gastrointestinal tract and kidneys. Although it was historically associated with hepatitis B viral (HBV) infections, the frequency of PAN cases associated with hepatitis B has decreased significantly over time, largely in part because of the widespread hepatitis B vaccination [36]. Although treatment of HBV-associated PAN requires antiviral treatment, glucocorticoids have been the cornerstone of therapy for idiopathic PAN, with cyclophosphamide reserved for more severe disease. TNF inhibitors, rituximab and tocilizumab have been reported to potentially have efficacy in PAN, but these data are limited to case series [37–39].

CONCLUSION

Biologics and novel targeted synthetic drugs are playing an increasingly important role as effective glucocorticoid-sparing medications for vasculitis. In particular, the management of GCA and AAV has evolved substantially in recent years with the completion of pivotal clinical trials. Tocilizumab represents the first effective glucocorticoid-sparing agent for GCA and avacopan may lead to a glucocorticoid-free remission induction regimen for AAV. However, additional studies in these and other vasculitides are needed to define the optimal role of biologics and other novel glucocorticoid-sparing therapies.

Acknowledgements

None.

Financial support and sponsorship

NSB is supported by the National Institutes of Health Ruth L. Kirschstein Institutional National Research Service Award (T32-AR- 007258). ZSW is funded by NIH/NIAMS (K23AR073334 and L30 AR070520). The National Institute of Health had no role in the design or authorship of this publication.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Broder MS, Sarsour K, Chang E, *et al.* Corticosteroid-related adverse events in patients with giant cell arteritis: a claims-based analysis. Semin Arthritis Rheum 2016; 46:246–252.
2. Morin C, Fardet L. Systemic glucocorticoid therapy: risk factors for reported adverse events and beliefs about the drug. A cross-sectional online survey of 820 patients. Clin Rheumatol 2015; 34:2119–2126.
3. Gale S, Wilson JC, Chia J, *et al.* Risk associated with cumulative oral glucocorticoid use in patients with giant cell arteritis in real-world databases from the USA and UK. Rheumatol Ther 2018; 5:327–340.
4. Gonzalez-Gay MA, Vazquez-Rodriguez TR, Lopez-Diaz MJ, *et al.* Epidemiology of giant cell arteritis and polymyalgia rheumatica. Arthritis Rheum 2009; 61:1454–1461.
5. Mahr AD, Jover JA, Spiera RF, *et al.* Adjunctive methotrexate for treatment of giant cell arteritis: an individual patient data meta-analysis. Arthritis Rheum 2007; 56:2789–2797.
6. Villiger PM, Adler S, Kuchen S, *et al.* Tocilizumab for induction and maintenance of remission in giant cell arteritis: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet 2016; 387:1921–1927.
7. Stone JH, Tuckwell K, Dimonaco S, *et al.* Trial of tocilizumab in giant-cell arteritis. N Engl J Med 2017; 377:317–328.
8. Langford CA, Cuthbertson D, Ytterberg SR, *et al.* A Randomized, double-blind trial of abatacept (CTLA-4Ig) for the treatment of giant cell arteritis. Arthritis Rheumatol 2017; 69:837–845.
9. Conway R, O'Neill L, Gallagher P, *et al.* Ustekinumab for refractory giant cell arteritis: a prospective 52-week trial. Semin Arthritis Rheum 2018; 48:523–528.
10. ■ Matza MA, Fernandes AD, Stone JH, Unizony SH. Ustekinumab for the treatment of giant cell arteritis. Arthritis Care Res 2020; doi: 10.1002/acr.24200. [Online ahead of print]

This open-label study of ustekinumab in giant cell arteritis was terminated early because of 77% of patients failing to achieve the primary endpoint of prednisone-free remission.

11. Hellmann DB. Giant-cell arteritis: more ecstasy, less agony. N Engl J Med 2017; 377:385–386.
12. ■■ Hellmich B, Agueda A, Monti S, *et al.* 2018 Update of the EULAR recommendations for the management of large vessel vasculitis. Ann Rheum Dis 2020; 79:19–30.

The updated EULAR guidelines for large-vessel vasculitis recommend tocilizumab in patients with GCA with refractory or relapsing disease or an increased risk of glucocorticoid toxicity. They recommend TNF inhibitors or tocilizumab in relapsing or refractory Takayasu arteritis.

13. Nakaoka Y, Isobe M, Takei S, *et al.* Efficacy and safety of tocilizumab in patients with refractory Takayasu arteritis: results from a randomised, double-blind, placebo-controlled, phase 3 trial in Japan (the TAKT study). Ann Rheum Dis 2018; 77:348–354.
14. Stone JH, Merkel PA, Spiera R, *et al.* Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 2010; 363:221–232.
15. Wallace ZS, Miloslavsky EM. Management of ANCA associated vasculitis. BMJ 2020; 368:m421.
16. Guillevin L, Pagnoux C, Karras A, *et al.* Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med 2014; 371:1771–1780.
17. ■ Smith R, Jayne D, Merkel PA. A randomized, controlled trial of rituximab versus azathioprine after induction of remission with rituximab for patients with ANCA-associated vasculitis and relapsing disease [abstract 806]. Arthritis Rheumatol 2019; 71:.

The RITAZAREM trial randomized patients with relapsing ANCA-associated vasculitis to either rituximab or azathioprine as maintenance therapy and found that rituximab was superior to azathioprine in preventing further relapse (HR 0.36; 95% CI 0.23–0.57).

18. Terrier B, Pagnoux C, Perrodeau E, *et al.* Long-term efficacy of remission-maintenance regimens for ANCA-associated vasculitides. Ann Rheum Dis 2018; 77:1150–1156.
19. Charles P, Terrier B, Perrodeau E, *et al.* Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2). Ann Rheum Dis 2018; 77:1143–1149.
20. ■■ Charles P, Perrodeau E, Samson M, *et al.* Long-term rituximab use to maintain remission of antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. Ann Intern Med 2020; 173:179–187.

The MAINRITSAN3 trial randomized patients from MAINRITSAN2 to receive an additional 18 months of rituximab or placebo; they found improved relapse-free survival at 28 months in the rituximab group (98 vs. 74%; $P = 0.008$).

21. ■ Jayne D, Blockmans D, Luqmani R, *et al.* Efficacy and safety of belimumab and azathioprine for maintenance of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled study. Arthritis Rheumatol 2019; 71:952–963.

Following induction therapy, this trial randomized patients with ANCA-associated vasculitis to belimumab or placebo in addition to azathioprine and glucocorticoids and found no significant difference in the maintenance of remission between the belimumab and placebo groups.

22. Wechsler ME, Akuthota P, Jayne D, *et al.* Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. N Engl J Med 2017; 376:1921–1932.
23. Langford CA, Monach PA, Specks U, *et al.* An open-label trial of abatacept (CTLA4-IG) in nonsevere relapsing granulomatosis with polyangiitis (Wegener's). Ann Rheum Dis 2014; 73:1376–1379.

24. Jayne DRW, Bruchfeld AN, Harper L, *et al.* Randomized trial of C5a receptor inhibitor avacopan in ANCA-associated vasculitis. J Am Soc Nephrol 2017; 28:2756–2767.

25. Merkel PA, Niles JL, Jimenez R *et al.* A randomized clinical trial of CCX168, an orally administered C5aR inhibitor for treatment of patients with ANCA-associated vasculitis. Abstract 2016 ACR/ARHP Annual Meeting. 2016.

26. Merkel PA, Jayne D, Yue H, *et al.,* on behalf of the ADVOCATE Study Group. A randomized, double-blind, active-controlled study of avacopan in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Ann Rheum Dis 2020; 79:8.

27. Goglin S, Chung SA. Current treatment of cryoglobulinemic vasculitis. Curr Treat Options Rheumatol 2016; 2:213–224.

28. Roccatello D, Saadoun D, Ramos-Casals M, *et al.* Cryoglobulinaemia. Nat Rev Dis Primers 2018; 4:11.

29. de Boysson H, Arquizan C, Guillevin L, Pagnoux C. Rituximab for primary angiitis of the central nervous system. J Rheumatol 2013; 40:2102–2103.

30. Salvarani C, Brown RD Jr, Huston J III, *et al.* Treatment of primary CNS vasculitis with rituximab: case report. Neurology 2014; 82:1287–1288.

31. Hatemi G, Mahr A, Ishigatsubo Y, *et al.* Trial of apremilast for oral ulcers in ■ Behcet's syndrome. N Engl J Med 2019; 381:1918–1928.

This phase 3 trial demonstrated a greater reduction in the number of oral ulcers with apremilast compared to placebo in patients with Behcet's syndrome.

32. Hatemi G, Silman A, Bang D, *et al.* Management of Behcet disease: a systematic literature review for the European League Against Rheumatism evidence-based recommendations for the management of Behcet disease. Ann Rheum Dis 2009; 68:1528–1534.

33. Muratore F, Pazzola G, Soriano A, *et al.* Unmet needs in the pathogenesis and treatment of vasculitides. Clin Rev Allergy Immunol 2018; 54:244–260.

34. Aksoy A, Yazici A, Omma A, *et al.* Efficacy of TNFalpha inhibitors for refractory vascular Behcet's disease: a multicenter observational study of 27 patients and a review of the literature. Int J Rheum Dis 2020; 23:256–261.

35. Mirouse A, Barete S, Monfort JB, *et al.* Ustekinumab for Behcet's disease. J Autoimmun 2017; 82:41–46.

36. Cui F, Shen L, Li L, *et al.* Prevention of chronic hepatitis B after 3 decades of escalating vaccination policy, China. Emerg Infect Dis 2017; 23: 765–772.

37. Ginsberg S, Rosner I, Slobodin G, *et al.* Infliximab for the treatment of refractory polyarteritis nodosa. Clin Rheumatol 2019; 38:2825–2833.

38. Krusche M, Ruffer N, Kotter I. Tocilizumab treatment in refractory polyarteritis nodosa: a case report and review of the literature. Rheumatol Int 2019; 39:337–344.

39. Loricera J, Blanco R, Hernandez JL, *et al.* Biologic therapy in ANCA-negative vasculitis. Int Immunopharmacol 2015; 27:213–219.

An update on the microbiome in vasculitis

Shahna Tariq and Alison H. Clifford

Purpose of review
To summarize recent evidence regarding the presence and potential role of the microbiome in systemic vasculitides.

Recent findings
Microbiomic descriptions are now available in patients with small, medium and large vessel vasculitis. The majority of studies have evaluated gastrointestinal inhabitants, with a smaller number of studies describing the nasal, pulmonary or vascular microbiomes. Most published studies are observational and cross-sectional. Dysbiosis is seen frequently in vasculitis patients with reduced microbial diversity observed in nasal, fecal and vascular samples compared with disease and/or healthy controls. Predominant bacteria vary, but overall, patients with vasculitis tend to have more pathogenic and less commensal bacteria in active disease. In the few longitudinal studies available, improvement or resolution of dysbiosis has been observed following vasculitis treatment and improved disease activity.

Summary
Dysbiosis and reduced microbial diversity has been identified in patients with small, medium and large vessel vasculitis. Although limited data suggests microbiomes may 'normalize' following immunosuppression, cause or effect cannot be determined. It is hypothesized that microbial disruption in a genetically susceptible individual may trigger excessive host immune activation and vasculitis; however, larger studies with longitudinal and translational design are needed to further our current understanding.

Keywords
antineutrophil cytoplasmic antibody-associated vasculitis, dysbiosis, giant cell arteritis, microbiome, vasculitis

INTRODUCTION

The microbiome refers to the trillions of microbes that live on and in our body. Due to a mutually beneficial relationship, it is believed that our microbiome evolved along with us – resident microbes likely play important roles in nutrition, prevention of invasion by pathogens, and in the continuous 'education' of our immune system [1]. Deviations from the healthy microbiome, broadly termed 'dysbiosis', may result in excessive immune activation and tissue damage. The field is rapidly evolving due to advances in sequencing technology that facilitate culture-independent analysis of these microbial communities. Dysbiosis is now implicated in many autoimmune and vascular diseases, including rheumatoid arthritis (RA) [2,3], systemic lupus erythematosus [3], inflammatory bowel disease (IBD) [4] and atherosclerosis [5]. In this review, we aim to summarize the recent evidence and hypotheses surrounding the role of the microbiome in systemic vasculitis.

THE ROLE OF MICROBES IN SMALL VESSEL VASCULITIS: ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are a group of diseases characterized by ANCA production, excessive neutrophil activation and small–medium vessel vasculitis [6]. Mucosal inflammation of the upper and lower respiratory tract is a striking feature in many AAV patients, raising suspicions for an infectious trigger [7]. *Staphylococcus aureus*, in particular, has

Division of Rheumatology, University of Alberta, Edmonton, Alberta, Canada

Correspondence to Alison H. Clifford, MD, FRCPC, Assistant Professor, Division of Rheumatology, University of Alberta, 8-130K Clinical Sciences Building, Edmonton, AB, Canada T6G 2G3. Tel: +1 780 492 1965; fax: +1 780 492 6088; e-mail: alison5@ualberta.ca

Curr Opin Rheumatol 2021, 33:15–23

DOI:10.1097/BOR.0000000000000758

KEY POINTS

- Dysbiosis and reduced microbial diversity is observed in vasculitis patients.
- In some studies, treatment of vasculitis is associated with 'normalization' of the healthy microbiome, but cause or effect is not determined.
- Common hypotheses suggesting a causal role for the microbiome include the possibility of a directly antagonistic relationship between pathogenic vs. commensal organisms that leads to chronic immune activation, or the existence of a gut-renal axis of inflammation.
- Studies evaluating larger groups of patients in a longitudinal fashion and translational experiments to understand possible disease mechanisms are needed.
- Whether the gut microbiome may influence or predict an individual's response to therapy or likelihood of adverse effects from immunosuppressive drugs is an area for future study.

been the subject of extensive study in granulomatosis with polyangiitis (GPA), with hypotheses including neutrophil priming, molecular mimicry [8▪], superantigen production, among others [9], proposed as possible pathogenic mechanisms. Although the efficacy of trimethoprim–sulfamethoxazole, and association of nasal carriage with relapse [10], supports a potential role for *S. aureus* in GPA [9], findings are not universal [11], suggesting this bacteria is not the singular trigger. Recently, nasal *Staphylococcus pseudintermedius*, typically a microbe of domestic animals, has also been cultured in GPA patients [12]; however, the significance of this is still unclear.

NASAL MICROBIOME IN ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIDES

In the first study of the collective nasal microbiome in GPA, Rhee *et al.* compared vasculitis patients (75% in remission) and healthy controls, using16S rRNA and internal transcribed spacer analysis. Nasal dysbiosis was identified in GPA patients, with reductions in *Propionibacterium acnes* and *Staphylococcus epidermidis* observed, but no differences in *S. aureus*. Use of nonglucocorticoid immunosuppression was associated with 'normalization' of the nasal microbiome independent of disease activity, suggesting that medication use may moderate nasal microbes [13▪]. Significantly, this idea is supported by the pharmacomicrobiomics literature (the study of interactions between the microbiome and drug efficacy/adverse events), where preliminary data also suggests that methotrexate may both influence and be influenced by our resident microbes [14▪▪]. Similarly to Rhee *et al.*, a German study identified a trend toward reduced bacterial diversity among nasal swabs from GPA patients compared with RA and healthy controls. Notably, *S. aureus* was increased in GPA compared with other groups [15]. In the United Kingdom, *S. aureus* was also significantly more abundant in the nasal cavity of GPA patients, whereas *S. epidermidis* was more abundant in healthy controls, suggesting an antagonistic relationship between species [16▪]. Mechanistically, the presence of *S. epidermidis* may protect against *S. aureus* by secreting Esp, a serine protease that inhibits bacterial adhesion and activates immune defenses to clear *S. aureus* [17].

Recently, longitudinal evaluation of the nasal microbiome in GPA (assessed every 3 months over an average of 6 years), demonstrated a higher *Staphylococcus* to *Corynebacterium* ratio prior to flare [18▪], and a fall in *S. epidermidis* and *P. acnes* populations during flare, with rise in *Corynebacterium tuberculostearicum* abundance. This work demonstrated that microbial communities fluctuate with time and disease activity; however, cause vs. effect is uncertain. Additional details of small vessel vasculitis microbiome studies using culture-independent techniques are available in Table 1.

OTHER NICHES IN ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIDES

A single study has assessed the lower respiratory tract microbiome in AAV, comparing pretreatment bronchoalveolar lavage (BAL) fluid from 16 predominantly microscopic polyangiitis patients and those with biopsy-proven sarcoidosis [19]. Alpha-diversity was negatively associated with disease activity in AAV; however, no significant differences between AAV and sarcoidosis were observed. When typical oral microbes (extrapolated from Human Microbiome Project data) were excluded from the analysis, differences did emerge, establishing the importance of considering oral microbiota contributions in future BAL studies.

Up to 80% of patients with IBD are ANCA-positive in the absence of clinical vasculitis [23], suggesting a possible shared pathogenesis of these diseases. Although the role of gut microbes in IBD continues to be a hot topic [4], this niche remains relatively unexplored in AAV. Preliminary data from Najem *et al.* describe the association of gut dysbiosis with significantly higher BVAS scores [20], whereas the

Table 1. Summary of microbiome studies in the small vessel vasculitides, performed using culture-independent techniques

Reference	Disease	Controls	Site	Technique	Study design	Major findings
Rhee *et al.* [13▪]	60 GPA in USA	41 Healthy controls	Nasal	Bacterial 16S rRNA (V1, V2) Fungal ITS1 gene sequencing	Cross-sectional	Reduced *Propionibacterium acnes* and *Staphylococcus epidermidis* in GPA No difference in *Staphylococcus aureus* observed Reduced abundance of fungal order *Malasseziales* with increased GPA disease activity Non-GC immunosuppression associated with 'healthy' nasal microbiome
Lamprecht *et al.* [15]	29 GPA in Germany 23 Remission 6 Active	21 RA 27 Healthy controls	Nasal	qPCR for *S. aureus*, *Haemophilus influenzae*, *Enterovirus*, *Rhinovirus* Bacterial 16s rRNA (V3, V4) UMERS	Cross-sectional	Reduced diversity and richness in GPA Relative abundance at family level differed with overall disease activity and ENT activity in GPA Increased *S. aureus* in GPA vs. RA or controls by qPCR UMERS identified no previously unknown pathogens
Wagner *et al.* [16▪]	56 GPA in UK 12 Active 44 Inactive	23 Disease controls 13 eGPA 10 MPA 15 Healthy controls 4 Household contacts 11 Healthy hospital personnel	Nasal	Nasal culture Bacterial 16S rRNA (V1, V2) Shotgun metagenomic sequencing Functional analysis SEED proteins	Cross-sectional	Increased *S. aureus* in active GPA Increased *S. epidermidis* in healthy controls *Staphylococcus pseudintermedius* relative abundance 13% Increased genes for chromisate synthesis and vitamin B12 pathways in GPA
Rhee *et al.* [18▪]	19 GPA in USA 9 Flare 10 Remission	None	Nasal	16S rRNA	Longitudinal	Prior to flare: increased ratio *Staphylococcus* to *Corynebacteria* During flare: reduced *S. epidermidis* and *P. acnes*, increased *Corynebacterium tuberculostearicum*
Fukui *et al.* [19]	16 New AAV in Japan 14 MPA 2 GPA	21 New biopsy+sarcoidosis Data from Human Microbiome Project	BAL	Bacterial 16S rRNA (V4)	Cross-sectional	Negative linear relationship between alpha-diversity and BVAS in AAV group No difference in alpha diversity in AAV vs. sarcoidosis Differences emerged when oral microbes excluded
Najem *et al.* [20]	49 AAV in USA 29 Active 20 Remission	14 Healthy controls	Fecal	Bacterial 16S rRNA (V1, V2)	Cross-sectional and longitudinal	Active AAV associated with gut dsybiosis Higher BVAS correlated with increased dysbiosis Immunosuppression and antibiotic use resulted in normalization of gut microbiome
Chen *et al.* [21]	98 Children hospitalized with IgAV in China	66 Healthy age, sex-matched children	Oral	Bacterial 16S rRNA (V1, V2)	Cross-sectional	Increased richness and diversity in IgAV Increased *Butyrivibrio* Reduced *Haemophilus* sp. *Prevotella nanceiensis* correlated with serum IgA
Wang *et al.* [22]	85 Children hospitalized with IgAV in China	70 Healthy age, sex-matched children	Fecal	Bacterial 16S rRNA (V2, V2)	Cross-sectional	Reduced diversity and richness in IgAV Reduced *Roseburia*, *Parasutterella*, *Dialister* in IgAV Increased *Enterococcus*, *Parabacteroides* in IgAV Negative correlation between *Bifidobacteria* and serum IgA Negative correlation between *Roseburia*, *Paraprevotella* and length of stay

AAV, antineutrophil cytoplasmic antibody-associated vasculitides; BAL, bronchoalveolar lavage; BVAS, Birmingham vasculitis activity score; eGPA, eosinophilic granulomatosis with polyangiitis; ENT, ear, nose, throat; GPA, granulomatosis with polyangitiis; GC, glucocorticoid; IgAV, IgA vasculitis; MPA, microscopic polyangiitis; qPCR, quantitative PCR; RA, rheumatoid arthritis; UMERS, unbiased nontargeted metagenomic sequencing.

gut microbiome in AAV patients in remission was similar to that of controls. Use of immunosuppressive drugs, glucocorticoids and antibiotics were all associated with reduced gut dysbiosis. With respect to possible mechanisms, Krebs *et al.* [24] previously demonstrated in a mouse model of AAV that Th17 cells may migrate from their place of residence in the bowel wall lamina propria to the kidney, where they contribute to glomerulonephritis. When mice were raised in germ-free conditions or treated with broad spectrum antibiotics, gut Th17 cells were significantly reduced, as was renal inflammation. Similarly, a recent case report describes the development of de novo biopsy-proven anti-myeloperoxidase (MPO) antibody-positive rapidly progressive glomerulonephritis in a patient 3 weeks following fecal transplant for recurrent *Clostridium difficile* infection [25], supporting the possibility of a gut-renal axis in susceptible humans.

MICROBIOME CONTRIBUTIONS TO IMMUNE COMPLEX VASCULITIS

Similarly, a role for the gastrointestinal microbiome has been suggested in IgA vasculitis (IgAV). With a peak onset between the ages of 4 and 6 years, seasonal variation, and a frequently self-limited nature, IgAV is felt likely to have an infectious trigger. Increased serum levels and tissue deposition of structurally abnormal IgA are a hallmark of this disease – when IgA1 becomes glycosylated, antigens at the hinge portion are exposed and stimulate immune activation and immune complex formation [26▪]. The same glycosylation is observed in IgA nephropathy (IgAN), a condition pathologically indistinguishable from IgAV in the kidney [27▪▪]. In a mouse model of IgAN, bowel commensals were shown to stimulate gut-associated lymphoid tissue production of glycosylated IgA1 and exacerbate nephritis, suggesting an interaction between host factors, gut microbes and IgA secretion [28].

In humans, the oral microbiome of children hospitalized with IgAV revealed increased microbial diversity and *Prevotella nanceiensis* positively correlated with serum IgA levels [21]. In contrast, reduced microbial diversity was identified in fecal samples of 85 patients with IgAV vs. healthy controls [22]. *Parabacteroides* and *Enterococcus* were more abundant in children with vasculitis, whereas *Dialister*, *Parasutterella* and *Roseburia* (a known producer of butyrate, which induces T-regulatory cells and has anti-inflammatory effects on the colonic mucosa) were reduced. Length of hospital stay was negatively correlated with the levels of *Roseburia* and *Paraprevotella*. Gut dysbiosis has also been demonstrated in patients hospitalized with IgAN [29▪].

In IgAN, a phase 2B study of targeted-release budesonide (designed to act specifically at Peyer's patches in the ileocolic junction) demonstrated a significant reduction in proteinuria and stabilization of glomerular filtration rate (GFR) as compared with supportive care alone, emphasizing the association between gut inflammation and nephritis [30]. Given the histopathologic similarities to IgAN, further descriptive and mechanistic studies of gut microbiome interaction with host cells in IgAV seem warranted.

THE MICROBIOME IN MEDIUM VESSEL VASCULITIS

Kawasaki disease is the most common cause of acquired cardiac disease in children in North America due to its propensity for coronary artery aneurysms. Recently, suspicions have been raised that Kawasaki disease may in fact be a form of IgAV that is mediated by gut inflammation [31▪]. In a mouse model of Kawasaki disease, intestinal permeability was found to be a critical, early component of the disease, leading to increased serum IgA levels and ultimately coronary artery aneurysm formation with IgA deposition in vessel walls. When intestinal permeability was blocked, vasculitis was ameliorated in mice [32▪▪]. Although limitations exist in extrapolating this data to human Kawasaki disease, autopsy data of children who died of ruptured coronary aneurysms from Kawasaki disease also demonstrated coronary artery IgA deposition [33]. In a genetically susceptible host, it is hypothesized that alterations in the gut microbiome as a result of age, diet, infectious triggers [34] and/or antimicrobials leads to immune system activation and ultimately Kawasaki disease [35]. In keeping with this, the highest incidence of Kawasaki disease is seen in 6–11-month olds, a time at which the gut microbiome is rapidly shifting due to maturation and the introduction of solid foods [35]. Gastrointestinal symptoms at Kawasaki disease onset are predictive of intravenous immunoglobulin (IVIG) resistance and associated with an increased risk of coronary artery aneurysms [36], and previous antibiotic use is associated with an increased risk for the development of Kawasaki disease (odds ratio 11.7) in a dose-dependent manner [37▪]. Culture-based approaches have shown higher levels of Gram-positive cocci (*Staphylococcus* and *Streptococcus*) and less *Lactobacillus* spp. in stool of Kawasaki patients but have failed to identify one specific pathogen for the condition [35].

Using metagenomic sequencing, Kinumaki *et al.* [38] longitudinally studied fecal samples of 28 Japanese Kawasaki disease patients. A single pathogen

Table 2. Summary of microbiome studies in medium vessel vasculitis, performed using culture-independent techniques

Reference	Disease	Controls	Site	Technique	Study design	Major findings/Comments
Kinumaki *et al.* [38]	28 Children hospitalized with KD, Japan	None	Fecal	Metagenomic sequencing Stool culture followed by 16S rRNA	Longitudinal Samples taken at hospital admission, and 4–6 months later	During acute phase, *Streptococcus* spp. increased Findings confirmed using stool culture and 16S rRNA In nonacute phase: increased *Roseburia*, *Ruminococcus*, *Faecalibacterium* seen
Khan *et al.* [39▪]	5 Children with KD, China	3 Healthy controls	Fecal	Bacterial 16S rRNA (V4)	Longitudinal Samples taken at acute illness, posttreatment and at revisit	In acute KD: increased *Streptococcus*, *Fusobacteria* and *Shigella* *Acinetobacter johnsonii*, *Anaerostipes butyraticus* and *Paludibacter* spp. found only in acute KD Posttreatment KD: *Roseburia* reappeared Posttreatment KD: prevalence of *Bacteroidetes* and *Firmicutes* increased over time, and *Fusobacterium* fell and disappeared at revisit

KD, Kawasaki disease.

could not be identified; however, *Streptococcus* (especially the mitis group) species were increased during the acute phase of the illness, whereas the nonacute phase of the illness was characterized by predominance of *Ruminococcus*, *Roseburia*, and *Faecalibacterium* (refer to Table 2 for medium vessel microbiome study details). Recently, Khan *et al.* [39▪] also assessed fecal samples from five Kawasaki disease patients in China obtained pre and posttreatment. Pathogenic bacteria dominated in samples collected during the acute phase of illness and subsequently fell posttreatment with IVIG and aspirin. Beneficial bacteria were more abundant in control samples, and were subsequently restored among treated Kawasaki disease patients, suggesting an association between gut dysbiosis and disease activity.

In May 2020, reports of a Kawasaki-like syndrome presenting in children following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection emerged [40▪]. In June 2020, an Italian study reported a 30-time increased incidence of Kawasaki disease during the 2-month period between February and April 2020, as compared with the previous 5 years, corresponding to their surge of SARS-CoV-2 cases [41]. Similar observations were noted by UK [40▪], French [42] and US [43,44] investigators, resulting in the new term, multisystem inflammatory syndrome in children (MIS-C). In contrast to typical Kawasaki disease, MIS-C has a predilection for children of African, rather than Asian, descent [42], and older children and adolescents. Enteropathy is striking in MIS-C, as is myocarditis [44]. Skin biopsy from a single patient with SARS-CoV-2 and MIS-C revealed leukocytoclastic vasculitis with IgA deposition [45▪], suggesting possible overlap with IgAV. Additional data regarding MIS-C, its association to SARS-CoV-2 and possible relationship to Kawasaki disease or other vasculitides are expected as information from the pandemic becomes available.

MICROBIOME IN GIANT CELL ARTERITIS

The cause of primary large vessel vasculitides remains elusive, but in giant cell arteritis (GCA), cyclical spikes in incidence [46], and known contributions of Th1 responses and IFNγ production [47], are suspicious for an underlying infectious trigger. Historically, varicella zoster virus (VZV) has been of special interest in GCA [48]. Although a high frequency of VZV antigen was detected in temporal artery and aorta samples of GCA patients by one group [49–52], results could not be replicated by other centers in the United States and Italy [53,54], suggesting that VZV is likely a mimic, not a cause of GCA.

Traditionally considered sterile, increasing evidence suggests that blood vessels may possess their own microbiome [55]. In 2013, the first study of the temporal artery microbiome using unbiased DNA sequencing was published [56]. No previously investigated pathogens and no novel microbes were identified. Skin contaminants were most abundant; however, results may have been limited by use of formalin fixation, and paraffin. Hoffman *et al.* [57▪] then described the microbiome of snap-frozen temporal arteries from biopsy-positive and biopsy-negative GCA patients. GCA samples differed from controls with respect to beta diversity, with no differences between biopsy-positive and negative patients appreciated. Fluorescence in-situ hybridization revealed bacterial rRNA localizing specifically

to the media, suggesting contamination was unlikely. The same group also described the microbial contents of the thoracic aorta [58▪]. Although temporal artery and aorta samples were processed simultaneously, significant differences between these microbial communities were detected, suggesting that, like other niches in the body, different vascular beds may contain different microbes. Again, diversity was significantly reduced in aortitis patients as compared with controls, but no differences noted between GCA and clinically isolated aortitis subtypes, perhaps reflecting a shared pathogenesis. Additional details of GCA and other large vessel vasculitis (LVV) microbiome studies are available in Table 3.

MICROBIAL CONTRIBUTIONS TO TAKAYASU'S ARTERITIS AND A WORD ON BEHCET SYNDROME

Although possessing a similar predilection for the aorta and major branches, Takayasu's arteritis (TAK) is distinguished from GCA by its younger age of onset and increased incidence in patients of Asian

Table 3. Summary of microbiome studies in large vessel vasculitis, performed using culture-independent techniques

Reference	Disease	Controls	Site	Technique	Study design	Major findings/Comments
Bhatt *et al.* [56]	12 Biopsy-proven GCA, USA	5 Controls in whom GCA excluded	Temporal artery (FF, PE)	Unbiased DNA sequencing	Cross-sectional	*Propionibacterium acnes*, *Escherichia coli* most abundant in both controls and GCA (16/17 samples) *Moraxella catarrhalis* most abundant in 1 control No previously investigated pathogens or unknown microbes identified
Hoffman *et al.* [57▪]	24 GCA, USA 9 +TAB 15 −TAB	23 Controls in whom GCA excluded	Temporal artery (snap-frozen)	Bacterial 16S rRNA (V3, V4) FISH with oligonucleotide probe for 16S rRNA	Cross-sectional	No differences in alpha diversity Beta diversity differed between GCA and controls No differences between TAB+ and TAB− GCA Reduced *Parasutterella*, *Bifidobacterium* in GCA Increased *Granulicatella*, *Streptococcus* in GCA Bacterial rRNA localized to media of artery in GCA and controls using FISH
Getz *et al.* [58▪]	26 Aortitis, USA 14 GCA 12 CIA	23 Noninflammatory controls	Thoracic aorta (snap-frozen)	Bacterial 16S rRNA (V3, V4)	Cross-sectional	Significant differences between temporal artery and thoracic aorta microbiomes Significant differences in alpha and beta diversity between aortitis and controls No differences in diversity in GCA vs. CIA aortas Increased *Phascolarctobacterium* in aortitis Decreased *Prevotella*, *Acinetobacter*, *Klebsiella*, *Staphylococcus*, *Corynebacterium* Increased pathways for oxidative phosphorylation and porphyrin metabolism, downregulated transcription factor pathways in aortitis
Desbois *et al.* [59]	20 TAK 10 Active 10 Remission	10 GCA 6 Active 4 Remission 16 Healthy controls	Blood	Bacterial 16S rRNA	Cross-sectional	Increased *Clostridia*, *Cytophagia*, *Deltaproteobacteria* in TAK vs. healthy controls Increased *Bacillus*, *Staphylococcus* in controls Active TAK associated with reduced *Staphylococcus* Increased *Bacteroidia* in TAK vs. GCA In TAK: increased porphyrin and chlorophyll pathways

CIA, clinically isolated aortitis; FF, formalin-fixed; FISH, fluorescence in-situ hybridization; GCA, giant cell arteritis; PE, paraffin-embedded; TAB−, temporal artery biopsy-negative; TAB+, temporal artery biopsy-positive; TAK, Takayasu's arteritis.

and South American, rather than Northern European, descent [60]. Of individual pathogens, the role of *Mycobacterium tuberculosis* (TB) in TAK has been most extensively studied to date. In TAK patients, the reported prevalence for latent (20–82%) and active (6.3–20%) TB infection ranges widely, as does the prevalence of TB DNA in TAK aortic samples (between 0 and 70%) [61]. Notably, TAK patients often respond favorably to antitumor necrosis factor therapy and have low rates of TB reactivation, suggesting this relationship is associative, not causal [62].

Relevant to the microbiome discussion, TAK and IBD may share a similar genetic predisposition (HLA-B52) and co-occur more frequently than would be expected by chance [63]. In TAK patients with IBD, bowel inflammation tends to precede vascular disease by an average of 7 years [64], raising the possibility that gut dysbiosis may stimulate immune dysregulation that results in both IBD and vasculitis. In support of this theory, Kanıtez *et al*. [65] performed random colon biopsies in 30 asymptomatic TAK patients, and found histologically proven colitis in 30%, more commonly those with active vs. inactive TAK.

In addition to IBD, similarities also exist between TAK and Behcet syndrome, an autoimmune disease characterized by gastrointestinal and vascular inflammation, with an HLA-B51 association. In Behcet's, like TAK, gastrointestinal manifestations usually precede vascular lesions, and respond to antitumor necrosis factor therapy [64]. Gut microbiome studies in Behcet's have revealed dysbiosis [66,67▪], with reduction in anti-inflammatory butyrate-producing bacteria and an increase in proinflammatory sulfate-reducing bacteria [67▪]. Fascinatingly, fecal transplant from Behcet's patients into a mouse model was shown to exacerbate uveitis, suggesting a causal link between gut bacteria and systemic inflammation [67▪]. A randomized trial to assess the efficacy of dietary interventions for control of Behcet's symptoms via manipulation of the gut microbiome is currently enrolling [68▪▪].

To date, preliminary data evaluating the blood microbiome in patients with TAK is published [59] (Table 3), but no descriptions of the gut microbiome in either TAK or GCA are available. Such studies are anticipated to shed light on possible common mechanisms.

CONCLUSION

Microbiome studies exploring the inhabitants of various niches of the human body, have, in most cases, revealed dysbiosis and reduced microbial diversity in vasculitis patients. In some cases, 'normalization' of the healthy microbiome has been documented with immunosuppression, but cause or effect has not been determined. It is hypothesized that in vasculitis patients, dysbiosis triggered by common life events such as infection or antibiotic use, stimulates an abnormal host immune response mediated by their particular genetic-susceptibility that leads to the development of systemic vasculitis. Thus far, studies in vasculitis have been predominantly descriptive and associative. Going forward, longitudinal studies in larger patient cohorts will be needed to help inform the sequence of events and distinguish cause from effect, whereas translational studies will be critical to further explore hypotheses and disease-specific mechanisms. In addition, we hope to see expansion in the field of pharmacomicrobiomics in vasculitis as a potential tool to better predict our patients' response to medications and risk for adverse events.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Turnbaugh PJ, Ley RE, Hamady M, *et al.* The human microbiome project. Nature 2007; 449:804–810.
2. Maeda Y, Takeda K. Host–microbiota interactions in rheumatoid arthritis. Exp Mol Med 2019; 51:1–6.
3. Konig MF. The microbiome in autoimmune rheumatic disease. Best Pract Res Clin Rheumatol 2020; 34:101473.
4. Nishida A, Inoue R, Inatomi O, *et al.* Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol 2017; 11:1–10.
5. Wun K, Theriault BR, Pierre JF, *et al.* Microbiota control acute arterial inflammation and neointimal hyperplasia development after arterial injury. PLoS One 2018; 13:e0208426.
6. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. Nat Rev Rheumatol 2019; 15:91–101.
7. Lamprecht P, Kerstein A, Klapa S, *et al.* Pathogenetic and clinical aspects of anti-neutrophil cytoplasmic autoantibody-associated vasculitides. Front Immunol 2018; 9:680.
8. ▪ Ooi JD, Jiang J-H, Eggenhuizen PJ, *et al.* A plasmid-encoded peptide from *Staphylococcus aureus* induces antimyeloperoxidase nephritogenic autoimmunity. Nat Commun 2019; 10:3392.

The study suggests that molecular mimicry may explain the association between *Staphylococcus aureus* and antineutrophil cytoplasmic antibody-associated vasculitides, by demonstrating that some *S. aureus* species contain a peptide homologous to an MPO T-cell epitope.

9. Tervaert JWC. Trimethoprim–sulfamethoxazole and antineutrophil cytoplasmic antibodies-associated vasculitis. Curr Opin Rheumatol 2018; 30:388–394.

10. Stegeman CA, Tervaert JW, Sluiter WJ, *et al.* Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. Ann Intern Med 1994; 120:12–17.
11. Tan BK, Crabol Y, Tasse J, *et al.* No evident association of nasal carriage of *Staphylococcus aureus* or its small-colony variants with cotrimoxazole use or ANCA associated vasculitis relapses. Rheumatology 2019; 59:77–83.
12. Kronbichler A, Blane B, Holmes MA, *et al.* Nasal carriage of *Staphylococcus pseudintermedius* in patients with granulomatosis with polyangiitis. Rheumatology 2018; 58:548–550.
13. ■ Rhee RL, Sreih AG, Najem CE, *et al.* Characterisation of the nasal microbiota in granulomatosis with polyangiitis. Ann Rheum Dis 2018; 77:1448–1453.

This is the first article to describe the collective bacterial and fungal inhabitants of nasal cavities in granulomatosis with polyangiitis (GPA) patients using culture-independent techniques, with intriguing results of normalization of the nasal microbiome with immunosuppression and disease quiescence.

14. ■■ Scher JU, Nayak RR, Ubeda C, *et al.* Pharmacomicrobiomics in inflammatory arthritis: gut microbiome as modulator of therapeutic response. Nat Rev Rheumatol 2020; 16:282–292.

This is an excellent review describing the potential role that pharmacomicrobiomics may play in understanding and possibly predicting an individual's likelihood of response to therapy, and potential for drug toxicities using examples in inflammatory arthritis.

15. Lamprecht P, Fischer N, Huang J, *et al.* Changes in the composition of the upper respiratory tract microbial community in granulomatosis with polyangiitis. J Autoimmun 2019; 97:29–39.
16. ■ Wagner J, Harrison EM, Pero MMD, *et al.* The composition and functional protein subsystems of the human nasal microbiome in granulomatosis with polyangiitis: a pilot study. Microbiome 2019; 7:137.

The study suggests a potential antagonistic relationship between *S. aureus* and *Staphylococcus epidermidis* in patients with GPA.

17. Iwase T, Uehara Y, Shinji H, *et al. Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. Nature 2010; 465:346–349.
18. ■ Rhee R, Sreih A, Bittinger K, *et al.* Longitudinal changes in the nasal microbiome of patients with granulomatosis with polyangiitis. Rheumatology 2019; 58(Suppl_2).

The abstract presents longitudinal microbiome data in GPA showing nasal microbial communities fluctuate over time with disease activity.

19. Fukui S, Morimoto S, Ichinose K, *et al.* Comparison of lung microbiota between antineutrophil cytoplasmic antibody-associated vasculitis and sarcoidosis. Sci Rep 2020; 10:9466.
20. Najem CE, Lee J-J, Tanes C, *et al.* Defining the gut microbiome in patients with ANCA associated vasculitis. Arthritis Rheumatol 2018; 70(Suppl 10).
21. Chen B, Wang J, Wang Y, *et al.* Oral microbiota dysbiosis and its association with Henoch-Schönlein Purpura in children. Int Immunopharmacol 2018; 65:295–302.
22. Wang X, Zhang L, Wang Y, *et al.* Gut microbiota dysbiosis is associated with Henoch Schönlein Purpura in children. Int Immunopharmacol 2018; 58:1–8.
23. Lee W-I, Subramaniam K, Hawkins CA, Randall KL. The significance of ANCA positivity in patients with inflammatory bowel disease. Pathology 2019; 51:634–639.
24. Krebs CF, Paust H-J, Krohn S, *et al.* Autoimmune renal disease is exacerbated by S1P-receptor-1-dependent intestinal Th17 cell migration to the kidney. Immunity 2016; 45:1078–1092.
25. Amlani A, Bromley A, Fifi-Mah A. ANCA vasculitis and hemophagocytic lymphohistiocytosis following a fecal microbiota transplant. Case Rep Rheumatol 2018; 2018:1–3.
26. ■ Oni L, Sampath S. Childhood IgA vasculitis (Henoch Schonlein Purpura) – advances and knowledge gaps. Front Pediatr 2019; 7:257.

This is an excellent overview of the current understanding of IgA vasculitis (IgAV) pathogenesis.

27. ■■ Coppo R. The gut-renal connection in IgA nephropathy. Semin Nephrol 2018; 38:504–512.

The review discusses the evidence supporting the existence of a gut-renal axis of inflammation in IgA nephropathy (IgAN), suggesting possible new targets for treatment.

28. McCarthy DD, Kujawa J, Wilson C, *et al.* Mice overexpressing BAFF develop a commensal flora–dependent, IgA-associated nephropathy. J Clin Invest 2011; 121:3991–4002.
29. ■ Hu X, Du J, Xie Y, *et al.* Fecal microbiota characteristics of Chinese patients with primary IgA nephropathy: a cross-sectional study. BMC Nephrol 2020; 21:97.

The study showed reduced bacterial diversity in patients with IgAN and an association between the presence of certain gut bacteria and worsening renal parameters, suggesting possible causal relationships.

30. Fellström BC, Barratt J, Cook H, *et al.* Targeted-release budesonide versus placebo in patients with IgA nephropathy (NEFIGAN): a double-blind, randomised, placebo controlled phase 2b trial. Lancet 2017; 389:2117–2127.
31. ■ Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. Nat Rev Rheumatol 2020; 16:391–405.

This is an excellent review of current thoughts on Kawasaki disease pathogenesis and observations from animal models of disease.

32. ■■ Noval Rivas M, Wakita D, Franklin MK, *et al.* Intestinal permeability and IgA provoke immune vasculitis linked to cardiovascular inflammation. Immunity 2019; 51:508–521.e6.

The study demonstrates a critical role for intestinal permeability in a mouse model of Kawasaki disease, suggesting a new possible target for therapy in Kawasaki disease, and possible disease overlap between Kawasaki disease and IgAV.

33. Rowley AH, Eckerley CA, Jack HM, *et al.* IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. J Immunol 1997; 159:5946–5955.
34. Rhim J-W, Kang HM, Han J-W, Lee K-Y. A presumed etiology of Kawasaki disease based on epidemiological comparison with infectious or immune-mediated diseases. Front Pediatr 2019; 7:202.
35. Esposito S, Polinori I, Rigante D. The gut microbiota-host partnership as a potential driver of Kawasaki syndrome. Front Pediatr 2019; 7:124.
36. Fabi M, Corinaldesi E, Pierantoni L, *et al.* Gastrointestinal presentation of Kawasaki disease: a red flag for severe disease? PLoS One 2018; 13:e0202658.
37. ■ Fukazawa M, Fukazawa M, Nanishi E, *et al.* Previous antibiotic use and the development of Kawasaki disease: a matched-pair case–control study. Pediatr Int 2020; 62:1044–1048.

The case–control study suggests a role for environmental exposures that alter the gut microbiome in stimulating vasculitis onset, by demonstrating that previous antibiotic use and c-section birth are associated with a significantly increased risk for subsequent Kawasaki disease.

38. Kinumaki A, Sekizuka T, Hamada H, *et al.* Characterization of the gut microbiota of Kawasaki disease patients by metagenomic analysis. Front Microbiol 2015; 6:824.
39. ■ Khan I, Li X-A, Law B, *et al.* Correlation of gut microbial compositions to the development of Kawasaki disease vasculitis in children. Future Microbiol 2020; 15:591–600.

The study longitudinally assessed the gut microbiome in Kawasaki disease patients, demonstrating shifts in pathogenic microbes pre and posttreatment.

40. ■ Riphagen S, Gomez X, Gonzalez-Martinez C, *et al.* Hyperinflammatory shock in children during COVID-19 pandemic. Lancet 2020; 395: 1607–1608.

This is the first report of the association of a novel, Kawasaki's-like hyperinflammatory syndrome in children with severe acute respiratory syndrome coronavirus 2 infection.

41. Verdoni L, Mazza A, Gervasoni A, *et al.* An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet 2020; 395:1771–1778.
42. Toubiana J, Poirault C, Corsia A, *et al.* Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. BMJ 2020; 369:m2094.
43. Leon MPD, Redzepi A, Mcgrath E, *et al.* COVID-19-associated pediatric multisystem inflammatory syndrome. J Pediatric Infect Dis Soc 2020; 9:407–408.
44. Chiotos K, Bassiri H, Behrens EM, *et al.* Multisystem inflammatory syndrome in children during the coronavirus 2019 pandemic: a case series. J Pediatric Infect Dis Soc 2020; 9:393–398.
45. ■ Schnapp A, Abulhija H, Maly A, *et al.* Introductory histopathological findings may shed light on COVID 19 paediatric hyperinflammatory shock syndrome. J Eur Acad Dermatol Venereol 2020.

The case report is the first to describe histopathology from a pediatric patient with COVID-19 hyperinflammatory syndrome, with findings of leukocytoclastic vasculitis with IgA and immune complex deposition.

46. Salvarani C, Gabriel SE, O'Fallon WM, Hunter GG. The incidence of giant cell arteritis in Olmsted County, Minnesota: apparent fluctuations in a cyclic pattern. Ann Intern Med 1995; 123:192–194.
47. Weyand CM, Liao YJ, Goronzy JJ. The immunopathology of giant cell arteritis. J Neuro-Ophthalmol 2012; 32:259–265.
48. Nagel MA, Gilden D. Update on varicella zoster virus vasculopathy. Curr Infect Dis Rep 2014; 16:407.
49. Gilden D, White T, Khmeleva N, *et al.* Prevalence and distribution of VZV in temporal arteries of patients with giant cell arteritis. Neurology 2015; 84:1948–1955.
50. Nagel MA, White T, Khmeleva N, *et al.* Analysis of varicella-zoster virus in temporal arteries biopsy positive and negative for giant cell arteritis. JAMA Neurol 2015; 72:1281–1287.
51. Gilden D, White T, Boyer PJ, *et al.* Varicella zoster virus infection in granulomatous arteritis of the aorta. J Infect Dis 2016; 213:1866–1871.
52. Gilden D, White TM, Nagae L, *et al.* Successful antiviral treatment of giant cell arteritis and Takayasu arteritis. JAMA Neurol 2015; 72:943–946.
53. Procop GW, Eng C, Clifford A, *et al.* Varicella zoster virus and large vessel vasculitis, the absence of an association. Pathog Immun 2017; 2:228–238.
54. Muratore F, Croci S, Tamagnini I, *et al.* No detection of varicella-zoster virus in temporal arteries of patients with giant cell arteritis. Semin Arthritis Rheum 2017; 47:235–240.
55. Clifford A, Hoffman GS. Evidence for a vascular microbiome and its role in vessel health and disease. Curr Opin Rheumatol 2015; 27:397–405.
56. Bhatt AS, Manzo VE, Pedamallu CS, *et al.* In search of a candidate pathogen for giant cell arteritis: sequencing-based characterization of the giant cell arteritis microbiome. Arthritis Rheumatol 2014; 66:1939–1944.

57. Hoffman GS, Getz TM, Padmanabhan R, *et al.* The microbiome of temporal ■ arteries. Pathog Immun 2019; 4:21–38.
The study demonstrated reduced microbial diversity in temporal arteries of giant cell arteritis patients, and visualized bacterial DNA localizing to the media of the arterial wall, suggesting the vascular wall may not be sterile.
58. Getz TM, Hoffman GS, Padmanabhan R, *et al.* Microbiomes of inflammatory ■ thoracic aortic aneurysms due to giant cell arteritis and clinically isolated aortitis differ from those of non-inflammatory aneurysms. Pathog Immun 2019; 4:105–123.
The study showed reduced microbial diversity in the aorta of vasculitis patients as compared with those with noninflammatory aortic disease, and highlighted the differences between the vascular microbiome of temporal arteries and thoracic aorta.
59. Desbois A, Ciocan D, Saadoun D, *et al.* A role for microbiota in the pathophysiology of Takayasu arteritis (TAK) and giant cell arteritis (GCA). Arthritis Rheumatol 2018; 70(Suppl 11).
60. Espinoza J, Ai S, Matsumura I. New insights on the pathogenesis of Takayasu arteritis: revisiting the microbial theory. Pathogens 2018; 7:73.
61. Pedreira ALS, Santiago MB. Association between Takayasu arteritis and latent or active *Mycobacterium tuberculosis* infection: a systematic review. Clin Rheumatol 2019; 39:1019–1026.
62. Castillo-Martínez D, Amezcua-Castillo LM, Granados J, *et al.* Is Takayasu arteritis the result of a *Mycobacterium tuberculosis* infection? The use of TNF inhibitors may be the proof-of-concept to demonstrate that this association is epiphenomenal. Clin Rheumatol 2020; 39:2003–2009.
63. Terao C, Matsumura T, Yoshifuji H, *et al.* Brief report: Takayasu arteritis and ulcerative colitis: high rate of co-occurrence and genetic overlap. Arthritis Rheumatol 2015; 67:2226–2232.
64. Akiyama M, Kaneko Y, Takeuchi T. Does microbiome contribute to HLA-B52-positive Takayasu arteritis? Mod Rheumatol 2019; 30:213–217.
65. Kanıtez NA, Toz B, Güllüoğlu M, *et al.* Microscopic colitis in patients with Takayasu's arteritis: a potential association between the two disease entities. Clin Rheumatol 2016; 35:2495–2499.
66. Consolandi C, Turroni S, Emmi G, *et al.* Behçet's syndrome patients exhibit specific microbiome signature. Autoimmun Rev 2015; 14:269–276.
67. Ye Z, Zhang N, Wu C, *et al.* A metagenomic study of the gut microbiome in ■ Behcet's disease. Microbiome 2018; 6:135.
The study describes specific shifts occurring in the gut microbiome of Behcet's patients and suggests a pathogenic role of gut dysbiosis by demonstrating that transplant of fecal material from BD patients into a mouse model-induced worsening uveitis in animals.
68. Pagliai G, Dinu M, Fiorillo C, *et al.* Modulation of gut microbiota ■■ through nutritional interventions in Behçet's syndrome patients (the MAMBA study): study protocol for a randomized controlled trial. Trials 2020; 21:511.
The article describes a novel study to manipulate the gut microbiome in Behcet's using a prospective, cross-over randomized trial of three different dietary interventions to assess for improvements in disease manifestations.

Cerebral vasculitis associated with drug abuse

David S. Younger

Purpose of review
To review understand the epidemiology, background, neuropharmacology, and histopathology of literature verified cases, and likely etiopathogenic mechanisms.

Recent findings
There are only a handful of histologically confirmed patients in the literature with cerebral vasculitis because of drug abuse.

Summary
There is little justification for invasive laboratory investigation given the ready availability of highly accurate vascular neuroimaging techniques to dictate management, which usually rests upon avoidance of further exposure and minimizing the secondary neurotoxic effects of the abused substances and polypharmacy use.

Keywords
central nervous system, cerebral vasculitis, stroke, substance abuse, vasculitis

INTRODUCTION

The vasculitides are heterogeneous clinicopathologic disorders that share the common feature of vascular inflammation [1]. The resulting disorder can vary depending on involvement of specific organs, caliber of blood vessels, the underlying inflammatory process, and individual host factors. The cumulative result is diminished blood flow, vascular alterations, and eventual occlusion with variable ischemia, necrosis, and tissue damage. An international classification [2▪▪] based upon the 2012 Revised Chapel Hill Consensus Conference of 2012 [3] is the most widely used for the nosology of primary and secondary systemic vasculitides. The category of single-organ vasculitis (SOV) [3] provides for limited expression of a systemic vasculitis, while vasculitis associated with a probable cause [3] acknowledges that vasculitis may be associated with a given cause, such as drug abuse.

As this article will show, drug abuse, as a category of vasculitis presents several challenges to nosology. First, its prototypical clinical manifestation as a CNS or cerebral vasculitis reminiscent of SOV. Second, the influence of polypharmacy that makes it difficult to isolate the responsible drug. Third, the contribution of infection that is common in intravenous drug users (IVDU), especially HIV-1 and opportunistic infections associated with AIDS that contribute to vasculitic brain lesions. Fourth, reliance on brain neuroimaging to establish the probable diagnosis of vasculitis rather than brain and meningeal tissue biopsy to establish the diagnosis of cerebral vasculitis with certainty. Fifth, the existence of only a handful of histologically proven cases in life or at postmortem examination that suggests that the disorder is decidedly rare or underreported.

EPIDEMIOLOGY

The epidemiology of systemic and neurovasclitis have been previously reviewed [4,5▪▪]. Estimates of primary systemic vasculitis in population studies cite a declining incidence from 2.3 per 100 000 between 1988 and 1998 to 1.1 per 100 000 [6] in Australia and the United Kingdom (UK), whereas estimates of its prevalence increased from 9.0 per 100 000 [7] to 30.7 per 100 000 [8] in the period 1990 and 2012 in the United States, suggesting the success of effective treatment. de Boysson *et al.* [9] in the French Cohort of patients with primary vasculitis of the CNS (PVCNS) reported improved survival with remission achieved in 95% of cases after initial immunosuppressive induction treatment, and

City University of New York Medical School, New York, New York, USA
Correspondence to David S. Younger, MD, MPH, MS, 333 East 34th Street 1J, New York, NY 10016, USA. E-mail: youngd01@nyu.edu

Curr Opin Rheumatol 2021, 33:24–33
DOI:10.1097/BOR.0000000000000766

KEY POINTS

- Drug abuse, as a category of vasculitis, presents several challenges to nosology.
- Polypharmacy rather than a single abused illicit substance is often present.
- Intravenous drug use and HIV/AIDS may contribute to vasculitic brain lesions.
- Most cases rely upon classical features of brain neuroimaging to establish the probable diagnosis of vasculitis rather than brain and meningeal tissue biopsy to establish the diagnosis with certainty.
- There are a handful of histologically proven cases in life or at postmortem examination suggesting that the disorder is decidedly rare or underreported.

prolonged remission without relapse in two-thirds after a mean of 57 months follow-up. However, the same cannot be stated in cerebral vasculitis associated with illicit drug use as there have been no population studies. It is nonetheless important to understand the contributing factors in this condition because of the mounting trend in lethal illicit drug use.

Polypharmacy versus monopharmacy

It is far simpler to approach cerebral vasculitis associated with use of illicit drugs by focusing on the predominant drug abused independent of other substances; however, this ignores the contribution of polypharmacy to the rise in lethal deaths for decades. Observations by the Office of the Chief Medical Examiner (OCME) of New York City between 1990 and 1998 found that drug addicts favor one or another class of illicit drugs and often participate in polysubstance abuse. This in turn accounts for rising trends in the increase in annual overdose death rates using the census population for age, sex, and race comparisons [10]. Opiates, cocaine, and alcohol were the most commonly used drugs in accidental overdose deaths accounting for 97.6%, with more than one-half used in combination. Changes in the rate of multidrug combination deaths account for changes in overdose death rates, whereas single drug overdose death rates remain relatively stable. Estimated annual trend data from Connecticut's OCME, standardizing the number of deaths per 100 000 population each year, and stratifying it further by polysubstance use for the period 2012–2018, found the rate of overdose deaths increased 221% from 9.9 per 100 000 in 2012, to 28.5 per 100 000 in 2018, with the majority occurring among persons age 35–64 years (66%), men (73%), and non-Hispanic whites (78.5%).

The investigation of the supply and demand of illicit drugs offers some insight into the factors influencing such lethal polypharmacy. The supply side argues that increasing drug availability results from the increased supply of addictive drugs for pain by pharmaceutical companies and the willingness of doctors to prescribe addictive analgesics. This was evident in the promotion and marketing of OxyContin (Purdue Pharma, Stamford, Connecticut, USA), a sustained-release oxycodone in 1996, with sales that grew from $48 million in 1996 to almost $1.1 billion in 2000 becoming the leading drug of abuse in the United States by 2004 [11]. As awareness of the dangers of misuse by physicians and patients grew, policy changes restricted their supply and form with a shift toward cheaper and illegal drugs, first heroin and then the more lethal synthetic fentanyl.

Research into the demand side has been more interesting, suggesting a driving force of increasing income inequality, and a changing economic and social landscape over several decades. Case and Deaton [12] found a marked increase in all-cause mortality of middle-aged white non-Hispanic men and women in the United States between 1999 and 2013 giving rise to the acronym, 'deaths due to despair,' reminiscent of alcohol-related suicides during the Great Depression. The authors noted a midlife mortality reversal confined to white non-Hispanics, with a commensurate fall in mortality rates of black non-Hispanics, Hispanics and those aged 65 years and above in every racial and ethnic group. The increase for whites was accounted by increasing death rates from drug and alcohol poisonings, suicide, chronic liver diseases and cirrhosis. Increases in midlife mortality parallel increases in self-reported midlife morbidity according to measures of self-assessed health status, pain, psychological distress, difficulty with activities of daily living, and alcohol use.

Long-term trends in drug-related deaths analyzed from estimates derived from the Centers for Disease Control and Prevention (CDC) offer new insights [13]. On an age-related basis, drug-related deaths rose by about 20% in 2002 to 25% in 2016, following a very different trend than those for other deaths of despair including alcoholism and suicide as referred to by Case and Deaton [12]. The age-adjusted mortality rate from drug-related causes and overdose in 2000 of 5.2 per 100 000, doubled in 10 years during which suicide and alcohol-related deaths fell. By 2017, the rate had quadrupled at 20.5 per 100 000 whereas age-adjusted deaths of despair

other than those drug-related, remained the same in 2017 as in 1975. Thus, the rise in drug overdoses reflects a fundamental change in the supply, addictiveness, and lethality of drugs.

Contribution of HIV and AIDS

Recognition of the propensity for cerebral vasculitis with HIV-1 infection and the AIDS has provided insights into the mechanisms of cerebral vasculitis in association with drug abuse. Early in the HIV/AIDs epidemic, it was clear that it included a significant proportion of individuals who engaged in intravenous drug user (IVDU). There is an extensive literature suggesting an independent contribution of IVDU to immune suppression, breakdown of the blood–brain barrier (BBB), microglial activation, and neuronal injury [14] with an additive or synergistic reinforcement of HIV-related brain damage by IVDU [15]. Two postulated periods in the neurobiology of HIV-1 when autoimmune disease manifestations appear to be significant for the development of cerebral vasculitis are shortly after seroconversion and before the spread of productive infection [16], and after initiation of HAART in association with the immune reconstitution syndrome (IRIS) [17].

HIV-seropositive, pre-AIDS

Postmortem series indicate an association of presymptomatic HIV-seropositive IVDU and cerebral vessel inflammation including true vasculitis. Gray *et al.* [16] studied two cohorts of 11 patients, one HIV-seropositive and non-AIDS, and the other HIV-seronegative heroin abusers, 10 patients of each died from heroin overdose and another of a fatal gunshot wound. Neuropathological studies showed varying degrees of vascular inflammation including 'true vasculitis' exemplified by dense vascular inflammation extending through the vessel wall, associated with leptomeningitis in 6 of the 11 HIV-seropositive AIDS-negative patients. Vascular inflammation was comparatively mild or absent and restricted to a few perivascular mononucleated cells associated with pigment deposition, without transmural vascular inflammation or meningitis in the HIV-seronegative cohort. A year later, Bell *et al.* [18] described the neuropathologic findings of 23 intravenous drug users from the Edinburgh HIV Autopsy Cohort who died suddenly after seroconversion but while still in the presymptomatic stage of HIV infection in comparison to 10 HIV-negative intravenous drug users, 12 nonintravenous drug user controls, and 9 patients with full-blown AIDS, who also died suddenly. Seven of the presymptomatic HIV-positive patients showed infiltration of T cells in the walls of veins in association with low-grade lymphocytic meningitis; seven others demonstrated isolated lymphocytic meningitis, and 1 patient had focal perivascular lymphocytic cuffing and macrophage collections throughout the central white matter tissue of the brain and in basal ganglia. Neuropathological examination in presymptomatic HIV-seropositive patients fail to reveal characteristic lesions of HIV encephalitis and none of the patients showed immunocytochemical evidence of p24 antigen in brain tissue. Nearly a decade later, Bell *et al.* [19] reiterated that in more than 50% of pre-AIDS cases so studied, the brain was characterized by a low-grade lymphocytic meningoencephalitis in which T-cell infiltration is present in leptomeninges and the perivascular compartment, with a very occasional HIV-p24-positive lymphocyte in the lymphocytic infiltrate, but no in brain parenchyma.

HIV/AIDS-associated immune reconstitution syndrome literature patients

The introduction of HAART changed the incidence, course, and prognosis of the neurological complications of HIV infection concomitant with almost undetectable viral load in plasma and a rise in circulating T lymphocytes [20]. One pathologically confirmed patient with cerebral vasculitis and IRIS has been described [21]. This HIV-seropositive homosexual man developed dysarthria and dysphagia after HAART with worsening and appearance of limb paresis after discontinuation of the medication. Treatment with corticosteroids preceded recommencement of HAART but there was worsening with discontinuation of corticosteroids. Biopsy of a hyperintense fronto-parietal lesion on T_2-weighted MRI showed small vessel lymphocytic vasculitis, with microglial activation in the surrounding parenchyma. A severe demyelinating leukoencephalopathy in association with intense perivascular infiltration by HIV-gp41 immunoreactive monocytes/macrophages and lymphocytes was described by Langford *et al.* [22] in seven postmortem patients. All were severely immunosuppressed and treated with HAART with presumed IRIS however, high not low levels of HIV replication; however, there was no consideration of cerebral vasculitis.

AMPHETAMINES AND RELATED AGENTS

Background

The earliest reports of misuse of amphetamine sulfate were in late 1930s when students used it to avoid sleep during examination periods [23]. This was followed by reports of death by those who

ingested the drug repeatedly as a stimulant for the same purpose [24], in a suicide attempt that resulted in a fatal intracerebral hemorrhage [25], or accidentally, when dexamphetamine and phenelzine were fatally ingested together decades later [26]. During the Second World War, amphetamine and methamphetamine was used clinically and illicitly but its abuse soared in San Francisco after 1962 wherein it was illegally produced and distributed [27]. In 2009, the United Nations Office on Drugs and Crime estimated that 16–51 million persons between the age of 15 and 64 years consumed amphetamine drugs, with more than half using methamphetamine [28], exceeding the combined consumption of all other drugs of abuse except cannabis [29].

The neuropathology and neuropharmacology of amphetamine, methamphetamine, and their derivatives have been reviewed [30]. Such drug agents constitute a large spectrum of agents [31] available in powder, capsule, tablet, and injectable fluid form that can be swallowed, snorted or taken intranasally, smoked or injected with highly variable purity and dosage equivalence. Their potent effects, which include elevation of blood pressure, pulse rate, and increased level of alertness, sometimes in association with insomnia, excitability, panic attacks, and aggressive behavior, can also be associated with seizure and stroke. Their effect of methamphetamine, are distributed throughout the brain [32]. Ecstasy refers to the different hallucinogenic amphetamine derivatives that contain 3,4-methylene-dioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDE) as the main components [33]. Ecstasy alters brain serotonin concentrations, and postsynaptic 5-HT_2 receptors play a role in the regulation of brain microvessels. The CNS toxic effects mitigate through blocking of the reuptake of dopamine and stimulation of the release of dopamine and norepinephrine, as well as, possible involvement upon serotonergic and endogenous opiate system. There can be dopamine receptor desensitization with marked reduction of dopamine transporters and drug levels, as well as other dopaminergic axonal markers. The neurotoxic effects of methamphetamine are mediated by multiple additional mechanisms including generation of free radicals, nitric oxide, excitotoxicity, mitochondrial dysfunction, apoptosis, and the induction of immediate early inflammatory genes and transcription factors. Methamphetamine is the most potent amphetamine and the most commonly abused. All forms of amphetamine administration increase the risk of stroke that may be ischemic, hemorrhagic and intraparenchymal [34], which may be up to four-fold that of nonusers, surpassing the rate of hemorrhagic stroke caused by cocaine use with odds ratios, respectively of 4.95 versus 2.33 [35]. Still, amphetamines and methamphetamine are the second commonest cause of all strokes after cocaine, occurring largely in persons younger than 45 years.

Pathologically verified cases

Cerebral vasculitis due to amphetamine, methamphetamine and related agents is exceedingly rare with only three histopathologically verified cases in the absence of other possible known causes [36,37]. This is surprising given the number of substances that could cause this disorder if there was an association. Amphetamine-related multiorgan arteritis including cerebral vasculitis was demonstrated by Citron *et al.* [37] in a highly publicized report of 14 Los Angeles multidrug abusers. The drug closest to a common denominator was methamphetamine using intravenously by all but two patients and exclusively by one. Acute vessel lesions of fibrinoid necrosis of the media and intima with infiltration by polymorphonuclear cells, eosinophils, lymphocytes and histiocytes, was followed by vascular elastic and vascular smooth muscle destruction resulting in lesions considered typical for polyarteritis nodosa (PAN). Two patients, one abbreviated D.G. and the other E.V., who injected methamphetamine via intravenous injection had arterial lesions in cerebral and cerebellar (D.G.) and brainstem pontine vessels (D.G. and E.V.); however, detailed histopathologic descriptions were not provided. Their report was followed by correspondence by Gocke [38] who contended that exposure to the Australia antigen of hepatitis B antigen was likely in their cohort conceivably associated with circulating immune complexes and complement activation. The authors [39] responded that no more than 30% of sera from drug abuse patients ultimately tested positive for the Australia antigen. Those with antigen-positive sera who had used drugs others than methamphetamine had no evidence of angiitis when on catheter angiography. Baden [40] commented that he had not observed a causal relation between drug abuse and necrotizing arteritis at the Office of Chief Medical Examiner of New York City for the past one-half century among thousands of autopsied drug abusers. Citron and Peters [39] responded that evidence of aneurysms so noted in 13 patients, was in their opinion ample evidence of arteritis.

Almost two decades later, cerebral vasculitis was demonstrated in a dubious report [36] of a 3-week postpartum woman who took her first over-the-counter Dexatrim diet pill in many months containing phenylpropanolamine, without a history of amphetamine abuse. This was followed 90 min later by sudden headache, nausea vomiting and

detection of subarachnoid blood on CT neuroimaging and a frontal lobe hematoma. Bilateral carotid angiography demonstrated diffuse segmental narrowing and dilatation of small, medium and large vessels and branches of the anterior and posterior circulation. Evacuation and histopathologic analysis of the hematoma was performed showing necrotizing vasculitis of small arteries and veins with infiltration of polymorphonuclear leukocytes particularly prominent in the intima with fragmentation of the elastic lamina and areas of vessel occlusion. It was unclear whether the findings were related to primary or drug-related CNS vasculitis. However, treatment with cyclophosphamide for 6 months was associated with almost complete resolution of cerebral angiographic abnormalities.

Etiopathogenic mechanisms

After the report of Citron *et al.* [37], Rumbaugh *et al.* [41] described the cerebral vascular changes of methamphetamine abuse in five rhesus monkeys given amphetamine in dose ranges used by human addicts. Two of the five monkeys developed generalized arterial spasm during a 2-week period following intravenous injection. Three of five animals demonstrated decreased caliber of named cerebral artery branches and flow of the contrast agent with normalization 1 day later whereas two others showing marked general decrease in small branches and large named vessels that improved in one animal and progressed in another. Histopathologic changes at postmortem examination included microaneurysmal enlargement of arteriolar segments, mononuclear perivascular cuffing of small arterioles, parenchymal necrosis, petechial hemorrhages, and swelling of brain tissue, with most of the hemorrhagic lesions centered on small-size arteriolar and capillary vessels. Although reminiscent of the clinical and histopathologic findings of Citron *et al.* [37], necrotizing arteritis and transmural inflammation were lacking. Five years later, Rumbaugh *et al.* [42] subjected monkeys to short-duration (2 weeks) (5 animals), medium-duration (1 month) (3 monkeys), and long duration (1 year) (3 monkeys) of thrice weekly (1.5 mg/kg body weight) of intravenous amphetamine and related agents including methamphetamine, secobarbital, methylphenidate, and placebo, with performance of cerebral angiography and documentation of the resulting histopathology. Their studies showed relatively severe vascular injury and brain damage from intravenous methamphetamine that included occlusions and slow blood flow in small cerebral vessels, respectively in two each of the five monkeys in the long-term administered drug, and in three each of those given drug for intermediate and short durations, with some animals and controls unaffected. There was less injury caused by secobarbital and methylphenidate. Vascular spasm because of subarachnoid blood was excluded by lack of blood at postmortem examination in the subarachnoid space in any of the animals.

COCAINE

Background

A classic review of cocaine and stroke describes its abuse potential [43]. Cocaine, which is derived from the leaves of the *Erythroxylum coca* plant found primarily in the eastern mountains of Peru, Ecuador, and Bolivia, is available for abuse as cocaine hydrochloride, a water-soluble white salt in crystal, granular, and white powder that can be sniffed and 'snorted' intranasally or injected parenterally. The 'free base' alkaloid form known as 'crack' derives its name from the cracking sound that occurs after dissolution of the hydrochloride salt in water, heated, and mixed with ammonia without or without baking soda. This chemical reaction converts cocaine hydrochloride to a volatile form of the drug, almost pure cocaine. Street cocaine or the noncrack form is highly variable in purity, and often cut with various agents. The alkaloid free-based form, which is inhaled or smoked, is accompanied by higher blood concentrations and more pronounced euphoria. When smoked as free-base, it is absorbed into the pulmonary circulation and transmitted to the brain in less than 10 s. After appearance in the bloodstream, cocaine is rapidly hydrolyzed to benzoylecgonine, which can be accurately tested in the urine; however, levels may persist for up to 27–36 h depending upon the route of administration and host cholinesterase activity. In recent years, with increasing availability and purity, and a drop in the price of cocaine from early 1970, new cohorts from all socioeconomic backgrounds and age groups have been attracted to this highly addictive drug, and use has continued to expand on a year-by-year basis.

Cocaine is a highly potent CNS stimulant that rapidly crosses the BBB because of its highly lipophilic properties. It is widely distributed through the brain with its major metabolites binding at receptors with varying affinities at presynaptic sites stimulating the release of DA from synaptic vesicles and blocking its reuptake resulting in enhanced neurotransmission. The investigation of single nucleotide polymorphisms (SNP) that encode amino acid substitutions in opioid receptors and ligands implicated in drug addiction, particularly those of the mu-opioid receptor (MUP-r) gene system (*OPRM1)* reveal interesting findings. The MUP-r system appears to release DA

from neural synapse when activated, whereas kappa receptors (KUP-r) instead lower extracellular DA levels supporting the observed variability in drug addiction among susceptible individuals [44].

Pathologically verified cases

Only 10 histologically verified patients have been described in the absence of other possible known causes [45–52]. In all but one patient, onset of neurological symptoms immediately followed cocaine use that was intranasal cocaine in six, intravenous in two, and acquired via unknown modality in one. Cerebral vasculitis was associated with cerebral hemorrhage in three patients, and ischemia in seven patients that typically presented with abrupt onset of headache and focal hemiparesis, confusion or agitation and grand mal seizures that progressed to stupor, coma, and death in three patients. Lumbar cerebrospinal fluid (CSF) analysis showed lymphocytic pleocytosis of 10–65 cells/μl with elevation of the protein content from 185 to 630 mg/dl, but was completely normal in two patients. Cerebral angiography performed in seven patients showed an avascular mass in the patient with a putaminal, abnormal large named vessel occlusions or segmental narrowing in three patients); poor filling and irregularities in vessel appearance in two, and normal in one patient. The disorder of cerebral vasculitis was established by brain and meningeal biopsy in life in seven patients; at postmortem examination in two, and by both in one patient. The underlying disorder of cerebral vasculitis was nonnecrotizing with transmural mononuclear cell inflammation affecting small arteries and veins in three patients or veins alone in three patients, and perivascular cuffing of small arteries and veins in another. In two patients, there was necrosis of small cerebral vessels associated with polymorphonuclear cell inflammation of small arteries and veins or large named vessels. Among three patients so studied at postmortem examination, nonnecrotizing small vessel vasculitis was noted in the brains of two patients without evidence of systemic involvement, whereas necrotizing large vessel vasculitis was found in both the brain and systemic organs. In all, treatment consisting of corticosteroids was administered to seven patients, five of whom improved and two who died of refractory seizures despite anticonvulsant medication or because of infection, coma, and decerebration.

Etiopathogenic mechanisms

Although cocaine-associated cerebral vasculitis has not been rigorously studied, several independent lines of experimental evidence suggest four possible etiopathogenic mechanisms in susceptible individuals. The first is the observed effects of cocaine in the induction of adhesion molecules and endothelial leukocyte migration across cerebral blood vessel endothelia walls particularly under inflammatory conditions, which may disturb the function of the blood–brain barrier. Cocaine increased the expression of the endothelial adhesion molecules intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and endothelial leukocyte adhesion molecule (ELAM)-1 on BMVEC with a peak effect on ICAM-1 expression between 6 and 18 h after treatment in human brain microvascular endothelial cells (BMVEC) cultures and increased monocyte migration in an in-vitro BBB model [53–56] constructed with BMVEC and astrocytes [57]. These effects of cocaine, exerted through a cascade of augmented expression of inflammatory cytokines and endothelial adhesion molecules, may contribute to the known cerebrovascular complications of cocaine abuse.

The second is the effect of cocaine on endothelial cell permeability and apoptosis as well as the induction of chemokines and cytokines. The immunomodulatory effects of cocaine on brain microvascular endothelial cells and its proinflammatory effects on induction of proinflammatory cytokines and chemokines was investigated using a human BBB model that included HIV-1 neuroinvasion [58]. Cocaine increased the in-vitro permeability of endothelial cells of the BBB model and induced apoptosis of mouse thymocytes in cultures of BMVEC and monocytes using an ELISA of generated accompanied by up-regulation of macrophage inflammatory protein (MIP)-1, MIP-1α, inducible protein (IP)-10, and interleukin (IL)-8 and TNF-α expression.

A third line of investigation is the observed synergy of cocaine in facilitating pathogenic retroviral neuroinvasion, which may confer an independent risk factor for cerebral vasculitis. Both in-vitro and in-vivo studies have provided valuable tools in exploring the role of cocaine in mediating HIV-associated neuropathogenesis. The importance of drug abuse in conjunction with HIV-1 has been underscored by the ability of cocaine to induce retroviral replication in mononuclear cells [59] and enhance gp120-induced neurotoxicity [60].

A fourth and more recent insight is the possible contribution of levamisole, an antihelminthic agent [61] first detected traces in cocaine bricks by the US Drug Enforcement Agency (DEA) in 2003 [62], and increasing in frequency from 44.1% of specimens in 2008 to 73.2% in 2009 [63], signaling a rising public health problem because of its highly addictive potential and its association with vasculitis.

Levamisole is 100- to 300-fold less potent than cocaine in blocking norepinephrine and dopamine uptake, and has a very low affinity for the serotonin transporter; and it does not trigger an appreciable substrate efflux. Nevertheless, the desired neuropharmacologic effects leads to its widespread contamination in cocaine production. Hofmaier *et al.* [64] have studied its pharmacology. Although the adulterant levamisole itself has only moderate inhibition of dopamine reuptake forming amphetamine-like metabolites, its metabolite, aminorex, exerts distinct psychostimulant effects, and the two substances 'kick in' after the cocaine effect 'fades out'.

Exposure to levamisole-adulterated cocaine is associated with a variety of well described hematological, skin, renal and pulmonary disorders [65]. It appears to induce antineutrophil cytoplasmic antibodies (ANCA) and small vessel vasculitis involving the ear lobes and the skin overlying zygomatic arch and lower extremities, often with purpuric plaques in a retiform pattern or central necrosis. Skin biopsy shows pathological involvement of superficial and deep dermal vessels associated with numerous neutrophils and eosinophils that surrounding and invade the walls of dermal vessels with extravasation of red blood cells, leukocytoclastic debris (nuclear dust), and fibrinoid necrosis on hematoxylin and eosin-stained tissue sections. Such findings are similar to children with chronic levamisole treated for nephrotic syndrome so noted in a minority of children who developed purpuric lesions of at least the ears and biopsies revealing cutaneous vasculitis [66]. Although organ involvement is not characteristic of levamisole-adulterated cocaine-induced autoimmune disease, there is an established association with proteinuria or hematuria, acute renal injury, focal necrotizing and crescentic pauci-immune glomerulonephritis in some cases, and increased titers to p-ANCA. As in other drug-induced vasculitides, pulmonary involvement can complete the triad of skin, kidney and lung disorder in the form of diffuse alveolar hemorrhages, idiopathic pulmonary hypertension, or other clinicopathologic presentations. There is a causal association of levamisole-associated multifocal inflammatory leukoencephalopathy in cocaine users [67]. Affected patients present with progressive mental change and ataxia associated with periventricular white matter lesions on brain MRI. Pathological studies of brain biopsy specimens reveal cerebral demyelination and perivascular inflammation similar to multiple sclerosis (MS).

The mechanisms underlying levamisole-adulterated cocaine-induced systemic disorder are not well understood but a causal relation to ANCA-associated disease is suggested by the correlation of the clinical disorder, and relapse with detectable autoantibodies, sensitivity to immune modulatory and immunosuppressive therapy, and predictable levamisole-induced histopathology. Levamisole potentiates the production of interferon and interleukins as well as increases T-cell activation and proliferation, neutrophil mobility, adherence, and chemotaxis and increases the formation of antibodies to antigens [61]. It acts as a hapten, triggering an immune response resulting in opsonization and leukocyte destruction. Levamisole may interact with neutrophil extracellular traps (NET) composed of a complex of deoxyribonucleic acid (DNA), histones, and neutrophil granules including myeloperoxidase (MPO), proteinase-3 (PR3), and human neutrophil elastase (HNE). Neutrophil extracellular traps release in response to stress and provide a source of antigen that can activate the immune system [65].

There has not been a postmortem-studied case of MIL, and for unclear reasons such patients fail to demonstrate cerebral vasculitis in brain biopsy tissue or symptomatic systemic vasculitic disorder, renal, hematologic, or pulmonary involvement in life

OPIOIDS

Background

The opioid drugs constitute a large number of agonists, antagonists, and mixed agonist-antagonists. Opioid overdose accounts for at least 16 000 deaths annually in the United States [68] and occurs across sex, ethnic, age, and geographic strata, and involves both medical and nonmedical opioid uses. According to the CDC, since 2003, opioid analgesic abuse [69] overdose deaths involving opioid analgesics exceeded those because of cocaine. For every unintentional overdose death related to an opioid analgesic, nine persons were admitted for substance abuse treatment, 161 reported drug abuse or dependency, and 461 reported nonmedical uses of opioid analgesic drugs [70]. Also known as diacetylmorphine, heroin was first synthesized by the Bayer Company in 1889 as a less addictive morphine sulfate substitute [71]; however, it has since become cheaper and more readily available. There is extensive literature relating to the outcome of heroin abuse and overdose [72] with a reported average mortality rate of 2% in regularly injecting persons, half of which is attributable to overdose and equal to 20 times the mortality rate expected in nondrug using peers.

Opioids or narcotic drugs have pharmacologic properties similar to those of morphine that include the derivatives morphine, hydrocodone,

oxycodone, hydromorphone, codeine, fentanyl, meperidine, methadone, and opium. Whereas the source of opioids is the exudate of seed from the poppy plant, heroin is derived from acetylation of morphine [21]. Heroin is administered intravenously, intranasally and subcutaneously. A higher bioavailability of heroin is present after heating on foil for inhalation compared with smoking after heating. Intravenous injection leads to extreme euphoria that peaks at 10 min followed by profound sedation and analgesia that lasts for up to 1 h. Opiate overdose produces the triad of coma, respiratory depression and miosis. The medical complications of long-term heroin exposure includes endocarditis, pulmonary complications of embolism, pneumonia and granulomatosis or fibrosis; nephropathy, immunosuppression, infection at the site of injection because of cellulitis, thrombophlebitis, bacteremia and hepatitis because of needle sharing [73]. It binds to endogenous opiate mu_1 receptors, which are responsible for most of the analgesic effects, and for the actions of the CNS and cardiovascular system leading to bradycardia, hypotension, and respiratory depression. Agonist actions at mu_2 receptors are responsible for respiratory depression, delayed gastrointestinal motility, miosis, and physical dependence. Agonist actions at kappa receptors lead to separate analgesia. Circulating serum morphine is transformed into morphine-3-glucuronide or morphine-6-glucuronide by the liver and the kidney. Most fatal and nonfatal overdoses occur when heroin is injected intravenously.

Pathologically verified cases

This author was unable to identify any pathologically confirmed cases of heroin-induced cerebral vasculitis reported in the literature, nor was cerebral vasculitis suggested as a likely occurrence in heroin abuse [74], heroin addiction [73,75] or acute overdose [76]. Moreover, detailed neuropathologic studies carried out on 134 victims of acute heroin intoxication including 18 and survived for periods of hours or days [77], and respectively demonstrated cerebral edema in conjunction with vascular congestion, capillary engorgement, and perivascular bleeding attributed to toxic primary respiratory failure; or ischemic nerve cell damage, showed no evidence of cerebral vasculitis, and only one focus of lymphocytic perivascular inflammation. The brains of 10 intravenous drug abusers who died from heroin overdoses, including one because of gunshot injury [16], likewise showed no evidence of cerebral vasculitis at postmortem examination, evidencing only a few perivascular mononuclear cells associated with pigment deposition.

Etiopathogenic mechanisms

The postulated mechanisms of opioid-related neuronal and CNS vascular injury include increased oxidative stress, induction of inflammatory cytokines, and increased permeability of the BBB especially in intravenous drug abuse. However, Ramage *et al.* [78] described increased deposition of hyperphosphorylated tau in entorhinal cortex and subiculum of the hippocampus, AT8-positive neurofibrillary tangles in entorhinal cortex, and increase in β-amyloid precursor protein (βAPP) in both the hippocampus and brainstem of drug abusers compared with controls. Several postulated causative mechanisms include repeated head injury, hypoxic–ischemic injury associated with opioid-induced respiratory depression, microglial associated cytokine release, and drug-associated neurotoxicity.

Clinical approach to cerebral vasculitis associated with illicit drug use

Patients with suspected cerebral vasculitis because of illicit drug use are at heightened risk for occlusive and hemorrhagic stroke of diverse cause. Such patients should be screened with one or more noninvasive studies to identify potential sites of inflammatory injury to cerebral vessels leading to luminal and mural changes and ischemia of brain tissue. Conventional MRI with T_2-fluid attenuation inversion recovery (FLAIR) and diffusion weighted imaging (DWI) distinguishes areas of acute, subacute and chronic brain ischemia. High-resolution (3-Tesla) (h) MRI with gadolinium-enhanced fat-saturation T_1 spin echo detects vessel wall changes. Both MR angiography (MRA) with time-of-flight (TOF) sequencing and brain computed tomography angiography (CTA) are ideal modalities to image vessel lumina and walls to detect aneurysms and potential sites of hemorrhage. Catheter angiography is the gold standard for the diagnosis of cerebral vasculitis when it reveals alternating areas of vascular dilatation and stenosis along multiple vessels reminiscent of a string of beads. The sensitivity of catheter angiography for detecting vasculitis varies from 40 to 100% with a specificity no higher than 40% depending upon the particular clinical, radiographic, and histopathologic definitions employed, and the caliber of cerebral vessels affected. Lumbar CSF analysis is performed in all suspected patients with cerebral vasculitis to confirm an elevated protein content typically more than 100 mg/dl and pleocytosis, and to exclude infection and malignancy. Adults with diagnostic catheter angiographic features of cerebral vasculitis who show relentless progression; and children with angiography-negative, biopsy-positive, SV-childhood PACNS

(cPACNS) [79] are candidates for meningeal and brain tissue biopsy to confirm the presence of vasculitis and direct further therapy with immunosuppressive therapy according to standard protocols [80▪▪]. Vasculitis is rarely found after extensive evaluation, and such patients need supportive management with particular attention to imminent illicit substance withdrawal and systemic toxicity.

CONCLUSION

Drug abuse is an extremely rare cause of histopathologically verified cerebral vasculitis. Nonetheless, the complications of illicit substance use on the cerebral circulation can be highly lethal with secondary vasculopathy, hemorrhage and aneurysm formation especially when the illicit substances are taken parenterally. A likely diagnosis rests upon the drug or combinations of illicit drugs abused, and the clinical and neuroradiologic findings of a presumptive case. However, HIV/AIDS, IVDU, and adulterants, such as levamisole, have introduced new aspects of causation and patterns of polypharmacy-related brain insults.

Acknowledgements

Sachiko Maharjan, BA, Patient Coordinator, Assisted in the preparation of the manuscript.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Younger DS. Overview of the vasculitides. Neurol Clin 2019; 37:171–200.
2. ▪▪ Jennette JC, Falk RJ, Gasim ADHM. Nomenclature and pathological features of vasculitides. Chapter 9. In: Younger DS, editor. The vasculitides. Vol 1. New York: Nova Science Publishers; 2019. pp. 171–192.

An important article that establishes the nomenclature of vasculitis.

3. Jennette JC, Falk RJ, Bacon PA, *et al.* 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013; 65:1–11.
4. Younger DS. Epidemiology of neurovasculitis. Neurol Clin 2016; 34:887–917.
5. ▪▪ Younger DS. Epidemiology of the vasculitides. Neurol Clin 2019; 37:201–217.

An important article on the current status of the neuro-epidemiology of vasculitis.

6. Omerod AS, Cook MC. Epidemiology of primary systemic vasculitis in the Australian Capital Territory and southeastern New South Wales. Intern Med J 2008; 38:816–823.
7. Lightfoot RW Jr, Michel BA, Bloch DA, *et al.* The American College of Rheumatology 1990 criteria for the classification of polyarteritis nodosa. Arthritis Rheum 1990; 33:1088–1093.
8. Jennette JC, Falk RJ, Bacon PA, *et al.* International Chapel Hill Consensus Conference nomenclature of vasculitides. Arthritis Rheum 2013; 65:1–11.
9. de Boysson H, Zuber M, Naggara O, *et al.* Primary angiitis of the central nervous system: description of the first 52 adult patients enrolled in the French COVAC' cohort. Arthititis Rheum 2012; 64(Suppl 10):S663–S664.
10. Coffin PO, Galea S, Ahern J, *et al.* Opiates, cocaine and alcohol combinations in accidental drug overdose deaths in New York City, 1990-98. Addiction 2003; 98:739–747.
11. Cicero T, Inciardi J, Munoz A. Trends in abuse of OxyContin and other opioid analgesics in the United States: 2002-2004. J Pain 2005; 6:662–672.
12. Case A, Deaton A. Rising morbidity and mortality in midlife among white non-Hispanic Americans in the 21st century. Proc Natl Acad Sci U S A 2015; 112:15078–15083.
13. Social Capital Project. Long-term trends in deaths of despair. SCR Report No. 4-19. US Joint Economic Committee, September 2019. Available at: https://www.jec.senate.gov/public/_cache/files/0f2d3dba-9fdc-41e5-9bd1-9c13f4204e35/jec-report-deaths-of-despair.pdf.
14. Kumar A. HIV and substance abuse. Curr HIV Res 2012; 10:365.
15. Bell JE, Donaldson YK, Lowrie S, *et al.* Influence of risk group and zidovudine therapy on the development of HIV encephalitis and cognitive impairment in AIDS patients. AIDS 1996; 10:493–499.
16. Gray F, Lescs MC, Keohane C, *et al.* Early brain changes in HIV infection: neuropathological study of 11 HIV seropositive, non-AIDS cases. J Neuropathol Exp Neurol 1992; 51:177–185.
17. Nachega JB, Morroni C, Chaisson RE, *et al.* Impact of immune reconstitution inflammatory syndrome on antiretroviral therapy adherence. Patient Prefer Adherence 2012; 6:887–891.
18. Bell JE, Busuttil A, Ironside JW, *et al.* Human immunodeficiency virus and the brain: investigation of virus load and neuropathologic changes in pre-AIDS subjects. J Infect Dis 1993; 168:818–824.
19. Bell JE, Arango JC, Robertson R, *et al.* HIV and drug misuse in the Edinburgh cohort. J Acq Immune Defic Syndr 2002; 31(Suppl 2):S35–S42.
20. Gray F, Keohane C. The neuropathology of HIV infection in the era of highly active antiretroviral therapy (HAART). Brain Pathol 2003; 13:79–83.
21. Van der Ven AJ, van Oostenbrugge RJ, Kubat B, *et al.* Cerebral vasculitis after initiation antiretroviral therapy. AIDS 2002; 16:2362–2364.
22. Langford TD, Letendre SL, Marcotte TD, *et al.*, HNRC Group. Severe, demyelinating leukoencephalopathy in AIDS patients on antiretroviral therapy. AIDS 2002; 16:1019–1029.
23. Benzedrine sulfate 'pep pills' [Editorial]. JAMA 1937; 108:1973–1974.
24. Smith L. Collapse with death following the use of amphetamine sulfate. AJAM 1939; 113:1022–1023.
25. Gericke O. Suicide by ingestion of amphetamine sulfate. JAMA 1945; 128:1098–1099.
26. Lloyd JT, Walker DR. Death after combined dexamphetamine and phenelzine. Br Med J 1965; 2:168–169.
27. Anglin MD, Burke C, Perrochet B, *et al.* History of the methamphetamine problem. J Psychactive Drugs 2000; 32:137–141.
28. United Nations Office on Drugs and Crime. UNODC 2009 World Drug Report. Vienne, Austria: United Nations; 2009.
29. United Nations Office on Drugs and Crime. UNIDC 2000 World Drug Report. New York, NY: United Nations; 2000.
30. Buttner A. Review: the neuropathology of drug abuse. Neuropathol Appl Neurobiol 2011; 37:118–134.
31. Christophersen AS. Amphetamine designer drugs - an overview and epidemiology. Toxicol Lett 2000; 112–113:127–131.
32. Kalasinsky KS, Bosy TZ, Schmunk GA, *et al.* Regional distribution of methamphetamine in autopsied brain of chronic human methamphetamine users. Forensic Sci Int 2001; 116:163–169.
33. Parrott A. Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. Psychopharmacology 2004; 173:234–241.
34. Fonseca AC, Ferro JM. Drug abuse and stroke. Curr Neurol Neurosci Rep 2013; 13:325.
35. Westover AN, McBride S, Haley RW. Stroke in young adults who abuse amphetamines or cocaine: a population-based study of hospitalized patients. Arch Gen Psychiatry 2007; 64:495–502.
36. Glick R, Hoying J, Cerullo L, *et al.* Phenylpropanolamine: an over-the-counter drug causing central nervous system vasculitis and intracerebral hemorrhage. Case report and review. Neurosurgery 1987; 20:969–974.
37. Citron BP, Halpern M, McCarron M, *et al.* Necrotizing angiitis associated with drug abuse. N Engl J Med 1970; 283:1003–1011.
38. Gocke DC. C.I. Angiitis in drug abusers [Letter]. N Engl J Med 1971; 284:112.
39. Citron B, Peters R. Angiitis in drug abusers. N Engl J Med 19711971; 284:111–113.
40. Baden M. Angiitis in drug abusers. N Engl J Med 1971; 284:111.
41. Rumbaugh CL, Bergeron RT, Scanlan RL, *et al.* Cerebral vascular changes secondary to amphetamine abuse in the experimental animal. Radiology 1971; 101:345–351.
42. Rumbaugh CL, Fang HC, Higgins RE, *et al.* Cerebral microvascular injury in experimental drug abuse. Invest Radiol 1976; 11:282–294.
43. Levine SR, Welch KM. Cocaine stroke. Stroke 1988; 19:779–783.
44. Kreek MJ, Bart G, Lilly C, *et al.* Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. Pharmacol Rev 2005; 57:1–26.

45. Bostwick DG. Amphetamine induced cerebral vasculitis. Hum Pathol 1981; 12:1031–1033.
46. Krendel DA, Ditter SM, Frankel MR, *et al.* Biopsy-proven cerebral vasculitis associated with cocaine abuse. Neurology 1990; 40:1092–1094.
47. Fredericks RK, Lefkowitz DS, Challa VR, *et al.* Cerebral vasculitis associated with cocaine abuse. Stroke 1991; 22:1437–1439.
48. Morrow PL, McQuillen JB. Cerebral vasculitis associated with cocaine abuse. J Forensic Sci 1993; 38:732–738.
49. Tapia JF, JM S. Case 27-1993-A 32- year-old man with the sudden onset of a right-sided headache and left hemiplegia and hemianesthesia. N Engl J Med 1993; 329:117–124.
50. Merkel PA, Koroshetz WJ, Irizarry MC, Cudkowicz ME. Cocaine-associated cerebral vasculitis. Semin Arthritis Rheum 1995; 25:172–183.
51. Martinez N, Diez-Tejedor E, Frank A. Vasospasm/thrombus in cerebral ischemia related to cocaine abuse. Stroke 1996; 27:147–148.
52. Diez-Tejedor E, Frank A, Gutierrez M, *et al.* Encephalopathy and biopsy-proven cerebrovascular inflammatory changes in a cocaine abuser. Eur J Neurol 1998; 5:103–107.
53. Fiala M, Gan XH, Zhang L, *et al.* Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis? Adv Exp Med Biol 1998; 437:199–205.
54. Fiala M, Gan X-H, Newton T, *et al.* Divergent effects of cocaine on cytokine production by lymphocytes and monocytes/macrophages. Adv Exp Med Biol 1996; 402:145–156.
55. Fiala M, Looney DJ, Stins M, *et al.* TNF- (opens a paracellular route for HIV-1 invasion across the blood-brain barrier. Mol Med 1997; 3:553–564.
56. Fiala M, Rhodes RH, Shapshak P, *et al.* Regulation of HIV-1 in astrocytes: expression of Nef-(and IL-6 is enhanced in coculture of astrocytes and macrophages. J Neurovirol 1996; 2:158–166.
57. Gan X, Zhang L, Berger O, *et al.* Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. Clin Immunol 1999; 91:68–76.
58. Zhang L, Looney D, Taub D, *et al.* Cocaine opens the blood-brain barrier to HIV-1 invasion. J Neurovirol 1998; 4:619–626.
59. Bagasra O, Pomerantz RJ. Human immunodeficiency virus type 1 replication in peripheral blood mononuclear cells in the presence of cocaine. J Infect Dis 1993; 168:1157–1164.
60. Buch S, Yao H, Guo M, *et al.* Cocaine and HIV-1 interplay in CNS: cellular and molecular mechanisms. Curr HIV Res 2012; 10:425–428.
61. Amery WR, Bruynseels JP. Levamisole, the story and the lessons. Int J Immunopharmacol 1992; 14:481–486.
62. Valentino AM, Fuentecilla K. Levamisole: an analytical profile. Microgram J 2005; 3:134–137.
63. Drug Intelligence brief: Cocaine containing levamisole adversely affecting drug users in the United States (DEA-10001-levamisode). Drug Enforcement Administration, Intelligence Production Unit. January 2010.
64. Hofmaier T, Luf A, Seddik A, *et al.* Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. Neurochem Int 2014; 73:32–41.
65. Nolan AI, Kuang-Yu J. Pathologic manifestations of levamisole-adulterated cocaine exposure. Diagn Pathol 2015; 10:48.
66. Rongioletti F, Chio L, Ginevi F, *et al.* Purpura of the ears: a distinctive vasculopathy with circulating autoantibodies complicating long-term treatment with levamisole in children. Br J Dermatol 1999; 140:948–951.
67. Hook CC, Kimmel DW, Kvols LK, *et al.* Multifocal inflammatory leukoencephalopathy with 5-fluorouracil and levamisole. Ann Neurol 1992; 31:262–267.
68. Beletsky L, Rich JD, Walley AY. Prevention of fatal opioid overdose. JAMA 2012; 308:1863–1864.
69. Centers for Disease Control and Prevention. CDC grand rounds: prescription drug overdoses-a U.S. epidemic. MMWR Morb Mortal Wkly Rep 2012; 61:10–13.
70. Substance Abuse and Mental Health Services Administration. Results from the 2009 national survey on drug use and health: Volume 1: Summary of national findings. Substance Abuse and Mental Health Services Administration. Rockville, MD, 2010.
71. De Ridder M. Heroin: new facts about an old myth. J Psychoactive Drugs 1994; 26:65–68.
72. Davoli M, Perucci CA, Forastiere F, *et al.* Risk factors for overdose mortality: a case-control study within a cohort of intravenous drug users. Int J Epidemiol 1993; 22:273–277.
73. Louria DB, Hensle T, Rose T. The major medical complications of heroin addiction. Ann Intern Med 1967; 67:1–22.
74. Caplan LR, Hier DB, Banks G. Current concepts of cerebrovascular disease. Stroke and drug abuse. Stroke 1982; 13:869–872.
75. Richter RW, Pearson J, Bruun B, *et al.* Neurological complications of addictions to heroin. Bull NY Acad Med 1973; 49:3–21.
76. Sporer KA. Acute heroin overdose. Ann Intern Med 1999; 130:584–590.
77. Oehmichen M, Meissner C, Reiter A, *et al.* Neuropathology in nonhuman immunodeficiency virus-infected drug addicts: hypoxic brain damage after chronic intravenous drug abuse. Acta Neuropathol 1996; 91:642–646.
78. Ramage SN, Anthony IC, Carnie FW, *et al.* Hyperphosphorylated tau and amyloid precursor protein deposition is increased in the brains of young drug abusers. Neuropathol Appl Neurobiol 2005; 31:439–448.
79. Benseler SM, deVeber G, Hawkins C, *et al.* Angiography-negative primary central nervous system vasculitis in children. Arthritis Rheum 2005; 52:2159–2167.
80. Younger DS. The clinical approach to patients with vasculitis. Chapter 1. In:
■■ Younger DS, editor. The vasculitidies, Volume 2. New York: Nova Science Publishers; 2019. pp. 3–17.
A very useful article on the clinical approach to vasculitis of the nervous system.

Aortitis: an update

Mustafa Erdogan

Purpose of review
Aortitis is the inflammation of the aorta due to various causes. Clinical presentations vary as well as the imaging findings. Exact pathogenetic mechanisms or triggering factors, as well as the best diagnostic and monitoring modalities and treatment strategies, are yet to be elucidated. We reviewed recent studies in aortitis and associated diseases.

Recent findings
Multiple cohort studies reporting long-term outcomes in patients with noninfectious aortitis were recently published. Comparative features of isolated aortitis were described. Six angiographic clusters for giant cell arteritis and Takayasu have been identified. New classification criteria have been proposed for IgG4-related disease by a data-driven method. The ultrasonographic slope sign and a halo score were described as specific imaging parameters in giant cell arteritis. The promising role of PET-computed tomography, not only in the diagnosis of aortitis but also in monitoring disease activity, has been noted. Results of in-vitro studies on Janus kinase (JAK)/signal transducers and activators of transcription and mammalian target of rapamycin (mTOR) pathways, comparative studies with leflunomide as an induction therapy, and a long-term follow-up study with tocilizumab may contribute to the management of Takayasu arteritis.

Summary
An impressive number of studies have addressed aortitis in recent years. However, there still is a lack of robust data on causes, monitoring disease activity by imaging and biomarkers, and drugs providing steroid-free remission in noninfectious aortitis.

Keywords
aortitis, giant cell arteritis, IgG4-related disease, PET-computed tomography, Takayasu's arteritis

INTRODUCTION

Aortitis is an infectious or noninfectious inflammatory disorder of the aortic wall (Table 1). Either cause can have similar clinical and imaging features. This is challenging for the clinician since the management strategy can widely differ. Inflammatory aortitis can present as an isolated disease or related to a systematic disease or a specific treatment [1]. While the epidemiology of the aortitis has not been adequately addressed the most common conditions associated with an aortitis are Takayasu arteritis and giant cell arteritis (GCA). The prevalence, clinical, and imaging features can vary depending on diagnostic methods and ethnicity, especially in the era of new imaging modalities [2]. Aortitis can be classified into four groups according to histopathological features: granulomatous, lymphocytic, neutrophilic, and mixed cellular [3]. In the case of neutrophilic aortitis, an infection should first be ruled out. The histopathological assessment is the gold diagnostic tool, and this almost always requires the availability of surgical or biopsy specimens. Specific diagnostic serum biomarkers are lacking, and imaging methods have been the primary diagnostic tool for years.

Nevertheless, no perfect imaging method has been identified yet. Imaging is a useful tool not only for diagnosis but also for monitoring disease activity despite some controversies [4]. This review will summarize the updated data on aortitis, mainly through published articles during the previous 12 months.

EPIDEMIOLOGY

To pinpoint the underlying disease in a patient with an aortic aneurysm can be difficult. Quimson *et al.* [5] compared the demographic, clinical, and

Department of Rheumatology, Basaksehir Cam ve Sakura City Hospital, Istanbul, Turkey

Correspondence to Mustafa Erdogan, Department of Rheumatology, Basaksehir Cam ve Sakura City Hospital, 34480 Istanbul, Turkey. E-mail: merdogan50@gmail.com

Curr Opin Rheumatol 2021, 33:34–40
DOI:10.1097/BOR.0000000000000762

KEY POINTS

- Isolated aortitis has a worse event-free survival rate than Takayasu arteritis and giant cell arteritis at long-term follow-up.
- JAK/signal transducers and activators of transcription signaling pathway and mTOR hyperactivity are involved in Takayasu arteritis pathogenesis. Thus, JAK inhibitors and mTOR inhibitors may have promising roles in treatment.
- Three new Takayasu arteritis clusters were defined: abdominal predominant, symmetrical aortic arch predominant, and asymmetrical focal disease.
- Patients with Takayasu arteritis and carotidynia or arm claudication, as well as patients with giant cell arteritis and posterior headache, should be carefully assessed for disease activity.
- Leflunomide and Tocilizumab may have promising roles in the treatment of Takayasu arteritis. However, there are controversies.

Table 1. Causes of aortitis

Inflammatory-associated
GCA (temporal arteritis)
Takayasu arteritis
IgG4-related disease
Behçet disease
Relapsing polychondritis
Cogan syndrome
Sarcoidosis
Idiopathic retroperitoneal fibrosis
Rheumatoid arthritis
Systemic lupus erythematosus
Spondyloarthropathies
ANCA-associated vasculitides
Polyarteritis nodosa
Immune checkpoint inhibitors
G-CSF
Inflammatory-idiopathic
Isolated aortitis
Chronic periaortitis
Inflammatory aortic aneurysm
Infectious
Salmonella spp.
Staphylococcus spp.
Streptococcus pneumoniae
Syphilis
Mycobacterial (i.e., *Mycobacterium tuberculosis*)
Fungal

ANCA, anti-neutrophil cytoplasmic antibody, GCA, giant cell arteritis, G-CSF, granulocyte colony-stimulating factor.

radiological characteristics of 262 patients who underwent surgical aortic aneurysm repairs retrospectively. Older age at the time of surgery [odds ratio (OR) 1.08 (95% confidence interval (CI) 1.03–1.13)], female sex [OR 2.36 (95% CI 1.01–5.51)], absence of coronary artery disease [OR 6.92 (95% CI 2.14–22.34)], larger aneurysm diameter [OR 1.74 (95% CI 1.02–2.98)], and presence of arterial wall thickening on imaging [OR 56.93 (95% CI 4.31–752.33)] predicted aortitis-related aneurysmal disease. They also reported a faster increase in the size of aneurysms, a higher rate of noncontiguous aortic aneurysms, and wall thickening in extra aortic arteries in the aortitis group. The lack of C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) data before surgery was a significant limitation of the study.

Several reports on outcome data of patients with large-vessel vasculitis (LVV) have been published. The French Study Group for LVV reported the long-term prognosis of 353 patients with noninfectious aortitis with a median follow-up time of 52 months. An event was defined as the occurrence of a new aneurysm, dissection, revascularization, or death. Aneurysms were identified in 42.5%, 25%, and 21% of patients with isolated aortitis, Takayasu arteritis, and GCA. Five-year event-free survival was 38% [interquartile range (IQR) (26; 55)], 67% [IQR (57; 71)], and 73% [IQR (64; 82)], for patients with isolated aortitis, Takayasu arteritis, and GCA, respectively. In multivariate analyses, an isolated aortitis [hazard ratio: 1.72 (95% CI: 0.72–2.01)] and the male sex [hazard ratio: 1.77 (95% CI: 0.6–1.44)] were independent risk factors for a new event occurrence [6▪].

Clifford *et al.* reported the clinical features of 196 patients with biopsy-proven noninfectious aortitis diagnosed following aortic root/ascending aorta or aortic arch surgery. The diagnoses were; 129 isolated aortitis, 42 GCA, 14 Takayasu arteritis, and 11 other diseases [7]. Among 65 patients with isolated aortitis and with serial imaging results, 14% were diagnosed as systemic vasculitis, and new vascular lesions developed in 45% in at least 6 months follow-up period. Nine patients (12%) with isolated aortitis died in a median of 52 months of follow-up.

A retrospective cohort study conducted in Israel based on a medical database reported increased mortality in the first 2 years and after 10 years of GCA diagnosis compared with the age-matched population [8]. On the contrary, Brekke *et al.* [9▪▪] found no significant increased mortality at any time

point. The mortality rates after 5 and 10 years were 20 and 50%. Cardiovascular disease (CVD) hazard ratio: 1.31 (CI 95%: 1.13–1.51) is the usual risk factor for mortality, while corrected analysis according to CVD risk factors could not be performed due to lack of data.

Egebjerg *et al.* [10] conducted a nationwide medical database survey of Takayasu arteritis in Denmark and compared the risk of cardiovascular events and mortality with a general age-matched population. The incidence of Takayasu arteritis was found 0.7/million/year. In patients with Takayasu arteritis, mortality was higher in the first 3 years after diagnosis [hazard ratio: 8 (95% CI: 3–21)], whereas minor and major cardiovascular event (CVE) was higher both before and after 3 years.

The seasonal occurrence of a disease may be an indirect evidence for an 'infectious cause hypothesis.' The seasonal occurrence of GCA has been debated with conflicting reports [11,12]. A recent study reported that summer months as a risk factor for biopsy-proven GCA [13▪]. However, owing to the lack of exact dates or distribution of first symptom dates, these results do not support the seasonal triggering hypothesis.

The literature on the rare types of aortitis, such as aortitis related to relapsing polychondritis, mostly relies on case reports and small retrospective studies. Last year we performed a systemic review and identified that aortic involvement in relapsing polychondritis has a high mortality rate (27% in a 24-month follow-up). It can be asymptomatic in 19% of the patients, so screening for aortic involvement is essential when a patient is diagnosed as relapsing polychondritis [14]. Patients with solid or hematological malignancies may have paraneoplastic or drug-induced autoimmune clinical conditions. Case reports for Granulocyte colony-stimulating factor (G-CSF) and immune checkpoint inhibitors related to aortitis are being increasingly reported [15–17]. Whether G-CSF is the main triggering or only the contributing factor in pathogenesis is a matter of debate because chemotherapeutic agents may also cause immunomodulatory effects. Clinicians should be aware of the drug-induced vasculitis in patients receiving chemotherapy. A new-onset fever, chest, or neck pain, and very high serum CRP levels are in the first weeks of the G-CSFs, and immune checkpoint inhibitors can indicate a drug-induced vasculitis.

DISEASE MECHANISM STUDIES

The search for the pathogenesis of aortitis has been problematic in general. In a study by Yoshizaki *et al.* [18], the expression level of IL-25 was increased in the aorta. IL-25 perhaps led to the development of IL-1, tumor necrosis factor, and IL-17A-mediated aortitis in IL-1 receptor antagonist deficient mice. Li *et al.* showed that the number and cytotoxic potential of natural killer cells were reduced in Takayasu arteritis patients. However, whether this was secondary to IL-6-related inflammation or had a specific role in the pathogenesis was not clear [19]. A study described the role of IL-33 in 52 patients with GCA, of whom 14 had aortitis. The authors found overexpression of IL-33 and its receptor, which cause an immunoregulatory effect via increased Th2 and Treg activity [20]. Although macrophages have a substantial role in Takayasu arteritis, which macrophage subtype has the leading role is unknown. Dos Santos *et al.* compared M1 and M2 macrophage expression in the aortic specimens of patients with Takayasu arteritis to patients with atherosclerotic disease and heart transplant donors. Overexpression of the CD206 (a marker for M2 macrophages) compared with CD68 (a marker for M1 macrophages) in the aorta from Takayasu arteritis patients was identified [21]. This finding, perhaps, may direct us to new molecular treatment strategies.

The role of differentiation of T cells into Th1 and Th17 cells plays a role in the pathogenesis of Takayasu arteritis. However, the mechanism is not clear [22]. Régnier *et al.* [23] investigated the role of Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway in the differentiation of T cells and interferon signature in patients with Takayasu arteritis. They also tested the effect of JAK/STAT inhibitors (JAKinibs) *in vitro* in cell cultures acquired from patients with Takayasu arteritis. They described that the JAK/STAT pathway and upregulated type 1 interferon signature has a proinflammatory effect in Takayasu arteritis. Another study identified a triggering mechanism related to the dysfunction of Th1 and Th17 cells in patients with Takayasu arteritis. The proposed mechanism is the hyperactivity of the mammalian target of rapamycin (mTOR) [24▪]. Consequently, JAKinibs and mTOR inhibitors may have a promising role in the treatment of Takayasu arteritis.

CLASSIFICATION

Clinical presentation and extent of vascular involvement vary among subgroups of patients with aortitis. A new classification system was proposed for Takayasu arteritis, based on angiographic disease patterns [25]. A cluster analysis was performed with Takayasu arteritis cohorts from India and North America. Three clusters were defined: abdominal predominant, symmetrical aortic arch

predominant, and asymmetrical focal disease. Among patients with serial imaging follow-up data, only one of the 92 patients changed cluster in a median of 3.2 years. Another study also showed that sequential vascular progress in Takayasu arteritis is often not observed [26▪▪].

Whether Takayasu arteritis and GCA are the same diseases with different clinical patterns has been debated, even after some clinical and epidemiological differences had been identified [27]. In a recent cluster analysis, six clusters with different distribution patterns between Takayasu arteritis and GCA were defined [28▪]. Patients with Takayasu arteritis compared with patients with GCA were significantly more likely to have involvement of the left carotid, left subclavian, abdominal, mesenteric, and renal arteries. In contrast, patients with GCA were more likely to have the descending thoracic aorta and axillary artery (left: 30.9 vs. 5.9%; right: 31.8 vs. 5.4%) involvement.

The IgG4-related disease is a lately recognized condition that can also involve the aorta. In the derivation and validation cohort of the new The 2019 American College of Rheumatology/European League against Rheumatism IgG4-related-disease criteria, aortic involvement was identified in 11% of the patients [29▪▪]. According to the new criteria, a patient with at least one inclusion criterium and .without any exclusion criteria should have at least 20 points to be classified as having an IgG4-related disease. The weighted points of 'diffuse thickening of the abdominal aortic wall' and 'circumferential, or anterolateral soft tissue around the infrarenal aorta or iliac arteries' were +4 and +8 points, respectively.

There were two other recent important studies about IgG4-related disease. The first one was a cluster analysis conducted to define subgroups [30]. One subgroup was the 'retroperitoneal fibrosis and/or aortitis group,' which accounted for 24% of the 493 patients in the derivation cohort. Significantly the median serum IgG4 level was lowest in this group compared with other groups. The second study classified patients with IgG4-related aortitis into four types according to the localization of involvement; type 1, thoracic aorta; type 2a, abdominal aorta; type 2b, abdominal aorta, and iliac artery; type 2c, iliac artery; type 3, thoracic and abdominal aorta; and type 4, other arterial sites [31▪]. The abdominal aorta was the most common site, followed by the iliac artery, thoracic aorta. The most common distribution type was 2b (83%).

DIAGNOSIS AND MONITORING METHODS

Cross-sectional imaging techniques, including computed tomography (CT) and MRI angiography (MRA), PET, and ultrasonography, replaced conventional angiography and have been the most critical diagnostic and monitoring tool in LVV [4]. Due to the wide variety of symptoms without specificity, there is still a lack of information about the correlation of imaging findings and exact disease activity or symptoms of LVV. Another issue is we still need a standardized definition of remission, disease activity, and a set of outcome measures [32].

A recent study investigated the relationship between measures of disease assessment, including patient-reported outcomes (PRO), physician global assessment (PhGlobal), laboratory, and imaging outcomes (PETVAS scoring) in LVV [33]. Disease activity was independently associated with PETVAS, CRP, and PtGlobal in multivariate analyses. Although PROs correlated with each other, they did not correlate with fluorodeoxyglucose-positron emission tomography (FDG-PET) findings, CRP, ESR, and physician-reported outcomes. PROs have a substantial role in the assessment of disease activity; hence it is still an area of research for skeptics to generate functional composite outcome measures consisting of PROs along with other activity measures.

The Giant Cell Arteritis and PET Study assessed the accuracy of a newer generation PET-CT as a first-line diagnostic tool for GCA [34▪▪]. Sixty-four patients underwent time-of-flight PET-CT within 72 h of starting glucocorticoids and before a temporal artery biopsy (TAB). PET-CT had comparable performance (sensitivity 92%, specificity 85%) with TAB and a high negative predictive value (98%). However, insufficient sensitivity was seen when compared with a clinical diagnosis. These results indicate that a negative PET-CT may obviate the need for a for TAB. Although another retrospective study indicated a lower diagnostic performance of PET-CT among patients with a negative TAB, the high proportion of patients with a history of corticosteroid treatment more than 3 days before imaging and the lack of standardization in diagnostic procedures may explain the lower specificity [35].

Kang *et al.* [36] compared the performance of PTX-3 and PETVAS to regional the maximum standardized uptake value (SUVmax) for monitoring disease activity in patients with Takayasu arteritis. PETVAS and Pentraxin-3 (PTX-3) were superior to detect activity than regional SUVmax, CRP, and ESR. PTX-3 also showed a better correlation with disease activity index and PETVAS than CRP and ESR.

A debated issue of PET-CT is imaging acquisition time. Quinn *et al.* [37] showed that delayed imaging after FDG injection (2-h time point) could reveal disease activity better than traditional imaging time (1-h time point), and clinically active disease was

significantly more common in patients in the delayed active group (OR: 1.94, 95% CI 1.13–3.53).

Distinguishing whether a symptom is related to activity or vascular damage is also challenging. A study compared the association of symptoms with vascular inflammation (assessed by FDG-PET) and damage (assessed by MRA) in LVV [38▪▪]. In patients with Takayasu arteritis, carotidynia indicated inflammation in the carotid arteries (strong association); arm claudication indicated inflammation (weak association) or damage (strong association) in subclavian arteries. On the other hand, in patients with GCA, posterior headache indicated inflammation or damage. An increased risk of major central nervous system events identified in patients with an increased burden of neck artery (carotid and vertebral arteries) disease. The specificity of PET-CT to distinguish inflammation from atherosclerosis or remodeling of the vessel wall can sometimes be difficult. Somatostatin Receptor PET may be a promising modality for better selectivity, although it needs to be confirmed by clinical studies [39].

Ultrasonography has been used widely as the initial diagnostic test in GCA being free of radiation, readily available, and inexpensive [4]. Slope sign in axillary arteries was suggested as a specific feature of GCA [40,41]. A cutoff for the slope sign (axillary to brachial intima media thickness (IMT) ratio) yielded 87% sensitivity and 89% specificity for detecting GCA [42]. In another study, adding an axillary assessment to the temporal artery, ultrasonography assessment increased the sensitivity of the ultrasonography from 52 to 71% without loss of specificity [43].

A new scoring system was proposed to assess the extent of vascular inflammation by counting the number of temporal artery segments and axillary arteries with a halo and calculating a composite halo score by the thickness of each halo [44▪]. A halo score of at least 10 was diagnostic with high specificity (>95%). A higher halo score of the temporal artery and the presence of halo sign in axillary arteries were associated with more systemic inflammation. Moreover, the halo counts scores were independent predictors of ocular ischemia. The presence of a halo sign in at least two arteries, and a halo score at least 3, predicted further ocular ischemia with an OR of 12 [95% CI = 1.430–100.705] and 9.8 [95% CI = 1.137–85.887], respectively. Contrast-enhanced ultrasonography (CEUS) was proposed as a modality that can detect activity in LVV by detecting vessel wall vascularization [45]. Although the standardization has been lacking, new studies confirmed the role of CEUS as a minimally invasive and reproducible monitoring tool [46,47]. The specificity is another concern, and whether it is related to the inability of CEUS to distinguish inflammation from remodeling need to be elucidated.

TREATMENT

glucocorticoids are the first-line treatments in LVV with or without adjunctive immunosuppressive treatment. In the updated EULAR management recommendations for LVV, Tocilizumab is recommended over conventional disease-modifying antirheumatic drugs (cDMARDs) and biologics as first-line adjunctive treatment in selected patients with GCA with a high level of evidence and agreement of experts. However, due to insufficient robust data, it is recommended only as a second-line treatment option in patients with the recalcitrant disease [48].

In a phase 3, placebo-controlled randomized study of tocilizumab in patients with refractory Takayasu arteritis (TAKT study), the primary endpoint (time to relapse) was not met, while the background glucocorticoids dose was tapered at a standard rate (10% per week) [49]. Recently, the long-term efficacy and safety were tested in an open-label extension study in patients who completed the TAKT study [50]. The median glucocorticoids dose could be reduced to less than 0.1 mg/kg/day in half of the patients, and significant improvement in health-related quality of life was identified. Due to the noncomparative study design, these results are not enough to conclude that tocilizumab has a sufficient glucocorticoids sparing effect in patients with Takayasu arteritis. Another study assessed the effect of tocilizumab in thirteen treatment naïve patients with Takayasu arteritis [51]. After seven infusions of tocilizumab along with glucocorticoids 0.7 mg/kg/day, six patients could discontinue glucocorticoids, and a significant decrease in disease severity (assessed through median National Institutes of Health (NIH) scale, ITAS-2010, and ITAS-A score) was noted. Tocilizumab can also be effective in patients with other types of aortitis, such as aortic involvement related to relapsing polychondritis [14]. Despite conflicting results, tocilizumab seems to be a promising treatment option in patients LVV regardless of associated disease. Although substantial treatment effects were observed with biologics, cDMARDs as induction or maintenance treatment in LVV are still an enigma due to insufficient robust data. A recent observational study favored leflunomide over cyclophosphamide as a more efficient and safer treatment in patients with Takayasu arteritis [52▪].

Although cDMARDs and biologic agents provided notable success in LVV, some patients with a recalcitrant LVV may still need further treatment.

Autologous hematopoietic stem cell transplantation (AHSCT) has been an opportunity to manage autoimmune diseases for decades. Despite the lack of controlled studies, the results of a retrospective study in six patients with Takayasu arteritis showed that AHSCT might be a part of the management of LVV [53].

Invasive vascular interventions may be needed to repair vascular damage in patients with LVV, but the timing and the kind of intervention were debated. Providing remission before aortic surgery is essential to prevent perioperative complications in LVV [54]. This was confirmed in a recent study on Behçet Syndrome, which also reported a promising role of prosthetic wrapping technique to prevent new aneurysm occurrence [55]. Finally, a study compared the outcomes of patients with Takayasu arteritis who were managed with different treatment strategies over a median 4.5 years, consist of; the percutaneous coronary intervention (PCI) group ($n = 18$), coronary artery bypass graft group ($n = 10$), and medical-therapy group ($n = 29$) [56]. Active disease and PCI were independent factors for the major cardiac event, which was defined as a composite of cardiac death, myocardial infarction, and coronary revascularization.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Gornik HL, Creager MAJC. Aortitis. Circulation 2008; 117:3039–3051.
2. Richards BL, March L, Gabriel SE. Epidemiology of large-vessel vasculidities. Best Pract Res Clin Rheumatol 2010; 24:871–883.
3. Stone JR, Bruneval P, Angelini A, *et al.* Consensus statement on surgical pathology of the aorta from the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology: I. Inflammatory diseases. Cardiovasc Pathol 2015; 24:267–278.
4. Dejaco C, Ramiro S, Duftner C, *et al.* EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice. Ann Rheum Dis 2018; 77:636–643.
5. Quimson L, Mayer A, Capponi S, *et al.* Rheumatology. Comparison of aortitis versus noninflammatory aortic aneurysms among patients who undergo open aortic aneurysm repair. Arthritis Rheumatol 2020; 72:1154–1159.
6. ■ Ferfar Y, Morinet S, Espitia O, *et al.* Long-term outcome and prognosis factors of isolated aortitis. Circulation 2020; 142:92–94.

The study defined the factors for large-vessel vasculitis-associated events (aneurysm, dissection, revascularization, or death). Long-term survival and clinical features of patients with isolated aortitis were compared with those with giant cell arteritis (GCA) and Takayasu arteritis.

7. Clifford AH, Arafat A, Idrees JJ, *et al.* Outcomes among 196 patients with noninfectious proximal aortitis. Arthritis Rheumatol 2019; 71:2112–2120.
8. Ben-Shabat N, Tiosano S, Shovman O, *et al.* Mortality among patients with giant cell arteritis: a large-scale population-based cohort study. J Rheumatol 2020; 47:1385–1391.
9. ■■ Brekke LK, Fevang BT, Diamantopoulos AP, *et al.* Survival and death causes of patients with giant cell arteritis in Western Norway 1972–2012: a retrospective cohort study. Arthritis Res Ther 2019; 21:154.

This was an outcome study in a large cohort of patients with GCA and a tightly matched control population. The long inclusion period (41 years) and follow-up time (median 8 years) reduced the risk of evaluating random time variations and missing late complications-related mortalities, respectively.

10. Egebjerg K, Baslund B, Obel N, Faurschou M. Mortality and cardiovascular morbidity among patients diagnosed with Takayasu's arteritis: a Danish nationwide cohort study. Clin Exp Rheumatol 2020; 38:91–94.
11. Smeeth L, Cook C, Hall AJ. Incidence of diagnosed polymyalgia rheumatica and temporal arteritis in the United Kingdom. Ann Rheum Dis 2006; 65:1093–1098.
12. Kisza K, Murchison AP, Dai Y, *et al.* Giant cell arteritis incidence: analysis by season and year in mid-Atlantic United States. Clin Exp Ophthalmol 2013; 41:577–581.
13. ■ Gokoffski KK, Chatterjee A, Khaderi SK. Seasonal incidence of biopsy-proven giant cell arteritis: a 20-year retrospective study of the University of California Davis Medical System. Clin Exp Rheumatol 2019; 37(Suppl 117):90–97.

The literature on aortic involvement in relapsing polychondritis mostly relied on case reports and case series. The study is an extensive search of all reports on aortitis related to relapsing polychondritis.

14. Erdogan M, Esatoglu SN, Hatemi G, Hamuryudan V. Aortic involvement in relapsing polychondritis: case-based review. Rheumatol Int 2019. [Epub ahead of print]
15. Taimen K, Heino S, Kohonen I, *et al.* Granulocyte colony-stimulating factor- and chemotherapy-induced large-vessel vasculitis: six patient cases and a systematic literature review. Rheumatol Adv Pract 2020; 4:rkaa004.
16. Sugai Y, Toyoguchi Y, Kanoto M, *et al.* Clinical and image features: large-vessel vasculitis after granulocyte colony stimulating factor administration. Acta Radiol 2020; 284185120931685. [Epub ahead of print]
17. Crout TM, Lennep DS, Kishore S, Majithia V. Systemic vasculitis associated with immune check point inhibition: analysis and review. Curr Rheumatol Rep 2019; 21:28.
18. Yoshizaki T, Itoh S, Yamaguchi S, *et al.* IL-25 exacerbates autoimmune aortitis in IL-1 receptor antagonist-deficient mice. Sci Rep 2019; 9:17067.
19. Li T, Gao N, Cui W, *et al.* Natural killer cells and their function in Takayasu's arteritis. Clin Exp Rheumatol 2020; 38(Suppl 124):84–90.
20. Desbois AC, Cacoub P, Leroyer AS, *et al.* Immunomodulatory role of interleukin-33 in large vessel vasculitis. Sci Rep 2020; 10:6405.
21. Dos Santos JP, Artigiani Neto R, Mangueira CLP, *et al.* Associations between clinical features and therapy with macrophage subpopulations and T cells in inflammatory lesions in the aorta from patients with Takayasu arteritis. Clin Exp Immunol 2020. [Epub ahead of print]
22. Savioli B, Abdulahad WH, Brouwer E, *et al.* Are cytokines and chemokines suitable biomarkers for Takayasu arteritis? Autoimmun Rev 2017; 16:1071–1078.
23. Régnier P, Le Joncour A, Maciejewski-Duval A, *et al.* Targeting JAK/STAT pathway in Takayasu's arteritis. Ann Rheum Dis 2020; 79:951–959.
24. ■ Zhang J, Zhao L, Wang J, *et al.* Targeting mechanistic target of rapamycin complex 1 restricts proinflammatory T cell differentiation and ameliorates takayasu arteritis. Arth Rheumatol 2020; 72:303–315.

The in-vitro study reported evidence for the promising role of Janus kinase/signal transducers and activators of transcription pathway inhibitors in Takayasu arteritis.

25. Goel R, Gribbons KB, Carette S, *et al.* Derivation of an angiographically based classification system in Takayasu's arteritis: an observational study from India and North America. Rheumatology 2020; 59:1118–1127.
26. ■■ Quinn KA, Gribbons KB, Carette S, *et al.* Patterns of clinical presentation in Takayasu's arteritis. Semin Arthritis Rheum 2020; 50:576–581.

In the study, five clinical presentation categories for patients with Takayasu arteritis were identified in two independent cohorts by a data-driven method.

27. Maksimowicz-McKinnon K, Clark TM, Hoffman GS. Takayasu arteritis and giant cell arteritis: a spectrum within the same disease? Medicine 2009; 88:221–226.
28. ■ Gribbons KB, Ponte C, Carette S, *et al.* Patterns of arterial disease in Takayasu's arteritis and giant cell arteritis. Arthritis Care Res 2020; 72:1615–1624.

1068 patients with a diagnosis of GCA and Takayasu arteritis from several international cohorts. The similarities and differences in global patterns of arterial involvement between patients with Takayasu arteritis and GCA have been described based on vascular imaging data.

29. ■■ Wallace ZS, Naden RP, Chari S, *et al.* The 2019 American College of Rheumatology/European League Against Rheumatism classification criteria for IgG4-related disease. Ann Rheum Dis 2020; 79:77–87.

This is a new classification criteria for IgG4-RD. The criteria was constructed by a three-step classification process and multcriterion decision analyses. The derivation and validation cohorts included 1879 patients with IgG4-related disease and mimickers.

30. Wallace ZS, Zhang Y, Perugino CA, *et al.* Clinical phenotypes of IgG4-related disease: an analysis of two international cross-sectional cohorts. Ann Rheum Dis 2019; 78:406–412.

31. ▪ Peng L, Zhang P, Li J, *et al.* IgG4-related aortitis/periaortitis and periarteritis: a distinct spectrum of IgG4-related disease. Arthritis Res Ther 2020; 22:103.
The study is the largest prospective cohort study that reported comparative analysis of patients with ($n = 89$) and without ($n = 498$) IgG4-related aortitis and preiaoritis.

32. Aydin SZ, Robson JC, Sreih AG, *et al.* Update on outcome measure development in large-vessel vasculitis: report from OMERACT. J Rheumatol 2019; 46:1198–1201.

33. Rimland CA, Quinn KA, Rosenblum JS, *et al.* Outcome measures in large vessel vasculitis: relationship between patient-, physician-, imaging-, and laboratory-based assessments. Arthritis Care Res 2020; 72: 1296–1304.

34. ▪▪ Sammel AM, Hsiao E, Schembri G, *et al.* Diagnostic accuracy of positron emission tomography/computed tomography of the head, neck, and chest for giant cell arteritis: a prospective, double-blind, cross-sectional study. Arth Rheumatol 2019; 71:1319–1328.
This is the first study conducted with a prospective design to assess PET-computed tomography as a first-line diagnostic tool for GCA.

35. Hay B, Mariano-Goulart D, Bourdon A, *et al.* Diagnostic performance of (18)F-FDG PET-CT for large vessel involvement assessment in patients with suspected giant cell arteritis and negative temporal artery biopsy. Ann Nucl Med 2019; 33:512–520.

36. Kang F, Han Q, Zhou X, *et al.* Performance of the PET vascular activity score (PETVAS) for qualitative and quantitative assessment of inflammatory activity in Takayasu's arteritis patients. Eur J Nucl Med Mol Imaging 2020. [Epub ahead of print]

37. Quinn KA, Rosenblum JS, Rimland CA, *et al.* Imaging acquisition technique influences interpretation of positron emission tomography vascular activity in large-vessel vasculitis. Semin Arthritis Rheum 2020; 50:71–76.

38. ▪▪ Michailidou D, Rosenblum JS, Rimland CA, *et al.* Clinical symptoms and associated vascular imaging findings in Takayasu's arteritis compared to giant cell arteritis. Ann Rheum Dis 2020; 79:262–267.
This is a prospective study that reported the relationships between vascular symptoms and imaging findings in patients with GCA and Takayasu arteritis. The study had standardized and blinded clinical and imaging assessing protocol.

39. Tarkin JM, Wall C, Gopalan D, *et al.* Novel approach to imaging active Takayasu arteritis using somatostatin receptor positron emission tomography/magnetic resonance imaging. Circ Cardiovasc Imaging 2020; 13:e010389.

40. Dasgupta B, Smith K, Khan AAS, *et al.* 'Slope sign': a feature of large vessel vasculitis? Ann Rheum Dis 2019; 78:1738.

41. Milchert M, Brzosko M, editors. Slide sign: a novel sonographic sign of extracranial giant cell arteritis. Third International Symposium and Workshop on GCA, PMR and LVV, Southend-on-Sea, Essex, UK; 2016.

42. Milchert M, Brzosko M, Bull Haaversen A, Diamantopoulos AP. Correspondence to 'Slope sign': a feature of large vessel vasculitis? Ann Rheum Dis 2019. [Epub ahead of print]

43. Hop H, Mulder DJ, Sandovici M, *et al.* Diagnostic value of axillary artery ultrasound in patients with suspected giant cell arteritis. Rheumatology 2020. [Epub ahead of print]

44. ▪ van der Geest KSM, Borg F, Kayani A, *et al.* Novel ultrasonographic Halo Score for giant cell arteritis: assessment of diagnostic accuracy and association with ocular ischaemia. Ann Rheum Dis 2020; 79:393–399.
This is the first study that suggested a scoring for halo sign in patients with GCA. A cutoff value for the halo score that predicts ischemic vision loss, confirming a firm diagnosis of GCA.

45. Germanò G, Macchioni P, Possemato N, *et al.* Contrast-enhanced ultrasound of the carotid artery in patients with large vessel vasculitis: correlation with positron emission tomography findings. Arth Care Res 2017; 69:143–149.

46. Wang Y, Wang YH, Tian XP, *et al.* Contrast-enhanced ultrasound for evaluating arteritis activity in Takayasu arteritis patients. Clin Rheumatol 2020; 39:1229–1235.

47. Li Z, Zheng Z, Ding J, *et al.* Contrast-enhanced ultrasonography for monitoring arterial inflammation in Takayasu arteritis. J Rheumatol 2019; 46:616–622.

48. Hellmich B, Agueda A, Monti S, *et al.* 2018 Update of the EULAR recommendations for the management of large vessel vasculitis. Ann Rheum Dis 2020; 79:19–30.

49. Nakaoka Y, Isobe M, Takei S, *et al.* Efficacy and safety of tocilizumab in patients with refractory Takayasu arteritis: results from a randomized, double-blind, placebo-controlled, phase 3 trial in Japan (the TAKT study). Ann Rheum Dis 2018; 77:348–354.

50. Nakaoka Y, Isobe M, Tanaka Y, *et al.* Long-term efficacy and safety of tocilizumab in refractory Takayasu arteritis: final results of the randomized controlled phase 3 TAKT study. Rheumatology 2020; 59:2427–2434.

51. Mekinian A, Saadoun D, Vicaut E, *et al.* Tocilizumab in treatment-naïve patients with Takayasu arteritis: TOCITAKA French prospective multicenter open-labeled trial. Arthritis Res Ther 2020; 22:218.

52. ▪ Dai X, Cui X, Sun Y, *et al.* Effectiveness and safety of leflunomide compared with cyclophosphamide as induction therapy in Takayasu's arteritis: an observational study. Ther Adv Chronic Dis 2020; 11:2040622320922019.
This is the largest study that compared leflunomide with cyclophosphamide as an induction therapy in Takayasu arteritis.

53. Laurent C, Marjanovic Z, Ricard L, *et al.* Autologous hematopoietic stem cell transplantation with reduced-intensity conditioning regimens in refractory Takayasu arteritis: a retrospective multicenter case-series from the Autoimmune Diseases Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant 2020; 55:2109–13.

54. Zheng T, Zhu S, Ou JF, *et al.* Treatment with corticosteroid and/or immunosuppressive agents before surgery can effectively improve the surgical outcome in patients with Takayasu's arteritis. J Invest Surg 2019; 32:220–227.

55. Mousa A, Sharabi A, Elkalla MA, *et al.* Prophylactic prosthetic wrapping for vascular anastomosis in patients with Behçet's aortic aneurysms: an experience from a resource-challenged setting. Int Angiol 2019; 38:484–493.

56. Wang H, Zhang Y, Shen Z, *et al.* Comparing the effects of different management strategies on long-term outcomes for significant coronary stenosis in patients with Takayasu arteritis. Int J Cardiol 2020; 306:1–7.

Intracranial vessel wall imaging

Serdar Arslan, Bora Korkmazer, and Osman Kizilkilic

Purpose of review
To give an overview regarding the potential usefulness of vessel wall imaging (VWI) in distinguishing various intracranial vascular diseases, their common imaging features, and potential pitfalls.

Recent findings
VWI provides direct visualization of the vessel wall and allows the discrimination of different diseases such as vasculitis, atherosclerosis, dissection, Moyamoya disease, and reversible cerebral vasoconstriction syndrome. Recent studies showed that concentric and eccentric involvement in the vessel wall, as well as the enhancement pattern were found important for the distinguishing these diseases and evaluating their activity.

Summary
Most of the imaging techniques currently used are based on luminal imaging. However, these imaging methods are not adequate to distinguish different diseases that can demonstrate similar radiological findings. VWI is being increasingly used as a noninvasive imaging method to offset this limitation.

Keywords
central nervous system vasculitis, MRI, vessel wall imaging

INTRODUCTION

Vessel wall imaging (VWI) is a new imaging technique which is used in the diagnosis of intracranial vascular diseases through MRI. This imaging method allows a more detailed evaluation regarding the vessel walls when compared with other conventional radiological modalities. This new imaging tool is being used in routine patient care in many centers in recent years [1].

VWI provides vascular wall-based imaging for intracranial arteries and has many advantages over conventional lumen-based angiography methods. Different intracranial vasculopathies can be distinguished by VWI. Both cerebrospinal fluid (CSF) and blood can be suppressed to provide a more detailed view of the vessel walls [2,3]. The goal of this review is to describe the technical requirements of VWI and to present the clinical applications of VWI in different vascular diseases. VWI features of diseases are summarized in Table 1.

TECHNICAL REQUIREMENTS AND IMAGING

An appropriate imaging protocol and optimized sequences are necessary for VWI [4]. To provide high spatial resolution, 3 Tesla or higher magnet strength is required. 3-Tesla magnets are used as standard imaging for VWI with all necessary technical requirements. Better image quality can be obtained with 7-Tesla MRI compared with 3-Tesla MRI. However, artifacts and the strict magnetic resonance security procedures are major disadvantages of ultra-high magnet scanners. Moreover, peripheral resolution is provided better with 32-channel or 64-channel head coils when compared with 8-channel or 12-channel head coils [2].

Intracranial arteries normally have very thin walls. This necessitates to perform VWI studies with the lowest possible *t* voxels to accurately determine the borders of the vascular wall and separate it from surrounding structures [5]. There must be at least two voxels inside to a structure in an image for measuring the size or thickness. Thus, the optimal voxel size should be half or less of the vessel wall (0.2–0.3 mm). Voxel size is 0.4–0.7 mm in three-dimensional VWI. Smaller voxel sizes are not used due to the increased acquisition time and motion artifacts.

Division of Neuroradiology, Department of Radiology, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey

Correspondence to Osman Kizilkilic, MD, Division of Neuroradiology, Department of Radiology, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Cerrahpaşa Mah., Kocamustafapaşa Cad. No: 34/E Fatih, Istanbul, Turkey. Tel: +90 2124143000;
e-mail: osmank@istanbul.edu.tr

Curr Opin Rheumatol 2021, 33:41–48
DOI:10.1097/BOR.0000000000000759

KEY POINTS

- VWI increases the accuracy of magnetic resonance angiography from 8.3 to 95.8% in the diagnosis of cerebral vasculitis.
- VWI is an effective method to determine the appropriate localization for biopsy.
- VWI can be used to evaluate the treatment response in the follow-up of patients with central nervous system vasculitis.
- There is a positive relationship between plaque enhancement in VWI and stroke recurrence.

Two-dimensional sequences were used initially for VWI. Two-dimensional sequence and they provided sufficient spatial resolution and signal-to-noise ratio with acceptable acquisition time. Both short and long axes of the vessels can be evaluated using sequences in different planes (axial, sagittal, and coronal). Moreover, it is highly dependent on correct positioning of the field of view.

Three-dimensional sequences are more frequently preferred in VWI. They provide multiple two-dimensional reformat images to be created in any plane by processing the data obtained with isotropic voxels. In this way, the partial volume averaging artifact arising from the oblique and tortuous course of the intracranial arteries can be eliminated. However, these sequences result in longer acquisition times [6,7], requiring 3–7 min to produce an image.

The blood signals inside the vessel lumen and the CSF signals outside the vessel wall must be suppressed for VWI. At least one T1-weighted pre and postcontrast sequence should be included in the imaging. Basically spin-echo imaging, preregional saturation pulse, and a double-inversion recovery-based sequence are used for suppression of blood and CSF [8].

Three-dimensional turbo spin-echo sequences with variable flip angle refocusing pulses are the most common imaging techniques used in VWI [3,4]. The names of these sequences vary based on the vendor. VISTA (volume isotropic turbo spin-echo acquisition; Philips Healthcare, Best, The Netherlands), CUBE (GE Healthcare, Milwaukee, Wisconsin, USA), and SPACE (sampling perfection with application optimized contrasts by using different flip-angle evolutions; Siemens, Erlangen, Germany) are main examples [2].

VASCULITIDES

Vasculitides represent a group of inflammatory diseases which primarily affect the vessel wall. They are categorized according to the location and size of the affected vessel [9]. Central nervous system (CNS)

Table 1. Vessel wall imaging features of intracranial diseases

Disease	Lesion pattern	Location	Vessel wall thickening pattern	Wall enhancement
CNS vasculitis	Asymmetric narrowings and dilatations	Multifocal areas, distal ICA and vertebral arteries	Concentric wall thickening	Almost always present
Giant cell arteritis	Asymmetric narrowings and dilatations	Multifocal areas, extracranial and intracranial arteries	Concentric wall thickening	Often present
Intracranial atherosclerosis	Multiple eccentric, focal wall thickening and remodeling	Multifocal areas, distal ICA and vertebral arteries	Eccentric wall thickening	Present or not present
After thrombectomy	Wall thickening	Thrombectomised segment	Eccentric or concentric wall thickening	Often present
Arterial dissection	Aneurysmal dilatation, intimal flap, intramural hematoma	Distal ICA and vertebral arteries	Eccentric wall thickening	Often present
Reversible cerebral vasoconstriction syndrome	Smooth wall thickening and segmental narrowing	Widespread	Concentric wall thickening	Often not present
Moyamoya disease	Marked narrowing, puff of smoke appearance	Distal ICA and proximal MCA	Concentric wall thickening	Present or not present
Intracranial aneurysm	Depends on the type of aneurysm	–	–	Presence of enhancement is associated with risk of rupture

CNS, central nervous system; ICA, internal carotid artery; MCA, middle cerebral artery.

involvement can occur in different ways. CNS vasculitis may occur as an isolated disease, or it may occur as part of systemic vasculitis, infection or a collagen – vascular disease.

Both clinical and radiological diagnosis of CNS vasculitis is difficult. The diagnosis of primary CNS vasculitis is a challenging process that requires excluding many other possible intracranial vasculopathies [10,11].

CNS vasculitis can be detected at any age, but it is predominantly seen in the 4th to 6th decades. Clinical presentation is nonspecific. Neurological symptoms in CNS vasculitis can manifest in a broad spectrum and it usually consists of headache, cognitive dysfunction, focal neurological deficit, or stroke.

Clinical evaluation, noninvasive [magnetic resonance angiography (MRA), computed tomography angiography (CTA)] or invasive [digital subtraction angiography (DSA)] imaging methods and histopathological evaluation are required for the diagnosis of CNS vasculitis [10]. CNS vasculitis has nonspecific features on conventional MRI. Subcortical white matter, deep gray matter, deep white matter, and cerebral cortex are more frequently affected areas on the MRI. The most common finding in CNS vasculitis is infarction. Infarcts occur both in the large and small vessel territories in the cortical and subcortical areas. T2/FLAIR hyperintensities are the most common finding in MRI. Mass-like lesions and leptomeningeal enhancement are detected in 10 and 15% of patients, respectively. Parenchymal or subarachnoid hemorrhage is rare [12,13].

DSA is the most important imaging method. However, its sensitivity varied between 27 and 30% in different studies [14]. False-negative results can be seen more frequently in CNS vasculitis with small vessel involvement. Moreover, asymmetric narrowings and dilatations with strings of bead appearance along the vessel course are considered as classical angiographic sign for CNS vasculitis and these findings can also be detected in other diseases such as intracranial atherosclerosis, reversible cerebral vasoconstriction syndrome (RCVS) and radiation vasculitis. Thus, the specificity of DSA is also very low [12]. Biopsy is the gold standard method for the diagnosis of CNS vasculitis. However, false-negative results may occur due to the segmental involvement of the vessels [14]. CTA and MRA are more successful in evaluating the proximal vascular involvement. However, they are not reliable in the evaluation of distal vascular involvement.

VWI is a promising imaging method in the diagnosis of CNS vasculitis. It has a more common indication for use, especially in patients with suspected vasculitis. Concentric thickening, homogeneous, and strong contrast enhancement can be detected in VWI (Fig. 1) [15–17]. VWI increases the accuracy of

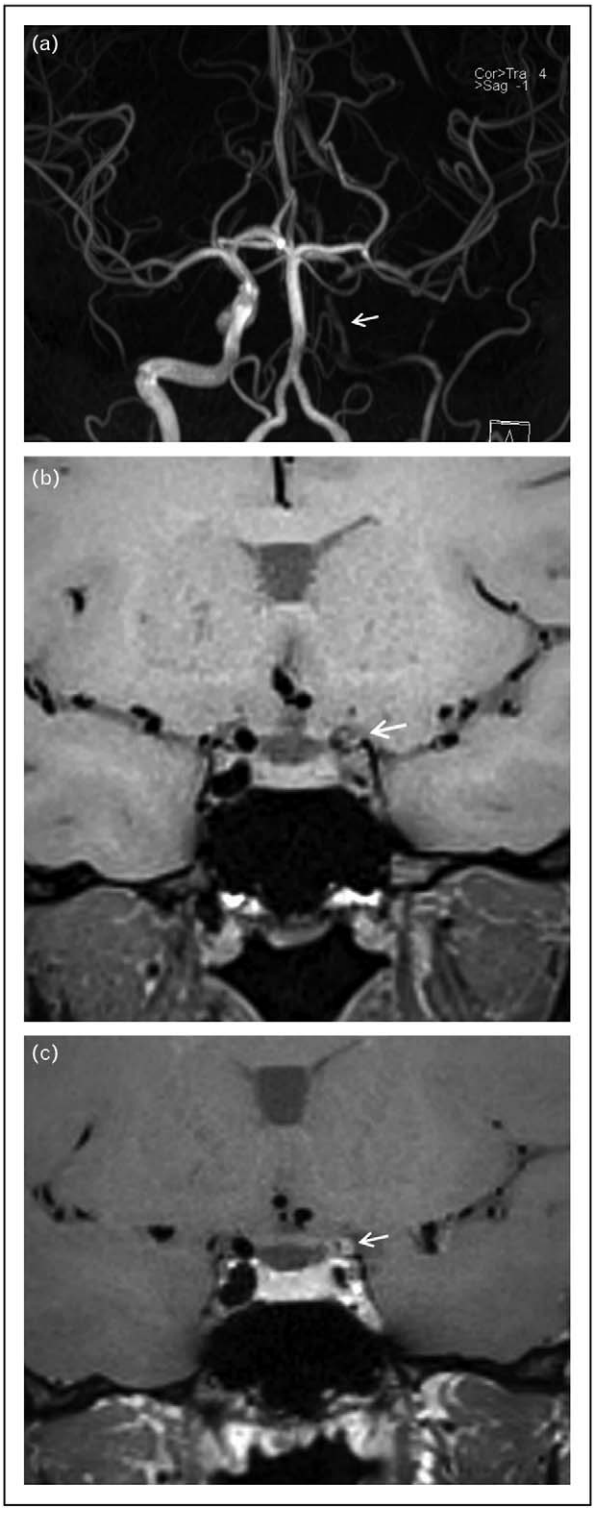

FIGURE 1. Central nervous system vasculitis. (a) A 33-year-old female patient presented with features suggestive of a transient ischemic attack. Time-of-flight magnetic resonance angiography shows a low-caliber flow in the left internal carotid artery. Vessel wall MRI performed (b) pre and (c) postgadolinium images show luminal narrowing and concentric enhancement in the left internal carotid artery supraclinoid segment.

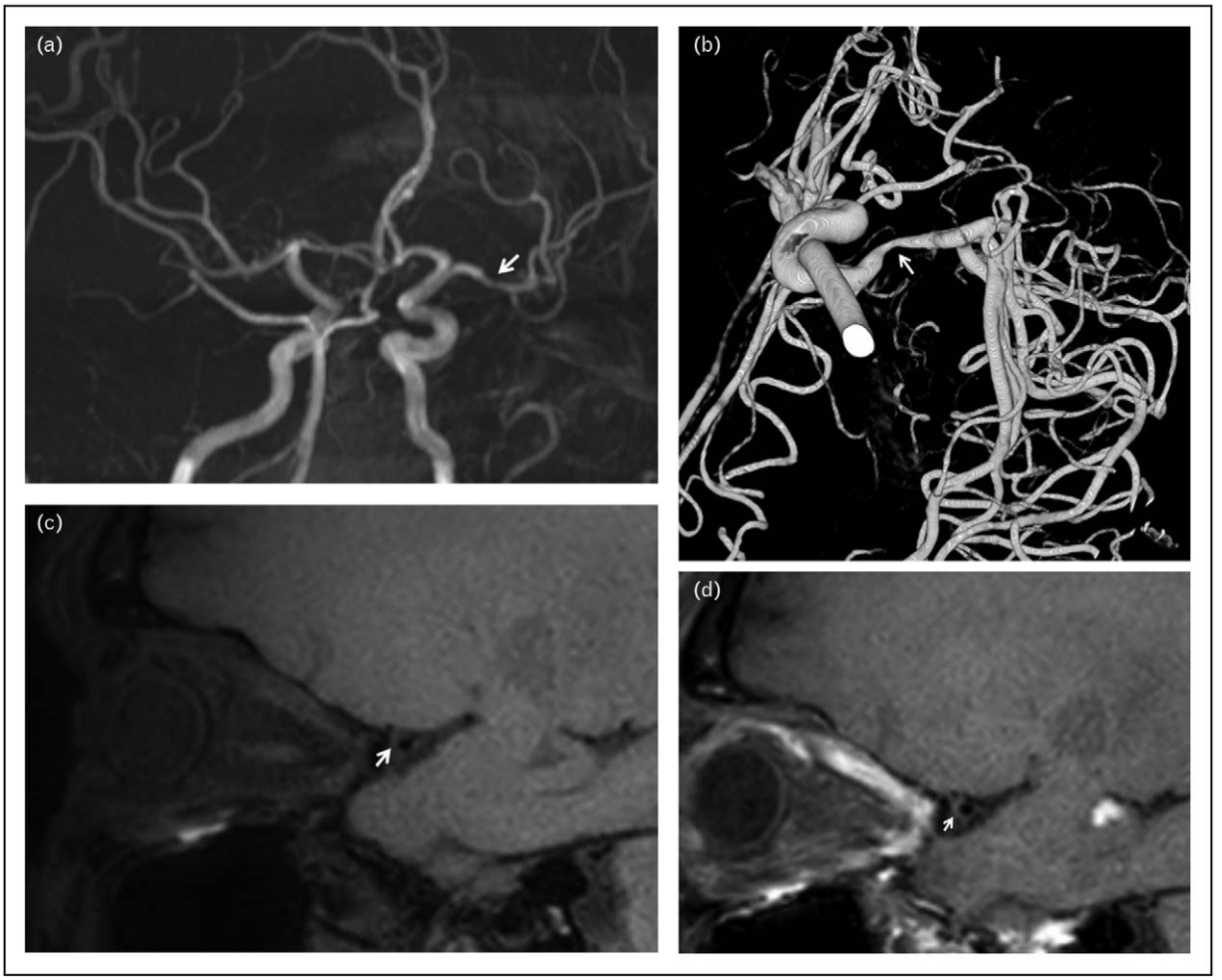

FIGURE 2. Central nervous system vasculitis. (a) A 53-year-old male patient presented with features suggestive of a transient ischemic attack. Time-of-flight magnetic resonance angiography and (b) three-dimensional digital subtraction angiography were performed and these demonstrated stenosis of the left middle cerebral artery M1 segment. (c) Pre and (d) postgadolinium images of vessel wall MRI show luminal narrowing and eccentric enhancement.

MRA from 8.3 to 95.8% in diagnosing of vasculitis [15]. Occasionally, eccentric enhancement may be observed in the vessel wall (Fig. 2). Usually, multiple vessel segments are involved [18]. Presence of contrast enhancement in the vessel wall cannot help to distinguish of the different types of vasculitis. Clinical status of the patients and the location of the involved vessel segment are more useful in differentiating of the different types of vasculitis [10]. A recent study by Destrebecq *et al*. showed that VWI enables detection of the nonocclusive intracranial vessel in CNS vasculitis responsible for brain ischemic lesions. Thus, concentric wall thickening and contrast enhancement pattern may be detected in multiple intracranial arteries which including the vessels responsible for the vascular territories corresponding to the clinical symptoms and parenchymal lesions [19▪].

VWI can help differentiate CNS vasculitis from other vascular diseases such as RCVS, intracranial atherosclerosis and dissection. In RCVS, which often mimics the CNS vasculitis, eccentric vascular wall thickening without contrast enhancement in the vessel wall is observed in the VWI. In intracranial atherosclerosis, unlike the vasculitis pattern, eccentric, and faint contrast enhancement can be detected [1,6,17].

VWI can be used in the evaluation of treatment response in patients with CNS vasculitis. Decrease in vascular wall thickness and contrast enhancement after treatment indicates regression [20]. Moreover, VWI is an effective method to determine the accurate localization of a biopsy [21].

Giant cell arteritis is the most common chronic inflammatory vasculitis of medium and large-sized vessels [22]. Polymorphic granulomatous infiltrate affecting all layers of the arterial vessel wall, especially in the tunica media, is observed. It can be detected as segmented and multifocal distribution

[23]. In a study comparing the diagnostic accuracy of three-dimensional versus two-dimensional contrast-enhanced VWI by Poillon *et al.* [24▪▪], it was shown that concentric wall thickening, mural enhancement, and perivascular inflammatory infiltration were detected in extracranial and intracranial arteries on the VWI. Moreover, in this study, three-dimensional contrast-enhanced VWI had higher accuracy rates with 80% sensitivity and 100% specificity for a diagnosis of giant cell arteritis.

INTRACRANIAL ATHEROSCLEROTIC DISEASE

Atherosclerosis is the leading cause of ischemic stroke, especially in the Asian population, African-Americans, and Hispanics. Intracranial atherosclerotic disease (IAD) is responsible for 25% cause of ischemic strokes [25]. Recent studies have reported that IAD is more common than previously thought and may be the most common cause of ischemic stroke cause worldwide [26].

VWI enables us to directly evaluate the plaque in the vessel wall in IAD. Components of the plaque (fibrous cap, lipid core, hemorrhage, and calcification), distribution, vessel wall remodeling, and enhancement can be evaluated [27,28]. Thus, it may be helpful in determining the exact cause in stroke patients with unknown cause [25]. VWI can also detect unstable nonstenotic plaques [28]. In a study by Schaafsma *et al.* [29▪], found that VWI has a substantial impact on etiologic classification in stroke patients. This method plays an important role in determining the cause in patients with ischemic stroke or transient ischemic attack. Thus, VWI can help to improve therapeutic decision in this patient group [29▪].

On imaging, an eccentric wall thickening can be detected in IAD (Fig. 3). Plaques tend to be multiple, focal, and may show enhancement [30]. The eccentric vascular wall involvement pattern is important in distinguishing IAD from other diseases, especially vasculitis and RCVS. A layered appearance can be detected in post contrast series. An enhancing fibrous capsule along the luminal surface, a nonenhancing core, and a thin peripheral enhancing layer may be detected [3]. Moreover, an increase in the size of the artery due to plaque 'positive remodeling' is an important finding in VWI. This is an adaptive remodeling pattern, resulting in less stenosis in the lumen.

Enhancement of the plaque in postcontrast T1WI is associated with a strong inflammatory response in the early period of ischemic stroke and decreases over time after the acute phase [31]. Song *et al.* [32▪] confirmed a positive relationship between plaque enhancement and stroke recurrence. Qiao *et al.* [33] reported that strong contrast enhancement in a plaque was associated with a vulnerable plaque. However, different studies have reported that 23% of nonculprit plaques may show enhancement [31]. So, further studies in larger populations are required to distinguish between a symptomatic and nonsymptomatic plaque.

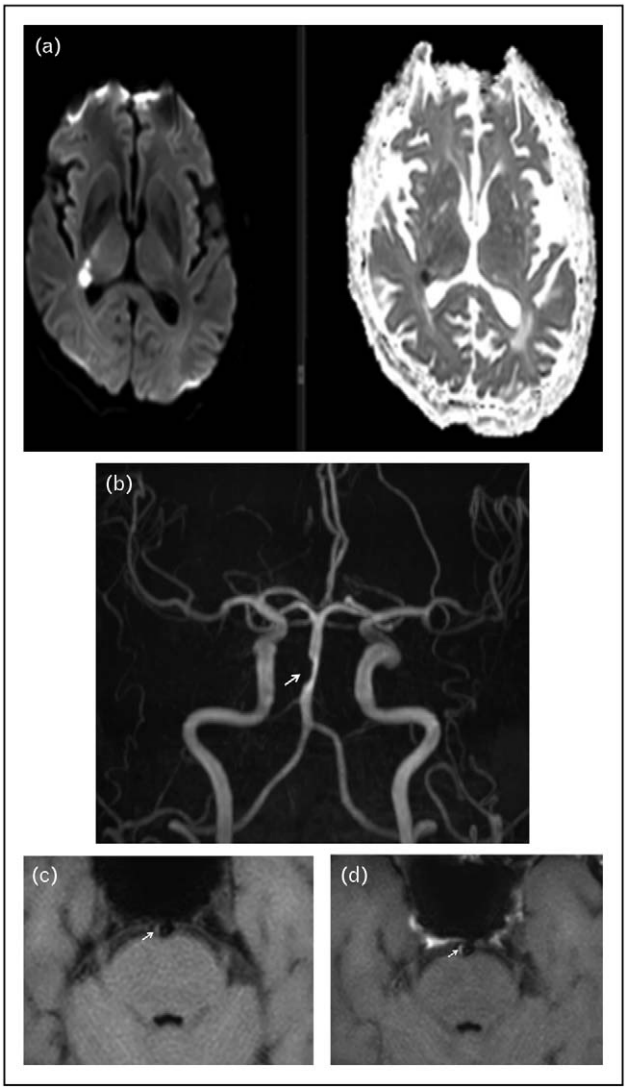

FIGURE 3. Atherosclerotic plaque. (a) A 65-year-old male patient presented with features suggestive of a stroke. Diffusion weighted imaging shows acute infarctions within the right thalamus and posterior limb of the internal capsule. (b) Time-of-flight magnetic resonance angiography shows focal narrowing of the mid-basilar artery. (c) Vessel wall MRI shows eccentric thickening of the basilar artery wall with intermediate signal intensity. (d) Contrast-enhanced vessel wall MRI shows enhancement of the plaque.

Vessel wall thickening and enhancement may be seen in patients undergoing mechanical thrombectomy [34]. These findings are less pronounced in patients undergoing clot aspiration than in those undergoing stent retriever thrombectomy.

INTRACRANIAL ARTERIAL DISSECTION

Intracranial arterial dissection is an important cause of stroke, especially in the young population. The spectrum of clinical presentation may be broad, ranging from headache to stroke or subarachnoid hemorrhage [35]. Imaging findings are nonspecific, such as stenosis, occlusion, irregularity in the vessel wall, and aneurysmatic dilatation. Thus, it is difficult to differentiate it from other diseases such as atherosclerosis, vasculitis, RCVS, and thromboembolic occlusion.

Intimal flaps, intramural hematomas, a double lumen, and an aneurysmal dilatation can be detected by angiographic methods. Especially, the string and pearl sign (irregular aneurysmal enlargement) is a typical finding in DSA [35,36].

VWI increases the detection rate of intimal flap significantly when combined with conventional imaging methods [37]. Moreover, the hyperintense intramural hematoma may be detected in the vessel wall. However, the hematoma intensity may change depending on the stage over time. T2* gradient echo or susceptibility weighted imaging sequences may be helpful to detect hematoma in the vessel wall. Eccentric enhancement may be detected due to inflammation, neovascularization or a false lumen in some cases (Fig. 4).

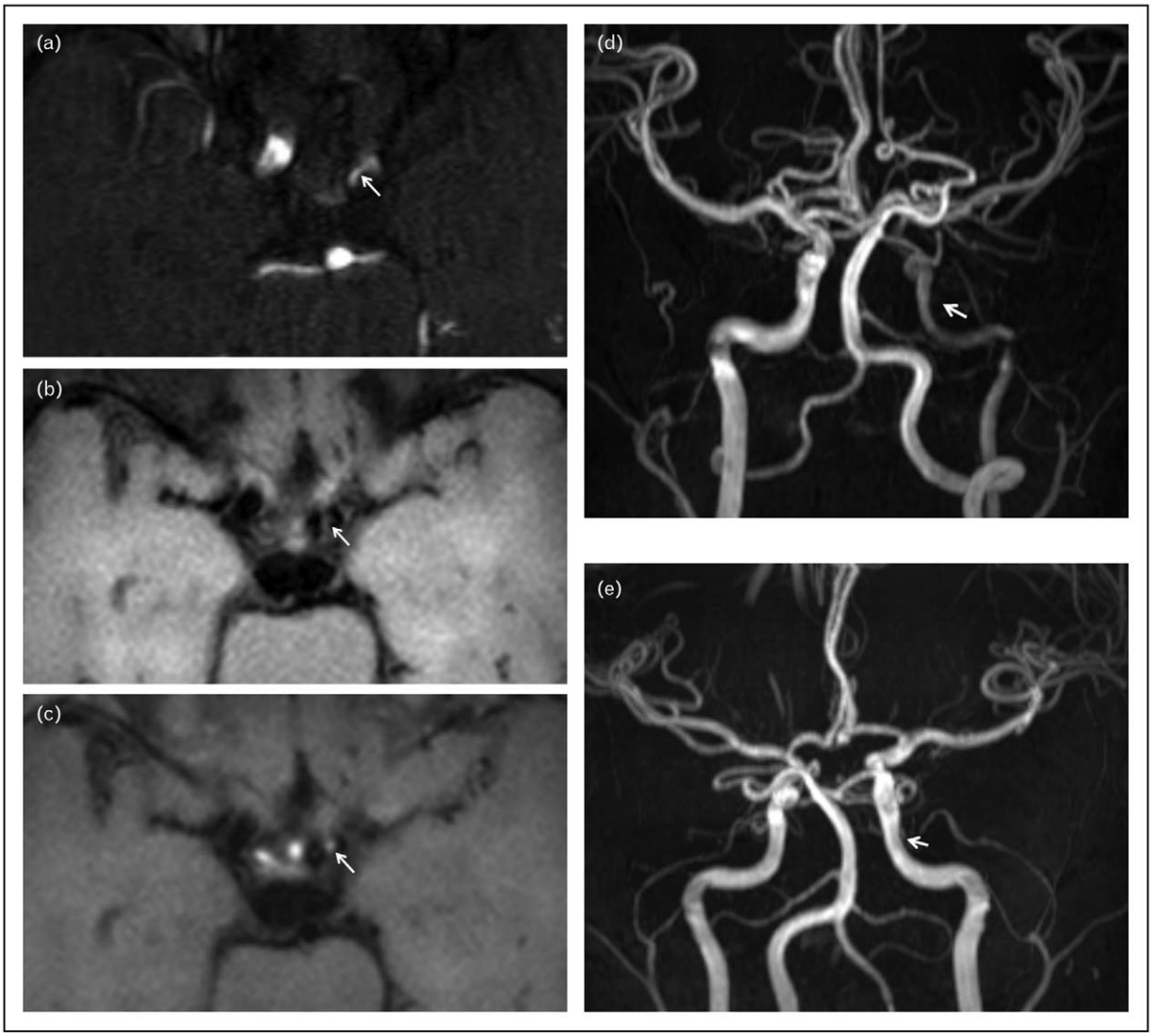

FIGURE 4. Arterial dissection. (a) An 11-year-old male patient presented with weakness in the right upper limb. Time-of-flight magnetic resonance angiography shows focal narrowing of the communicating segment of the left internal carotid artery. Axial vessel wall (b) pre and (c) postgadolinium images show mild eccentric contrast enhancement. (d) Maximum intensity projection image from three-dimensional time-of-flight magnetic resonance angiography shows a low-caliber flow in the left internal carotid artery. (e) In the control time-of-flight magnetic resonance angiography of the same case, after 5 months, normal flow of internal carotid artery is observed due to regression of the dissection.

OTHER VASCULOPATHIES

RCVS is typically characterized by severe headache, with or without focal neurological symptoms. RCVS is one of the most important diseases in the differential diagnosis of CNS vasculitis. Unlike CNS vasculitis, RCVS shows smooth and minimal thickening in the vessel wall. It shows segmental narrowing of the arteries without contrast enhancement. Moreover, the narrowing in the vessel calibration usually shows regression within 3 months [38].

Moyamoya disease is an idiopathic vaso-occlusive condition that affects the terminal segments of bilateral internal carotid artery and the proximal middle cerebral arteries, leading to progressive stenosis-occlusion. It is characterized by noninflammatory and nonatherosclerotic vasculopathy. The classic ' puff of smoke ' collateral appearance may not be detected at early stages of the disease [39]. There are different reports in the literature about enhancement in the vascular wall in the Moyamoya disease; however, the general opinion is that there is marked narrowing and concentric enhancement in VWI. A study by Kathuveetil *et al.* showed that wall thickening and enhancement are not common in Moyamoya disease. However, marked enhancement of the vessel wall in Moyamoya disease is related with symptomatic disease and increased risk of stroke [40▪].

One other indication for use of VWI in intracranial aneurysms is to detect the ruptured aneurysm in a patient with multiple aneurysms and a subarachnoid hemorrhage [41]. In addition, the risk of rupture in stable aneurysms can be evaluated with VWI. Edjlali *et al.* [42] and Larsen *et al.* [43] reported that the contrast enhancement in the wall of nonruptured aneurysms is associated with the increased risk of rupture. Another study with a large series showed that the lack of contrast enhancement in the aneurysm wall indicates the stability of the aneurysm [44].

PITFALLS

VWI is being increasingly used as a diagnostic tool in daily practice. However, it is important to know the limitations and potential pitfalls besides the many advantages of VWI. VWI is sensitive to motion artifacts due to relatively long acquisition times. The enhancement of the venous structures adjacent to arteries or low-velocity flow within the vessel lumen resulting in loss of flow voids can mimic vessel wall enhancement [1]. Moreover, the vasa vasorum of intracranial arteries may cause concentric arterial wall thickening and enhancement, leading to false positive interpretations for the presence of a CNS vasculitis [1].

CONCLUSION

VWI is an important radiological imaging method that allows direct evaluation of the vascular wall rather than just the visualization of the vessel lumen. It enables us to distinguish various diseases that show similar findings with conventional radiological methods. Therefore, in clinical practice, VWI should be a part of vascular imaging in addition to conventional imaging methods.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Lindenholz A, van der Kolk AG, Zwanenburg JJM, Hendrikse J. The use and pitfalls of intracranial vessel wall imaging: how we do it. Radiology 2018; 286:12–28.
2. Harteveld AA, van der Kolk AG, van der Worp HB, *et al.* High-resolution intracranial vessel wall MRI in an elderly asymptomatic population: comparison of 3T and 7T. Eur Radiol 2017; 27:1585–1595.
3. Mandell DM, Mossa-Basha M, Qiao Y, *et al.* Intracranial vessel wall MRI: principles and expert consensus recommendations of the American Society of Neuroradiology. AJNR Am J Neuroradiol 2017; 38:218–229.
4. Lindenholz A, Harteveld AA, Zwanenburg JJM, *et al.* Comparison of 3T intracranial vessel wall MRI sequences. AJNR Am J Neuroradiol 2018; 39:1112–1120.
5. Harteveld AA, Denswil NP, Van Hecke W, *et al.* Data on vessel wall thickness measurements of intracranial arteries derived from human circle of willis specimens. Data Brief 2018; 19:6–12.
6. Alexander MD, Yuan C, Rutman A, *et al.* High-resolution intracranial vessel wall imaging: imaging beyond the lumen. J Neurol Neurosurg Psychiatry 2016; 87:589–597.
7. Tan HW, Chen X, Maingard J, *et al.* Intracranial vessel wall imaging with magnetic resonance imaging: current techniques and applications. World Neurosurg 2018; 112:186–198.
8. Zhu XJ, Wang W, Liu ZJ. High-resolution magnetic resonance vessel wall imaging for intracranial arterial stenosis. Chin Med J 2016; 129:1363–1370.
9. Jennette JC. Overview of the 2012 revised international chapel hill consensus conference nomenclature of vasculitides. Clin Exp Nephrol 2013; 17:603–606.
10. Abdel Razek AA, Alvarez H, Bagg S, *et al.* Imaging spectrum of CNS vasculitis. Radiographics 2014; 34:873–894.
11. Berlit P, Kraemer M. Cerebral vasculitis in adults: what are the steps in order to establish the diagnosis? Red flags and pitfalls. Clin Exp Immunol 2014; 175:419–424.
12. Birnbaum J, Hellmann DB. Primary angiitis of the central nervous system. Arch Neurol 2009; 66:704–709.
13. Hajj-Ali RA, Singhal AB, Benseler S, *et al.* Primary angiitis of the CNS. Lancet Neurol 2011; 10:561–572.
14. John S, Hajj-Ali RA. CNS vasculitis. Semin Neurol 2014; 34:405–412.
15. Mossa-Basha M, Shibata DK, Hallam DK, *et al.* Added value of vessel wall magnetic resonance imaging for differentiation of nonocclusive intracranial vasculopathies. Stroke 2017; 48:3026–3033.
16. Obusez EC, Hui F, Hajj-Ali RA, *et al.* High-resolution MRI vessel wall imaging: spatial and temporal patterns of reversible cerebral vasoconstriction syndrome and central nervous system vasculitis. AJNR Am J Neuroradiol 2014; 35:1527–1532.
17. Swartz RH, Bhuta SS, Farb RI, *et al.* Intracranial arterial wall imaging using high-resolution 3-Tesla contrast-enhanced MRI. Neurology 2009; 72: 627–634.

18. Jung SC, Kang DW, Turan TN. Vessel and vessel wall imaging. Front Neurol Neurosci 2016; 40:109–123.

19. ■ Destrebecq V, Sadeghi N, Lubicz B, *et al.* Intracranial vessel wall MRI in cryptogenic stroke and intracranial vasculitis. J Stroke Cerebrovasc Dis 2020; 29:104684.

The article highlights the vessel wall imaging (VWI) enables detection of the intracranial vessel in central nervous system vasculitis responsible for brain ischemic lesions.

20. Pfefferkorn T, Linn J, Habs M, *et al.* Black blood MRI in suspected large artery primary angiitis of the central nervous system. J Neuroimaging 2013; 23:379–383.

21. Zeiler SR, Qiao Y, Pardo CA, *et al.* Vessel wall MRI for targeting biopsies of intracranial vasculitis. AJNR Am J Neuroradiol 2018; 39:2034–2036.

22. Salvarani C, Cantini F, Hunder GG. Polymyalgia rheumatica and giant-cell arteritis. Lancet 2008; 372:234–245.

23. Klink T, Geiger J, Both M, *et al.* Giant cell arteritis: diagnostic accuracy of MR imaging of superficial cranial arteries in initial diagnosis-results from a multicenter trial. Radiology 2014; 273:844–852.

24. ■■ Poillon G, Collin A, Benhamou Y, *et al.* Increased diagnostic accuracy of giant cell arteritis using three-dimensional fat-saturated contrast-enhanced vessel-wall magnetic resonance imaging at 3 T. Eur Radiol 2020; 30:1866–1875.

This is the first study that compares the diagnostic accuracy of three-dimensional versus two-dimensional contrast-enhanced VWI in the diagnosis of giant cell arteritis.

25. Saver JL. Cryptogenic stroke. N Engl J Med 2016; 375:e26.

26. Qureshi AI, Caplan LR. Intracranial atherosclerosis. Lancet 2014; 383:984–998.

27. Suri MF, Qiao Y, Ma X, *et al.* Prevalence of intracranial atherosclerotic stenosis using high-resolution magnetic resonance angiography in the general population: the atherosclerosis risk in communities study. Stroke 2016; 47:1187–1193.

28. Wu F, Ma Q, Song H, *et al.* Differential features of culprit intracranial atherosclerotic lesions: a whole-brain vessel wall imaging study in patients with acute ischemic stroke. J Am Heart Assoc 2018; 7:e009705.

29. ■ Schaafsma JD, Rawal S, Coutinho JM, *et al.* Diagnostic impact of intracranial vessel wall MRI in 205 patients with ischemic stroke or TIA. AJNR Am J Neuroradiol 2019; 40:1701–1706.

The study presented impact of VWI on the etiologic classification of ischemic stroke. Thus, VWI can improve therapeutic decision in the stroke patients.

30. Dieleman N, Yang W, Abrigo JM, *et al.* Magnetic resonance imaging of plaque morphology, burden, and distribution in patients with symptomatic middle cerebral artery stenosis. Stroke 2016; 47:1797–1802.

31. Gupta A, Baradaran H, Al-Dasuqi K, *et al.* Gadolinium enhancement in intracranial atherosclerotic plaque and ischemic stroke: a systematic review and meta-analysis. J Am Heart Assoc 2016; 5:e003816.

32. ■ Song X, Zhao X, Liebeskind DS, *et al.* Incremental value of plaque enhancement in predicting stroke recurrence in symptomatic intracranial atherosclerosis. Neuroradiology 2020; 62:1123–1131.

The study highlights that plaque enhancement not only correlates with recent ischemic stroke but is also associate with stroke recurrence.

33. Qiao Y, Zeiler SR, Mirbagheri S, *et al.* Intracranial plaque enhancement in patients with cerebrovascular events on high-spatial-resolution MR images. Radiology 2014; 271:534–542.

34. Power S, Matouk C, Casaubon LK, *et al.* Vessel wall magnetic resonance imaging in acute ischemic stroke: effects of embolism and mechanical thrombectomy on the arterial wall. Stroke 2014; 45:2330–2334.

35. Debette S, Compter A, Labeyrie MA, *et al.* Epidemiology, pathophysiology, diagnosis, and management of intracranial artery dissection. Lancet Neurol 2015; 14:640–654.

36. Metso TM, Metso AJ, Helenius J, *et al.* Prognosis and safety of anticoagulation in intracranial artery dissections in adults. Stroke 2007; 38:1837–1842.

37. Wang Y, Lou X, Li Y, *et al.* Imaging investigation of intracranial arterial dissecting aneurysms by using 3 T high-resolution MRI and DSA: from the interventional neuroradiologists' view. Acta Neurochir 2014; 156:515–525.

38. Calabrese LH, Dodick DW, Schwedt TJ, Singhal AB. Narrative review: reversible cerebral vasoconstriction syndromes. Ann Intern Med 2007; 146:34–44.

39. Wang M, Yang Y, Zhou F, *et al.* The contrast enhancement of intracranial arterial wall on high-resolution MRI and its clinical relevance in patients with Moyamoya vasculopathy. Sci Rep 2017; 7:44264.

40. ■ Kathuveetil A, Sylaja PN, Senthilvelan S, *et al.* Vessel wall thickening and enhancement in high-resolution intracranial vessel wall imaging: a predictor of future ischemic events in Moyamoya disease. AJNR Am J Neuroradiol 2020; 41:100–105.

The study presented that VWI findings such as wall thickening and enhancement may predict future ischemic events in patients with Moyamoya disease.

41. Hsu CC, Suthiphosuwan S, Huynh T, *et al.* High-resolution MRI vessel wall imaging in acute aneurysmal subarachnoid hemorrhage: spatiotemporal pattern and clinicoradiologic implications. Clin Neuroradiol 2019. [Online ahead of print]

42. Edjlali M, Guedon A, Ben Hassen W, *et al.* Circumferential thick enhancement at vessel wall MRI has high specificity for intracranial aneurysm instability. Radiology 2018; 289:181–187.

43. Larsen N, Fluh C, Saalfeld S, *et al.* Multimodal validation of focal enhancement in intracranial aneurysms as a surrogate marker for aneurysm instability. Neuroradiology 2020. [Online ahead of print]

44. Texakalidis P, Hilditch CA, Lehman V, *et al.* Vessel wall imaging of intracranial aneurysms: systematic review and meta-analysis. World Neurosurg 2018; 117:453–458.e1.

Coronavirus 2019: clinical and neuropathological aspects

David S. Younger

Purpose of review
To understand the role of postinfectious autoimmune vascular inflammation in the pathogenesis of coronavirus disease 2019-related neurological illness caused by the novel severe acute respiratory syndrome coronavirus 2 virus and its effects on the brain in children and adults.

Recent findings
There are a very small number of postmortem neuropathological series of coronavirus disease 2019-related cerebrovascular and parenchymal disease. However, they fall into at least three major categories, with the majority manifesting those of terminal hypoxia, and others demonstrating inflammatory vascular leptomeningeal, cerebral and brainstem interstitial changes suspicious for encephalitis in a minority of cases. It remains uncertain whether these histopathological features have a relationship to post-infectious inflammatory immune mechanisms and microscopic vasculitis in adults as it appears to be in affected children with multisystem inflammatory syndrome.

Summary
The reasons for this dichotomy are unclear but may related to inherent and epigenetic factors that remain poorly understood. Treatment addressing postinfectious mechanisms of pulmonary, systemic, and nervous system injury may avert early mortality.

Keywords
autoimmunity, coronavirus disease 2019, neurological disease, pediatric multisystem inflammatory syndrome, severe acute respiratory syndrome coronavirus 2, vasculitis

INTRODUCTION

The earliest reports of clusters of patients with pneumonia of unknown origin linked to exposure at a seafood and wet animal market in Wuhan (Hubei Province, China) [1▪] were rapidly identified as a new beta coronavirus named severe or novel acute respiratory syndrome-coronavirus-2 (SARS-nCoV-2 or SARS-CoV-2). These single-stranded RNA enveloped viruses have the largest known RNA genome, ranging from 26.2 to 31.7 kilobases that encodes an important spike glycoprotein that mediates viral entry and determines the range of potential host-cell tropism and disease pathogenesis, hence it has been a major source of vaccine interest [2]. Six coronavirus species cause human disease [3] types widely prevalent in the population that are associated with the common cold symptoms and two others, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), the causal agent of the SARS outbreaks in 2002 and 2003 of Guangdong Province, China [4], and the Middle East Respiratory Syndrome or Middle East respiratory syndrome coronavirus (MERS-CoV), responsible for outbreaks in 2012 [5] are zoonotic beta coronaviruses and linked to fatal illness [6]. SARS-CoV-1 and SARS-CoV-2 use angiotensin-converting enzyme 2 (ACE 2) receptor binding site to infect ciliated bronchial epithelial cells and type II pneumocystis, which explains the affinity of pulmonary involvement.

EPIDEMIOLOGY

With five of seven human coronavirus isolated in this century, coronaviruses have assumed an important place in 21st century [7]. SARS-CoV-2 also originated in bats and reached humans via badgers, Himalayan palm civets and raccoon dogs, showing a similar capacity to infect humans, first by jumping

City University of New York, Medical School, Neuroscience Division, New York, New York, USA

Correspondence to David S. Younger, MD, DrPH, MPH, MS, 333 East 34th Street, 1J, New York, NY 10016, USA. E-mail: youngd01@nyu.edu; website: http://www.davidsyounger.com

Curr Opin Rheumatol 2021, 33:49–57
DOI:10.1097/BOR.0000000000000769

KEY POINTS

- Novel coronaviruses are likely continue to proliferate.
- With them comes the foreseeable risk of rising fatality and neurological complications.
- Adults with severe acute respiratory syndrome coronavirus 1 and the coronavirus disease 2 have shown CNS inflammatory vasculopathy but not frank vasculitis.
- Children with multisystem inflammatory syndrome appear to be a heightened risk for Kawasaki disease.
- Immunotherapy aims at modulating or preventing a postinfectious autoimmune inflammatory response.
- Long-awaited vaccination for severe acute respiratory syndrome-coronavirus-2 is underway in different platforms but may pose uncertain risks in healthy recipients and others with asymptomatic infection.

across species from bat reservoirs. A decade later, MERS-CoV originated in bats utilizing camels as intermediate hosts to human. A zoonotic origin of SARS-CoV-2 was confirmed with viral isolation from reservoirs in bats that infected, as intermediate hosts, the Malayan pangolin and other wildlife used for food in China [8,9]. All three outbreaks confirm the high infectivity and lethality of the coronaviruses and the serious public health threat they pose.

There are animal models that convincingly demonstrate the capacity of coronaviruses to enter the central nervous system (CNS) across the blood brain barrier (BBB). Older immunodeficient BALB/c mice exhibit a clinical syndrome, with increasing age as a risk factor [10]. Transgenic K18-hACE2 mice infected with SARS-CoV [11] invoke infiltration of macrophages and lymphocytes to the lungs and a local release of proinflammatory cytokines reminiscent of the cytokine storm postulated in SARS-CoV-2.

An analysis of 425 initial cases of coronavirus (2019-CoV)–infected pneumonia of the Wuhan, Hubei Province of China, from December 2019 to January 2020 lends understanding of the associated epidemiology [12]. Early laboratory confirmed cases of were identified through surveillance of pneumonia of unknown cause with fever ($\geq$38 °C), radiographic evidence of pneumonia, low or normal white-cell count or low lymphocyte count, and no symptomatic improvement after antimicrobial treatment for 3–5 days after standard therapy; and substantiated by WHO laboratory assays [13] that extracted 2019-CoV RNA by real-time PCR (RT-PCR) using specific primers and probes in upper and lower respiratory tract specimens. Epidemic curves showed an exponential growth rate of 0.10/day [95% confidence interval (CI), 0.050–0.16] with a doubling time of 7.4 days (95% CI, 4.2–14) and a reproductive rate (R_0) of 2.2 (95% CI, 1.4–3.9), meaning that on average, each patient is spread infection to 2.2 others. The goal of control measures is now to reduce the reproductive number to less than 1 to prevent exponential growth by interrupting human-to-human transmission in small communities through quarantining, careful infection control; tracing, testing and isolation of affected contacts, and use of social distancing and facial masks in the general population.

SUSCEPTIBILITY TO INFECTION

Adults

All individuals are generally susceptible to coronavirus disease 2019 (Covid-19). A convenience sampling of Chinese individuals returning to work from Covid-19 [14] identified females, the elderly, residents with chronic diseases, and children as perceived higher risk and in need of special attention in healthcare management. In small case series, the clinical characteristics of pregnant women with confirmed Covid-19 infection are similar to nonpregnant adult but may be more susceptible to infection versus the general population [15] and should have greater health counseling, screening, and follow-ups to ensure maternal and fetal safety. The risk of severe infection and mortality increases with age, and mortality heightens by comorbid cardiovascular disease, hypertension, diabetes, pulmonary disease, and cancer.

A multicenter retrospective Cox-proportional-hazards regression analysis of 147 critically ill Chinese patients with Covid-19 [16] revealed that age older than 65 years, thrombocytopenia at ICU admission, acute respiratory distress syndrome (ARDS), and acute kidney injury independently predicted higher 60-day mortality. Epidemiological data reflect lower susceptibility among children compared with adults, and milder severity of disease compared to adults however, the large proportion of asymptomatic children makes epidemic surveillance more difficult.

Children

The susceptibility of children to SARS-CoV-2 infection and development of Covid-19 illness is uncertain however; one particularly severe affliction has been noted. Incident cases of fever and mucocutaneous manifestations resembling Kawasaki disease [17], a rare vasculitis of childhood that causes coronary-artery aneurysms, emerged in Europe [18▪▪] during school closures after UK pediatricians alerted

the National Health Service to an unusual inflammatory illness. Two contemporaneous reports in the New England Journal of Medicine describe the epidemiology and clinical features of the US disorder [19▪▪,20]. With approximately 1000 cases of so-called multisystem inflammatory disorder in children (MIS-C) worldwide, the incidence is considerably lower than SARS-CoV-2 for individuals less than 21 years of age. Epidemic curves of laboratory-confirmed SARS-CoV-2 infection among persons less than 21 years of age in New York State show a peak in the number of MIS-C cases that follows the peak in the number of laboratory-confirmed SARS-CoV-2 infections by 31 days (from March to 10 May 2020), with an incidence of 322 in 100 000 persons compared with two per 100 000 cases of MIS-C for the same age of less than 21 years [19▪▪]. Among children with laboratory confirmed MIS-C manifesting prototypical febrile hyperinflammatory syndrome of dermatologic, mucocutaneous, gastrointestinal manifestations and cardiac dysfunction, 48% of patients 0–5 years of age, 43% of patients 6–12 years of age, and 12% of those 13–20 years of age present with typical or suggestive of Kawasaki disease (KD). In view of the overlapping clinical features and the lack of a diagnostic test for either KD or MIS-C, attributing a causal relationship remains enigmatic for several reasons. First, the epidemiology of the two disorders does not follow the same trend in all cases. While KD has been virtually identical in all countries in the world for the past 50 years or more, with 80% of cases occurring in children <5 years of age and with a peak incidence at ~10 months of age, MIS-C typically affects older children. Second, although SARS-CoV-2 in not a definite cause of MIS-C, the appearance of MIS-C during outbreaks of COVID-19 in Europe and the US, although not Asia, is highly suggestive. Third, although children with MIS-C may display some of the clinical features of KD such as fever, dilation of conjunctival blood vessels, rash and redness of the oropharynx, they are not specific for any one diagnosis and can be observed in other childhood infectious diseases. Yet, while the etiology of KD remains largely elusive, there is mounting interest in identifying infectious agents that trigger the cascade that causes the observed cytokine storm with high serum IL-6 levels, coronary aneurysms, and necessary inotropic support to maintain cardiac output and avert shock. The question therefore remains whether MIS-C and KD are the same entity and if SARS-CoV-2 viral infection is one trigger among many for KD. There is a trial recruiting for the collection of clinical data and tissue samples to characterize MIS-C and its relationship to KD (ClinicalTrials.gov Identifier: NCT04538495).

NEUROLOGICAL MANIFESTATIONS AND COMPLICATIONS

The neurologic manifestations of SARS-CoV-2 infection results from a variety of mechanisms including virus-induced hyperinflammatory and hypercoagulable states, and direct virus infection of neurons. There is still a paucity of rigorous case observations of acute, subacute and chronic clinical and laboratory neurological involvement in SARS-CoV-2 infection. Three large case series totaling 425 hospitalized patients illustrate the current state of knowledge including 214 retrospectively studied cases from January to February 2020 in China's Wuhan Provence [21]; 58 prospectively studied Covid-19 cases between March and April 2020 in France [22]; and 153 cases surveyed during April 2020 in the United Kingdom [23]. The retrospective, observational study of Wuhan cases was carried out at three centers early in the pandemic [21] noting 36.4% CNS manifestations among them dizziness (16.8%) and headache (13.1%); and 8.9% overall peripheral nervous system manifestations affecting taste (5.6%) and smell (5.1%). There was no mention of brain neuroimaging, lumbar cerebrospinal fluid (CSF) analysis, neuromuscular biopsy findings, or the prognostic contribution to mortality of any particular neurological syndrome.

Among the 58 consecutive French patients seen somewhat later in the pandemic at one hospital with Covid-19-related ARDS [22], investigators noted neurological manifestations in 84%, including encephalopathy, prominent agitation, confusion and corticospinal tract signs each in two-thirds of cases. Two of 13 patients who underwent brain MRI had single acute ischemic strokes, and 13 showed perfusion abnormalities with leptomeningeal involvement in two-thirds. Nonspecific findings seen on electroencephalography suggested encephalopathy. Examination of CSF samples obtained from seven patients showed no cells; two patients had oligoclonal bands identical to electrophoretic serum patterns; and all were negative or SARS-CoV-2 by RT-PCR assay.

Among 153 UK patients enrolled in a surveillance study of the acute neurological and psychiatric complications of Covid-19 in the month of April 2020 [23], two-thirds (62%) of patients overall presented with a cerebrovascular event including stroke (74%), intracerebral hemorrhage (12%), and CNS vasculitis (1%); and a third of patients presented with mental status changes that included encephalopathy (23%) and encephalitis (18%), and the remainder (59%) suggested new presentation of psychosis, dementia, and affective disorder. Altered mental status was the second most common neurological manifestation, affecting patients both older

and younger than age 60 years, while the commonest neurological presentation, that of acute cerebrovascular events, preferentially affected older individuals more often than younger counterparts (82 versus 18%).

CLINICOPATHOLOGICAL CORRELATION

Severe acute respiratory syndrome coronavirus 1

Cases of the 2002 SARS-CoV-1 epidemic have shown neurological manifestations including seizures and encephalitis [24,25]. Complementing these reports, among four patients who died suddenly of dissecting aneurysms, ectopic pregnancy, and cerebral hemorrhage [26] there was positive staining by murine mAbs specific for SARS-CoV-1 nucleoprotein and probes specific for a SARS-CoV-1 RNA polymerase gene fragment for immunohistochemistry. In-situ hybridization of the cerebrum at postmortem included localized perivasculitis of cerebral veins.

Middle East respiratory syndrome coronavirus

There are no published data regarding human postmortem neuropathological findings of MERS-CoV yet the disorder is still a relevant threat for populations in the Middle East with high lethality (close to 35%) [27]. However, three reported living patients manifested initial fever followed by coma, ataxia, focal motor deficits, and peripheral nerve symptoms [28▪▪] and four of 23 other patients treated at a single hospital reported delayed neurological symptoms up to 3 weeks consistent with concomitant Bickerstaff's encephalitis overlapping with Guillain–Barré syndrome, ICU-acquired weakness, and toxic or infectious neuropathies [29▪▪].

Severe acute respiratory syndrome-coronavirus-2

The postmortem findings of Covid-19 illness have been described in 391 patients succumbing to Covid-19 illness [30▪▪–32▪▪,33,34▪▪,35▪▪,36–50,41], which is miniscule in relation to the number of confirmed cases and reported deaths in the United States and worldwide. Nevertheless, they are both very revealing and important in understanding the likely pathogenic mechanisms associated with SARS-CoV-2 infection. What was initially thought to be a self-limited disease almost exclusively involving the lungs now is being recognized as one that involves multiple organ systems including the brain with the unique capacity for both invasive and post-infectious dysimmune phases that evolve in an overlapping fashion over a relatively short period.

Younger (51) recently summarized the neuropathological findings of Covid-19 illness in the first 50 cases with detailed brain findings [30▪▪–32▪▪, 33,34▪▪,35▪▪]. Older age, male gender, increased serum cytokine and pro-coagulation markers, and critical care hospitalization for $\leq$10 days prior to death characterized the cohort [49]. Serum cytokine and procoagulant were consistently elevated in those so studied. The vast majority of patients were critically ill and managed in an intensive care unit (ICU) where the immediate causes of death was generally ascribed to cardiopulmonary failure. SARS-CoV-2 staining in brain tissue by polymerase chain reaction was negative in all cases [31▪▪,32▪▪,34▪▪,35▪▪], while a third of cases (36%) (30-34) showed focal or diffuse cortical and brain leptomeningeal or interstitial inflammation, characterized mainly as T-cell-mediated based upon flow cytometry. Six patients [32▪▪] between the ages of 58 and 82 years, who presented with somnolence (in 3 with an average Glasgow Coma Scale [GCS] of 11.3) or no neurological symptoms and a normal GCS of 15), (in the other 3) without preponderant comorbidities, showed histopathological features of encephalitis. These included localized perivascular and interstitial infiltrates with neuronal cell loss and axonal degeneration involving brainstem nuclei and tracts without territorial infarctions, or evidence of virus infiltration. Sparse T-cell infiltrates with clusters of macrophages and axonal injury tr)acking along vessels resembling acute disseminated encephalomyelitis (ADEM) were noted in two other cases, including one with neuronophagia and microglial nodules [34▪▪], and another with expression of angiotensin converting enzyme (ACE)2 receptor along capillary endothelia cells [31▪▪].

Younger's analysis [51] of critically ill Covid-19 cases reveal several important findings and implications. First, hypoxia-ischemia evident does not account for all relevant neuropathological features of severe Covid-19. Second, patients presenting with elevated levels of circulating interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-a, suggests activation of innate and adaptive immunity indicative of a cytokine storm. Together with increased serum D-dimer and markers of hypercoagulability in 42% of cases, affected patients are at risk for thrombotic and hemorrhagic parenchymal tissue infarction so noted in nine (18%) of cases. Third, the findings of 16% of cases with ADEM-like features or indolent brainstem encephalitis suggests the need for a high index of suspicion in patients presenting with altered sensorium, early brainstem signs including those with fluctuating vital signs and early ventilator dependence.

There were several limitations of this small cohort analysis of literature cases. First, case series were often small and unselected often with missing demographic data and causes of death. Second, there were often contradictory conclusions about the significance of inflammatory vascular brain changes; moreover, there were all critically ill patients and there were no comparisons to control patients with sepsis. Third, it was uncertain whether negative in-situ SARS-CoV-2 RNA PCR results in those so studied makes a secondary inflammatory immune mechanisms of injury more likely.

An updated clinicopathologic analysis of 141 Covid-19 cases shown in Table 1 that comprised 91 additional cases, including 31 cases [39,40] excluded from the series of Younger [51] for lack of description neuropathology, and 60 histopathologically documented cases [36–38] show four notable findings.

The first was the increased number of positive SARS-CoV-2 genome by PCR testing, accounting for 13 (48%) of 27 examined brains in the study by Matschke and colleagues [36]; in 4 (80%) of 5 brain tissue specimens studied by Hanley and coworkers [37]; and in 8 (38%) of 21 brain specimens examined by Puelles and coinvestigators [40]. Remarkably, SARS-CoV-2 presence did not correlate with the severity of neuropathological findings [36]. It remains unclear whether a comparably low viral genome levels detectable by qRT-PCR in brain tissue could be blood-derived.

A second finding was the increase in leptomeningeal and interstitial brainstem inflammation characterized as cytotoxic T-cells in 34 (79%) cases according to Matschke and colleagues [36], coinciding with the localization of SARS-CoV-2 viral proteins in cranial nerves and interstitial areas of the lower brainstem. The detection of SARS-CoV-2 RNA specifically in olfactory bulb neurons and glial cells in 4 (57%) of 7 patients in another study cohort [38], but not in any other brain regions, lends support to a route of viral entry via the olfactory system and the importance of anosmia as an early clinical sign of Covid-19. While activated microglia localize to the olfactory bulb and medulla oblongata in Covid-19 brain tissues suggesting a point of viral entry, similar findings noted in control brains of patients who deceased under septic condition [38]. Considering the capability of SARS-CoV-2 to infect human gastrointestinal enterocytes as well as pneumocytes, it bears consideration whether the vagus nerve derived from the medulla could be another route of entry to the brain.

A third finding was the detection of microglial activation and sparse perivascular and leptomeningeal T-cell infiltrates in Covid-19 brains, as well as in controls with sepsis or systemic inflammation in a small series [38] suggesting a histopathological correlate of critical illness-related encephalopathy rather than a disease-specific finding.

Fourth, Matschke and colleagues [36] were interested in the neuronal cell types prone to SARS-CoV-2 infection, thereby screening gene expression datasets for signatures related to viral entry and persistence. The authors noted high expression of ACE2 in oligodendrocytes, and increased expression of transmembrane serine proteases 2 and 4 (TMPRSS2 and TMPRSS4) in neurons that respectively encode proteins implicated in host viral entry (ACE2) and pruning of the viral-decorating spikes (TMPRSS2).

There are several implications of these findings in regards the immunoinflammatory and neurotoxic response of SARS-CoV-2 to neurons that has recently been captured in a study by Ramani and coworkers [52▪▪] who employed a brain organoid model to examine whether SARS-CoV-2 directly targets neurons and can lead to productive infection and neurotoxicity. Cells from mock organoids displayed a healthy nucleus that is labelled with 4′,6-diamidino-2-phenylindole (DAPI) compared to SARS-CoV-2 exposed organoids that display increased terminal deoxynucleotidyl transferase dUTP nick end labeling) staining (TUNEL) which detects the DNA breaks formed when DNA fragmentation occurs in the last phase of apoptosis. While most of the SARS-CoV-2-positive cells are TUNEL-positive, some were caspase-positive displaying pT231 Tau localization at the cell soma not observed in mock organoids. pT231-tau is highly neurotoxic and acts as an early driver of tauopathy in neurodegenerative diseases such as Alzheimer disease. This model offers insight into the fore mentioned findings in the most recent Covid-19 series. For example, if neurons are indeed a target for SARS-CoV-2, a basal (low) level of ACE2 expression may be sufficient for viral entry into neurons. This could explain why SARS-CoV-2 has a broad spectrum of target organs and cell types as suggested by Puelles and colleagues [40]. In so much as Tau abnormalities in SARS-CoV-2 positive neurons could result from infection, it could also result from triggering of a cascade of downstream effects that results in immune-inflammation, neuronal stress, and direct neurotoxicity, all of which warrant future investigations. Moreover, as organoids are an experimentally tractable human in vitro system and convenient to culture as well as infection, organoid models can serve as a test-bed for anti-SARS-CoV-2 therapeutic agents.

Immunotherapy

The Covid-19 pandemic is proving to be associated with high-case fatalities in both children and adults

Table 1. Updated clinical and neuropathologic findings of 141 Covid-19 fatalities

Observation	Number of cases	Reference
Sex		
Male	72	[28▪▪–32▪▪,33,34▪▪,36,38]
Female	31	[28▪▪,30▪▪,31▪▪,33,34▪▪,36]
Age		
<21	0	
1–49	1	[33]
50–64	17	[30▪▪,33,34▪▪,36]
>65	66	[29▪▪,30▪▪,32▪▪,33,34▪▪,36,38]
Serum cytokine and procoagulant levels		
Elevated	21	[28▪▪,29▪▪]
Normal	0	
Duration of hospital illness to death (days)		
0–1	5	[31▪▪,32▪▪,33]
1–10	18	[29▪▪–31▪▪,33]
>10	14	[30▪▪,31▪▪,33,36]
Place of death		
Hospital	86	[28▪▪–32▪▪,33,34▪▪,36]
Nursing Home	6	[34▪▪]
Home	5	[34▪▪]
Cause of death		
Massive intracranial hemorrhage	3	[30▪▪,32▪▪]
Pulmonary embolism	2	[30▪▪,33]
Cardiopulmonary failure	50	[30▪▪,33,34▪▪,36]
Multisystem organ failure	6	[29▪▪,34▪▪,38]
SARS-CoV-2 RNA reactivity in brain sections		
Positive	24	[34▪▪,35▪▪,38]
Negative	61	[28▪▪,31▪▪,34▪▪,36,38]
Neuropathology		
Acute microscopic ischemic infarcts	10	[28▪▪,34▪▪]
Acute microscopic hemorrhagic infarcts	3	[28▪▪,29▪▪,35▪▪]
Petechial hemorrhage	3	[30▪▪]
Focal perivascular parenchymal T-cell infiltrates	8	[28▪▪,29▪▪,32▪▪,35▪▪]
Diffuse perivascular parenchymal T-cell infiltrates	2	[29▪▪,32▪▪]
Leptomeningeal inflammation	41	[30▪▪,31▪▪,34▪▪]
Interstitial brainstem inflammation	10	[30▪▪,34▪▪]
Capillary endothelium expression of ACE2 receptor	1	[29▪▪]
Microglial nodules	1	[32▪▪]
Hypoxic ischemia changes and neuronal loss	27	[28▪▪,31▪▪,32▪▪,33,36]
No abnormalities	3	[36,37]
Associated findings:		
Chronic infarction	8	[31▪▪,34▪▪]
Alzheimer disease	5	[30▪▪,31▪▪]
Lewy body disease	3	[31▪▪,36]
Primary brain tumor	1	[31▪▪]
Multiple sclerosis	1	[36]
Metastatic cancer	1	[34▪▪]

due to a dysregulated, postinfectious autoimmunity response, analogous to the cytokine storm of severe viral influenza illness [53]. Recognizing the importance of a given patient's immune response to the SARS-CoV-2 exposure, patients have been recruited to participate in studies to examine B-cell and T-cell repertoire and immune responses during the acute and resolved phases of Covid-19 infection at home and in the hospital (ClinicalTrials.gov Identifier: NCT04362865). Four immunotherapeutic approaches are being used to stem the Covid-19 pandemic by targeting the immune system, in keeping with the multiplier effect of infection, immunity, and inflammation known as I-Cubed (I^3) [54]. The oral antimalarial drug hydroxychloroquine was the first highly publicized agent recognized for its immune-mediated mechanisms of chemotaxis, phagocytosis and superoxide production by neutrophils to inhibit SARS *in vitro*. It was administered in an open-label nonrandomized clinical trial of 20 adults and minors with severe Covid-19 illness with improvement, and later made widely available as prophylaxis [55]. An observational study of 1446 hospitalized adult patients at a New York City hospital with Covid-19 illness did not show a significant association between hydroxychloroquine use and intubation or death (hazard ratio, 1.04, 95% CI, 0.82–1.32), with similar findings in multiple sensitivity analyses [56].

The biological agent remdesivir, a nucleotide analogue prodrug that inhibits viral RNA polymerases is showing the greatest promise in reducing fatality due to its vitro activity against SARS-CoV-2 by inhibiting the activity of RNA-dependent RNA polymerase [57]. A clinical protocol allowing expanded access to remdesivir (ClinicalTrials.gov Identifier: NCT04323761), and several clinical studies have begun recruiting subjects in an randomized, open-label, controlled clinical trials, in collaboration with the WHO (ClinicalTrials.gov Identifier: NCT04330690). A phase III randomized study comparing the safety and efficacy and antiviral activity of two remdesivir regimens with respect to clinical status is recruiting subjects (ClinicalTrials.gov Identifier: NCT04292899).

Convalescent plasma transfusion of SARS-CoV-2-specific IgG and neutralizing antibodies have been administered in uncontrolled case series to critically ill adult patients with Covid-19 with clinical improvement [58]. These preliminary findings suggest a role for transfusion therapy in the treatment of critically ill patients with Covid-19. A pilot prospective study collecting plasma to measure neutralizing antibodies to SARS-CoV-2 in recovered subjects is recruiting subjects (ClinicalTrials.gov Identifier: NCT04344977).

Treatment with 2 g/kg high-dose intravenous immune globulin (IVIg) therapy administered to three adult patients over 4–5 consecutive days in the early stages of clinically apparent SARS-CoV-2 viremia, alone (one patient) or in association with antiviral and antibacterial antibiotics showed clinical stabilization and were uneventfully discharged from the hospital [59]. Early administration of IVIg is first-line therapy in children with KD that appears to be missed or delayed during the Covid-19 pandemic etiologically related to SARS-CoV-2 infection prompted a single-center, randomized, open-label, controlled study in Peking China to evaluate the safety of IVIg in conjunction with standard care for severe 2019-nCov pneumonia has not started recruiting subjects (ClinicalTrials.gov identifier NCT04261426). However, no similarly available studies have been announced in the United States.

An anecdotal prospective analysis of 55 children and adults treated with maintenance (400 mg/kg monthly) and high-dose (2 g/kg) IVIg therapy to treat diverse acquired and postinfectious autoimmune neurological disorders, found no new cases of SARS-CoV-2 stratified by a single home infusion service via phone interviews at the height of the Covid-19 pandemic (when it would have been impermissible for a nurse to enter the home) [60▪▪]. This uncontrolled observation suggests that Ig therapy delivered via intravenous, subcutaneous, or intramuscular routes may yet have an important role in Covid-19 illness prevention among vulnerable individuals. However, a longer period of follow-up of this cohort will be necessary to confirm these observations, as are further controlled studies to identify the dose and frequency of IVIg treatment to confer prophylactic efficacy.

Efforts to develop a safe and efficacious 2019-nCoV vaccine were underway in early 2020. Whole, live-attenuated or inactive whole virus vaccines represent a classic strategy for viral vaccinations similar to the Ebola vaccine platform employing an adenoviral vector. However, live virus vaccines often require extensive additional testing to confirm their safety. This is especially an issue for coronavirus vaccines, given the findings of increased infectivity following immunization with live or killed whole virus SARS coronavirus vaccines. Subunit vaccines for both SARS coronaviruses rely on eliciting an immune response against the spike protein to prevent its docking with the host ACE2 receptor. There are also advanced nucleic acid vaccine platforms for Covid-19. More recently, new modifications and formulations have improved nucleic acid performance in humans, with an expectation that this approach might eventually lead to the first licensed

human nucleic acid vaccine. There are now at least half-dozen candidates, including live viruses, recombinant protein subunits, and nucleic acids that may ultimately offer promise as preventive vaccines. However, each require additional manufacturing steps and formal toxicology testing before submitting a regulatory package to national regulatory agencies to be able to commence the clinical development, proceeding through first with phase 1 clinical trials for safety and immunogenicity, and later, phase 2 and phase 3 trials for both safety and efficacy [61]. However, the induction of protective immunity comes with a possibility of adverse effects. A preponderant emergence of post-vaccination vasculitis [62▪] led to formal guidelines for case definition [63].

CONCLUSION

Given their high prevalence and wide distribution, prominent genetic diversity, genomic recombination, and human–animal interface activities in certain parts of the world, the Covid-19 pandemic and other novel coronaviruses will likely continue to proliferate [64]. This depends upon multiple factors not the least of which is superspreading that occurs when single patients infect a disproportionate number of contacts across continents enhanced by travel [65]. With them comes the foreseeable risk of rising fatality and expected neurological complications. Adults with SARS-CoV-1 and the CoV-2 show inflammatory vasculopathy, encephalitis, and silent infarctions at postmortem examination, with variable SARS-CoV-2 RNA genomes by PCR. Children with MIC are purported to have a clinical syndrome that may resemble KD, however histopathology in life or at postmortem has not been documented in these cases. This has led to innovative treatments aimed at viral eradication and immunotherapy directed at heightened postinfectious inflammatory response termed I-cubed that expresses the multiplier effect of infection, immunity and inflammation in the context of genetics and other environmental exposures.

Acknowledgements

Sachiko Maharjan, Clinical Coordinator; assisted in preparation of the manuscript.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

1. Zhu N, Zhang D, Wang W, *et al.* A novel coronavirus from patients with ▪ pneumonia in China, 2019. N Engl J Med 2020; 382:727–733.
An early article of the pandemic virus in China.
2. Qiang XL, Xu P, Fang G, *et al.* Using the spike protein feature to predict infection risk and monitor the evolutionary dynamic of coronavirus. Infect Dis Poverty 2020; 9:33.
3. Su S, Wong G, Shi W, *et al.* Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016; 24:490–502.
4. Zhong NS, Zheng BJ, Li YM, *et al.* Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Re**public of China, in February 2003. Lancet 2003; 362:1353–1358.
5. Zaki AM, van Boheemen S, Bestebroer TM, *et al.* Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367:1814–1820.
6. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17:181–192.
7. Bulut C, Kato Y. Epidemiology of COVID-19. Turk J Med Sci 2020; 50(SI-1):563–570.
8. Lam TT, Jia N, Zhang YW, *et al.* Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature 2020; 583:282–285.
9. Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr Biol 2020; 30:1–6.
10. Subbarao K, Roberts A. Is there an ideal animal model for SARS? Trends Microbiol 2006; 14:299–303.
11. McCray PB, Pewe L, Wohlford-Lenane C, *et al.* Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol 2007; 81:813–821.
12. Li Q, Guan X, Wu P, *et al.* Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020; 382: 1199–1207.
13. He S, Chen S, Kong L, Liu W. Analysis of risk perceptions and related factors concerning COVID-19 epidemic in Chongqing, China. J Community Health 2020; 1–8. [Online ahead of print]
14. Qiao J. What are the risks of COVID-19 infection in pregnant women? Lancet 2020; 395:760–762.
15. Xu J, Yang X, Yang L, *et al.* Clinical course and predictors of 60-day mortality in 239 critically ill patients with COVID-19: a multicenter retrospective study from Wuhan, China. Crit Care 2020; 24:394.
16. Baker AL, Lu M, Minich LL, *et al.* Associated symptoms in the ten days before diagnosis of Kawasaki disease. J Pediatr 2009; 154:592–595.e2.
17. Whittaker E, Bamford A, Kenny J, *et al.* Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA 2020; e2010369. [Online ahead of print]
18. Dufort EM, Koumans EH, Chow EJ, *et al.* Multisystem inflammatory syndrome ▪▪ in children in New York state. N Engl J Med 2020; 383:347–358.
An important article documenting this unusual vasculitic syndrome in children due to postinfectious autoimmunity.
19. Feldstein LR, Rose EB, Horwitz SM, *et al.* Multisystem inflammatory syndrome ▪▪ in U.S. children and adolescents. N Engl J Med 2020; 383:334–346.
An important article documenting this unusual vasculitic syndrome in children due to postinfectious autoimmunity.
20. Mao L, Jin H, Wang M, *et al.* Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020; 77:1–9.
21. Helms J, Kremer S, Merdji H, *et al.* Neurologic features in severe SARS-CoV-2 infection. N Engl J Med 2020; 382:2268–2270.
22. Varatharaj A, Thomas N, Ellul MA, *et al.* Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. Lancet Psychiatry 2020; 7:875–882. [published correction appears in Lancet Psychiatry. 14 July 2020]
23. Hung ECW, Chim SSC, Chan PKS, *et al.* Detection of SARS coronavirus RNA in the cerebrospinal fluid of a patient with severe acute respiratory syndrome. Clin Chem 2003; 49:2107–2108.
24. Lau K, Yu W, Chu C, *et al.* Possible central nervous system infection by SARS coronavirus. Emerg Infect Dis 2004; 10:342–344.
25. Ding Y, He L, Zhang Q, *et al.* Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis virus transmission pathways. J Pathol 2004; 203:622–630.
26. Arabi YM, Harthi A, Hussein J, *et al.* Severe neurologic syndrome associated with Middle East respiratory syndrome corona virus (MERS-CoV). Infection 2015; 43:495–501.
27. Kim JE, Heo JH, Kim HO, *et al.* Neurological complications during treatment of middle east respiratory syndrome. J Clin Neurol 2017; 13:227–233.

28. Bryce C, Grimes Z, Pujadas E, *et al.* Pathophysiology of SARS-Cov-2: ■■ targeting of endothelial cells renders a complex disease with thrombotic microangiopathy and aberrant immune response. The Mount Sinai COVID-19 autopsy experience. Med Rxiv 2020.

The article provides valuable information of Covid-19 neuropathology.

29. Reichard RR, Kashani KB, Boire NA, *et al.* Neuropathology of COVID-19: a ■■ spectrum of vascular and acute disseminated encephalomyelitis (ADEM)-like pathology. Acta Neuropathologica 2020; 140:1–6.

This article provides valuable information of Covid-19 neuropathology.

30. von Weyhern CH, Kaufmann I, Neff F, Kremer M. Early evidence of pro- ■■ nounced brain involvement in fatal COVID-19 outcomes. Lancet 2020; 395:e109.

This article provides valuable information of Covid-19 neuropathology.

31. Solomon IH, Normandin E, Bhattacharyya S, et al. Neuropathological Features ■■ of Covid-19 [published online ahead of print, 2020 Jun 12]. N Engl J Med 2020; NEJMc2019373.

This article provides valuable information of Covid-19 neuropathology.

32. Al-Dalahmah O, Thakur KT, Nordvig AS, *et al.* Neuronophagia and microglial ■■ nodules I a SARS-CoV-2 patient with cerebellar hemorrhage. Acta Neuropathol Commun 2020; 8:147.

This article provides valuable information of Covid-19 neuropathology.

33. Kantonen J, Mahzabin S, Mayranpaa MI, *et al.* Neuropathological features of four autopsied COVID-19 patients. Brain Pathol 2020. doi: 10.1111/bpa.12889.

34. Matschke J, Lütgehetmann M, Hagel C, *et al.* Neuropathology of patients with ■■ COVID-19 in Germany: a post-mortem case series. Lancet Neurol 2020; 19:919–929.

The article provides valuable information of Covid-19 neuropathology.

35. Hanley B, Naresh KN, Roufosse C, *et al.* Histopathological findings and viral ■■ tropism in UK patients with severe fatal COVID-19: a post-mortem study. Lancet Microbe 2020; 1:e245–e253.

The article provides valuable information of Covid-19 neuropathology.

36. Deigendesch N, Sironi L, Kutza M, *et al.* Correlates of critical illness-related encephalopathy predominate postmortem COVID-19 neuropathology. Acta Neuropathol 2020; 140:583–586.

37. Schaller T, Hirschbühl K, Burkhardt K, *et al.* Postmortem Examination of Patients With COVID-19. JAMA 2020; 323:2518–2520.

38. Puelles VG, Lütgehetmann M, Lindenmeyer MT, et al. Multiorgan and Renal Tropism of SARS-CoV-2 [published online ahead of print, 2020 May 13]. N Engl J Med 2020; NEJMc2011400.

39. Ackermann M, Verleden SE, Kuehnel M, *et al.* Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med 2020; 383:120–128.

40. Buja LM, Wolf DA, Zhao B, *et al.* The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. Cardiovasc Pathol 2020; 48:107233.

41. Carsana L, Sonzogni A, Nasr A, *et al.* Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. Lancet Infect Dis 2020. S1473-3099(20)30434-5.

42. Copin MC, Parmentier E, Duburcq T, *et al.* Time to consider histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. Intensive Care Med 2020; 46:1124–1126.

43. Fox SE, Akmatbekov A, Harbert JL, *et al.* Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. Lancet Respir Med 2020; 8:681–686.

44. Lax SF, Skok K, Zechner P, *et al.* Pulmonary arterial thrombosis in COVID-19 with fatal outcome: results from a prospective, single-center, clinicopathologic case series. Ann Intern Med 2020; M20–M2566.

45. Magro C, Mulvey JJ, Berlin D, *et al.* Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res 2020; 220:1–13.

46. Menter T, Haslbauer JD, Nienhold R, *et al.* Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. Histopathology 2020; 77:198–209.

47. Wichmann D, Sperhake JP, Lütgehetmann M, *et al.* Autopsy findings and venous thromboembolism in patients with covid-19: a prospective cohort study. Ann Intern Med 2020; 173:268–277.

48. Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 Autopsies, Oklahoma, USA. Am J Clin Pathol. 2020 May 5;153(6):725-733. Erratum in: Am J Clin Pathol. 2020 May 5;153(6):852.

49. Younger DS. Postmortem Neuropathology in Covid-19. Brain Pathol. 2020 Oct 23:e12915. doi: 10.1111/bpa.12915. Epub ahead of print. PMID: 33098141.

50. Ramani A, Müller L, Ostermann PN, *et al.* SARS-CoV-2 targets neurons of 3D human brain organoids. EMBO J 2020; 39:e106230.

51. Netland J, Meyerholz DK, Moore S, *et al.* Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008; 82:7264–7275.

52. Younger DS. Eleven themes in the history of systemic and nervous system ■■ vasculitides. Neurol Clin 2019; 37:149–170.

A landmark article in the history of vasculitis.

53. Guo XJ, Thomas PG. New fronts emerge in the influenza cytokine storm. Semin Immunopathol 2017; 39:541–550.

54. Younger DS. I-cubed (infection, immunity, and inflammation) and the human microbiome. Neurol Clin 2016; 34:863–874.

55. Biot C, Daher W, Chavain N, *et al.* Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. J Med Chem 2006; 49:2845–2849.

56. Geleris J, Sun Y, Platt J, *et al.* Observational study of hydroxychloroquine in hospitalized patients with Covid-19. N Engl J Med 2020; 382: 2411–2418.

57. Jean SS, Lee PI, Hsueh PR. Treatment options for COVID-19: the reality and challenges. J Microbiol Immunol Infect 2020; 53:436–443.

58. Shen C, Wang Z, Zhao F, *et al.* Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 2020; 323:1582–1589.

59. Cao W, Liu X, Bai T, *et al.* High-dose intravenous immunoglobulin as a therapeutic option for deteriorating patients with coronavirus disease 2019. Open Forum Infect Dis 2020; 7:ofaa102.

60. Younger DS. Post-infectious sequela of SARS-COV-2 infection in adults and ■■ children: an overview of available agents and clinical responsiveness. Arch Neurol Neurol Disord 2020; 3:e102.

An important update in the immunotherapy approach to treat coronavirus disease 2019.

61. Chen WH, Strych U, Hotez PJ, *et al.* The SARS-CoV-2 vaccine pipeline: an overview. Curr Trop Med Rep 2020; 1–4. [Online ahead of print]

62. Younger DS. Cervical and lumbosacral Radiculoplexus neuropathy following ■ influenza vaccination. World J Neurosci 2019; 9:255–161.

An important article demonstrating postvaccination vasculitic illness.

63. Hadden RDM, Collins MP, Zivkovic SA, *et al.* Vasculitic peripheral neuropathy: case definition for collection analysis, and presentation of immunization safety data. Vaccine 2017; 35:1567–1578.

64. Cui J, Li F, Shi ZL. Vasculitic peripheral neuropathy: case definition for collection analysis, and presentation of immunization safety data. Origin and evolution of pathogenic coronaviruses 2019; 17:181–192.

65. Wong G, Liu W, Liu Y, *et al.* MERS, SARS, and ebola: the role of superspreaders in infectious disease. Cell Host Microbe 2015; 18:398–401.

Rheumatology in the era of precision medicine: synovial tissue molecular patterns and treatment response in rheumatoid arthritis

Amit Lakhanpal[a], *Melanie H. Smith*[a], *and Laura T. Donlin*[b,c]

Purpose of review
A critical unmet need in rheumatoid arthritis (RA) is the identification of biomarkers that predict which of the available medications will be most effective for an individual in order to lower disease activity sooner than is afforded by the current treat-to-target approach. Here we will discuss recent reports examining the potential for synovial tissue molecular, cellular, and spatial profiling in defining objective measures of treatment response and therein developing personalized medicine for RA.

Recent findings
Recent high-dimensional molecular profiling of RA synovium has provided unprecedented resolution of the cell types and pathways in tissues affected by rheumatic diseases. Heightened attention to tissue architecture is also emerging as a means to classify individual disease variation that may allow patients to be further stratified by therapeutic response. Although this wealth of data may have already pinpointed promising biomarkers, additional studies, likely including tissue-based functional drug response assays, will be required to demonstrate how the complex tissue environment responds.

Summary
Molecular, cellular, and more recently spatial profiling of the RA synovium are uncovering fundamental features of the disease. Current investigations are examining whether this information will provide meaningful biomarkers for individualized medicine in RA.

Keywords
individualized medicine, molecular profile, precision medicine, rheumatology

INTRODUCTION

Heterogeneity in the manifestation of rheumatic diseases imparts extensive difficulty in diagnosis and treatment. This includes patients with rheumatoid arthritis (RA) who vary clinically in the severity and distribution of affected joints; the presence of autoantibodies; and their response to treatment. Biomarkers for stratifying patients according to treatment responsiveness are of considerable interest. In the absence of such guidance, treatment often involves an iterative approach to find an effective therapy, during which irreversible damage may advance uncontrolled. Given the ease of access to blood samples, many studies have sought to identify circulating biomarkers for better diagnosis and prognostication (Table 1). However, even with complex multipanel approaches, there has not been a finding of sufficient magnitude or reliability to augment routine clinical practice. Further, although susceptibility genes for RA have been established, we are only beginning to understand the many effects of genetic variation on treatment response (Table 2).

Here, we will discuss recent studies aimed at testing whether certain molecular and cellular patterns in RA joint tissue might predict treatment response, and therein could be used for individualized medicine strategies. We will also present studies suggesting that further experimental assays are needed to define how

[a]Division of Rheumatology, Department of Medicine, [b]Arthritis and Tissue Degeneration Program and the David Z. Rosensweig Genomics Research Center, Hospital for Special Surgery and [c]Weill Cornell Medical College and Graduate School, New York, New York, USA

Correspondence to Laura T. Donlin, 515 E 71st St, 4th floor, New York, NY 10021, USA. Tel: +1 212 774 2743; e-mail: donlinl@hss.edu

Curr Opin Rheumatol 2021, 33:58–63
DOI:10.1097/BOR.0000000000000767

KEY POINTS

- Efficacy of RA treatment is limited by our inability to predict who will respond to which therapeutic.
- Although many potential serum biomarkers and response-associated SNPs have been identified, none have been shown to be robust predictors of therapeutic response.
- Characterization of synovial tissue samples may elucidate differences in underlying abnormalities that will allow us to individualize therapy and advance the possibility of precision medicine in RA.
- Functional assays using perturbation of synovial or blood-derived cells may further elucidate dominant pathologic pathways that are effective therapeutic targets across RA patient tissues or for a subset of individuals.
- Prediction of therapeutic responses may require integration of multiple sources of data: genetic, epigenetic, histologic, and synovial gene expression.

identifiable patient-specific tissue patterns impact response to various medications.

PIVOTING BIOMARKER FOCUS TOWARDS THE JOINT

Although RA has extra-articular manifestations, such as cardiovascular and interstitial lung disease [21,22], and findings suggest loss of self-tolerance may initiate at mucosal surfaces [23], the primary target tissue is the synovium. In light of this understanding, pathologic features within the synovium may hold the key to identifying biomarkers that clinicians can use to predict treatment response. Accordingly, considerable recent emphasis has been placed on high-dimensional molecular profiling of tissue sampled directly from the diseased joint. Across a large number of patients, these efforts may develop an overall disease framework, within which per-patient variations of clinical significance may be identified. This approach is well rooted in the historical discoveries of tumor biomarkers where molecular profiling led to the discovery of oncogenic driver mutations. These definitive objective molecular biomarkers have led to precision medicine testing, tailored therapeutics, and most recently circulating blood biomarkers in the form of the tumor cell-free DNA. Although somatic DNA lesions are not necessarily the biomarkers we anticipate for RA, this discovery trajectory is a reminder of the potential importance of starting with the target tissue itself, which may over time lead to the identification of a circulating biomarker.

Table 1. Blood biomarkers: high hopes, but limited utility

Seropositivity predicts response to rituximab [1] and abatacept [2,3], but has had mixed results in predicting response to TNF inhibitors [4].
As reviewed by Nouri and colleagues [5], multiple studies have assessed possible serum biomarkers for the prediction of response to IL-6 receptor blockade. However, results need to be independently replicated with larger sample sizes to establish clinical utility.
Although there are no serum biomarkers in clinical use to predict response to methotrexate monotherapy, a recent pilot study identified four molecules (C-reactive protein, leptin, TNF-RI and VCAM-1) that, in combination, may predict response to methotrexate [6].
The only commercially available multibiomarker disease activity (MBDA) test for RA (VECTRA DA) has shown some efficacy in determining disease activity and risk of progression; however, has been less useful in guiding management decisions [7] and may not be correlate as well with other markers of disease activity, such as CDAI or DAS28-CRP after cytokine-directed treatment [8].

SYNOVIAL TISSUE ATTRIBUTES AND DRUG RESPONSIVENESS

There is extensive literature over the years probing the cellular composition of the synovium in affected RA joints, particularly the features of the immune cell infiltrates. From early work defining the lymphocytic infiltrate [24], technologic advances for high-dimensional identification of cell types, molecular pathways, and histologic patterns have begun to provide a more comprehensive understanding [25▪,26,27,28▪▪,29–32]. Moving forward, this detailed tissue framework may be used to categorize synovial tissue from individual patients and test if any features relate to treatment response.

Prior to these contemporary technologies, studies used synovial biopsies combined with methods such as histopathologic scoring and gene expression (via microarray or bulk RNA-sequencing). Reports examining these features in relation to treatment with conventional synthetic and biologic disease-modifying antirheumatic drugs (csDMARDs and bDMARDs, respectively) have been extensively reviewed [33]. Some of the more recent studies in this domain are described here.

Several reports from patients in the Pathobiology of Early Arthritis Cohort have recently connected synovial histology, gene expression, and clinical characteristics with RA progression and response to treatment [34▪,35▪,36▪]. Synovial histology was found to be classifiable into three types – lympho-myeloid, diffuse-myeloid, and pauci-immune fibroid – which correlated to profiles of synovial gene expression defined either by

Table 2. The promise of genetics and pharmacogenomics

Both pathway-focused and unbiased genome-wide genetic association studies have sought connections between genetic variants and the response to steroids [9], methotrexate [10,11], TNF inhibition [12,13], and IL-6 inhibition [14–17]. Other recent advances that may help realize the possibility of personalized medicine include:
Epigenetic modifications, including DNA methylation, play a central role in determining gene expression and cellular phenotypes. In a small study of RA patients started on methotrexate, Nair and colleagues [18] showed that changes in DNA methylation in four CpG positions between baseline and 4 weeks into therapy were associated with therapeutic response at 6 months.
Integration of genetic information with epigenetics and immunophenotyping may provide additional insight as shown by Spiliopoulou and colleagues [19]. They introduced a new approach for localizing genetic effects of a response to TNF inhibition using GWAS-identified SNPs, heritable immune cell traits and whole blood expression and methylation.
The emerging field of pharmacomicrobiomics, which studies the interactions between drugs and the microbiome, has the potential to further elucidate variables that may influence therapeutic response in RA [20].

GWAS, genome-wide association study; RA, rheumatic arthritis; TNF, tumor necrosis factor.

previously determined immune cell expression or by unsupervised clustering on the observed data. Both the histologic type and gene expression profiles were found to correlate with response to csDMARD, with changes between pretreatment and posttreatment gene signature mostly correlating with baseline disease severity [34▪]. By including synovial gene expression data in addition to clinical features, the area under the curve for predicting DMARD responsiveness at 12 months was improved [36▪].

Another study using synovial biopsies and synovial histopathology classification into the same three groups looked at 37 established RA patients before and after anti-tumor necrosis factor (TNF) treatment. Here the authors found that both myeloid histopathologies were substantially more likely to respond to certolizumab than the pauci-immune histology [37]. Focusing on B-cell infiltration of the synovium, Pitzalis and colleagues compared synovial biopsies between 165 treatment-naïve early RA patients and 164 established RA patients who had not responded to TNF inhibition [38]. When compared with treatment-naïve patients, those patients who did not respond to TNF inhibition were more likely to have synovial B-cell enrichment.

Mass cytometry on $CD4^+$ T cells from inflamed RA synovium discovered an expanded population that is PD-1^{hi}/CXCR5$^-$, described as 'peripheral helper' cells, that promote B-cell activity [39]. Subsequently, in a study of 11 RA patients who had failed csDMARD treatment, synovial biopsies taken before and after anti-TNF therapy underwent transcriptomic analysis with deconvolution for cell-type distribution inference, which revealed that the absence of the PD-1^{hi}/CXCR5$^-$ peripheral helper T cells in the synovium correlated significantly with better response to TNF inhibition [40].

Microarray expression data from synovial biopsies has also been integrated with genome-wide association study (GWAS) data to identify predictors of response to TNF inhibition [41]. Using synovial biopsies of active RA patients starting anti-TNF therapy, Julià and colleagues found modules of co-expressed genes that correlated with treatment response. They then compared those modules with the list of genes related to single nucleotide polymorphisms (SNPs) found to be associated with response to TNF inhibition from a GWAS and identified two co-expression modules that associated with response to specific TNF inhibitors.

In the future, applications of newer techniques which have enabled spatially resolved transcriptomics in synovial tissue, as applied to RA [42] and psoriatic arthritis [43], and other multiparameter histologic techniques, may elucidate both the dominant pathways in synovial microenvironments and their effects on therapeutic response.

FUNCTIONAL DRUG RESPONSE ASSAYS FOR PATIENT SYNOVIAL TISSUE

Synovial biopsy data alone is limited as a static snapshot, wherein inferences regarding the underlying dynamics and responses to interventions can only be drawn indirectly. By contrast, assays of functional response in various tissues to therapeutic perturbations have been attempted with the aim of characterizing the dynamics of the pathologic state, and potentially prefiguring the response of those tissues to therapies. Stimulation assays on whole blood or peripheral blood mononuclear cells (PBMCs) have been widely used to describe responses of circulating immune cells to particular immunogenic stimuli. Recent advances in high-throughput assays and computational techniques for integrating data between these has allowed for more informative characterization [44], and helped uncover dominant cytokine responses that can lead to therapeutic decisions [45▪▪]. Perturbation assays on extracts from rheumatoid synovium that vary in the extent to which synovial architecture is replicated have also been reported.

At one extreme, disaggregated cells are isolated and cultured in two dimensions, such as with rheumatoid joint fibroblast-like synoviocytes in [46]

where features including TNFα-stimulated cytokine production, migration speed, and invasiveness were found to be affected by treatments including methotrexate, hydroxychloroquine, and artesunate. Dissociated RA synovial tissue has also been recently cultured in ex-vivo drug assays [47▪]. Kuo and colleagues showed that treatment of the dissociated synovium with anti-inflammatory therapies (anti-TNF antibodies, tofacitinib, naproxen, and dexamethasone) resulted in a negative enrichment in genes associated with an inflammatory macrophage population, indicating these cells may be one of the targets of common RA therapies. The response, however, to each medication resulted in a distinct macrophage expression pattern, which may ultimately affect outcomes differentially. Whether these initial studies lay the groundwork for individualized medicine assays for RA patients will require more detailed mechanistic understanding of drug responses and broad testing across patient tissues.

An organoid-like system that employs microfluidic devices has been used to model the effect of potential therapeutics on synovial fibroblast interaction with bone [48]. Further morphologic verisimilitude in three-dimensional cultures and analysis with continuous light-scatter imaging has recently been reported [49], with synovial fibroblasts recapitulating a tight surface versus loose sub-surface architecture within which the effect of TNF on motility and proliferation could be measured. A three-dimensional organoid co-culture of synovial fibroblasts and endothelial cells in [30] revealed aspects of fibroblast differentiation that were instructed by the endothelial cells. The potential to use such systems to screen drug candidates is clear, although the ability to detect useful compounds whose effect depends on disease-specific interactions between multiple cell types would require extending the system to include more of the tissue-associating and disease-associating cell types.

At the most-organized end of the spectrum, whole tissue explants have been isolated and maintained to monitor their response to stimuli. Specifically, to elucidate features of response to JAK/STAT inhibition, McGarry and colleagues [50] obtained seven rheumatoid synovial explants and cultured them with tofacitinib. They defined changes in gene expression (including mitochondrial genes involved in apoptosis), metabolic state, and cytokine secretion after treatment, all of which had the effect of reducing synovial fibroblast invasion and outgrowths in the explants. Although not a particular focus of that study, it is reasonable to test the theory that the effect size in treated synovial explants might predict disease response to treatment in the patient, allowing personalization of the treatment strategy without the lengthy trial-and-error approach that is presently the norm.

CONCLUSION

The increasing availability of high-throughput molecular and spatial technologies has enabled more extensive characterization of the pathologic activity in the rheumatoid synovium. The next challenge is developing data analysis tools to extract generalizable biologic and clinical insights from this wealth of data. This is a very active field in which a variety of complementary approaches have recently been reviewed by Ma and colleagues. [51]. Dimensional reduction of the high-throughput data by projection onto the most informative axes has been employed and optimized extensively in individual modalities, and naturally extended to combinations of data (e.g. single-cell RNA sequencing and proteomic data). Beyond integrating different data types within a single experiment, barriers to combining data with differences in experimental and biological factors have been addressed by algorithms such as Harmony [52] and Seurat3 [53]. Improvements in these algorithms and their usability will hopefully drive discovery of novel cell types and interactions in the rheumatoid synovium that may eventually guide exploration of more accessible signals of disease activity and responsiveness to treatment.

As we have discussed, an important unmet clinical question is whether any given RA patient is more or less likely to respond to a specific treatment, not only because of the related delay in disease control for individual patients, but also because new agents which may in fact be highly effective in a subset of the disease can fail to show efficacy in unselected trial populations, leading to rejection of what could be useful therapeutics. The additional insight into disease pathogenesis derived from studies of the sort discussed here can be incorporated into more narrowly tailored clinical trial designs as described by [54▪] and is in the process of being realized for agents including rituximab, tocilizumab, and etanercept in the R4-RA and STRAP trials.

Acknowledgements

None.

Financial support and sponsorship

We acknowledge our funding sources NIH R01 AI148435 (LTD) and NIH AMP Consortium UH2 AR067691 (AL, MHS, LTD), Carson Family Trust (LTD) and Leon Lowenstein Foundation (LTD).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Isaacs JD, Cohen SB, Emery P, *et al.* Effect of baseline rheumatoid factor and anticitrullinated peptide antibody serotype on rituximab clinical response: a meta-analysis. Ann Rheum Dis 2013; 72:329–336.
2. Harrold LR, Litman HJ, Connolly SE, *et al.* Effect of anticitrullinated protein antibody status on response to abatacept or antitumor necrosis factor-α therapy in patients with rheumatoid arthritis: a US National Observational Study. J Rheumatol 2018; 45:32–39.
3. Gottenberg JE, Courvoisier DS, Hernandez MV, *et al.* Brief report: association of rheumatoid factor and anti-citrullinated protein antibody positivity with better effectiveness of abatacept: results from the Pan-European Registry Analysis. Arthritis Rheumatol 2016; 68:1346–1352.
4. Mulhearn B, Barton A, Viatte S. Using the immunophenotype to predict response to biologic drugs in rheumatoid arthritis. J Pers Med 2019; 9:46.
5. Nouri B, Nair N, Barton A. Predicting treatment response to IL6R blockers in rheumatoid arthritis. Rheumatology (Oxford) 2020.
6. Hambardzumyan K, Bolce RJ, Wallman JK, *et al.* Serum biomarkers for prediction of response to methotrexate monotherapy in early rheumatoid arthritis: results from the SWEFOT Trial. J Rheumatol 2019; 46:555–563.
7. Fleischmann R, Connolly SE, Maldonado MA, Schiff M. Brief report: estimating disease activity using multi-biomarker disease activity scores in rheumatoid arthritis patients treated with abatacept or adalimumab. Arthritis Rheumatol 2016; 68:2083–2089.
8. Reiss WG, Devenport JN, Low JM, *et al.* Interpreting the multibiomarker disease activity score in the context of tocilizumab treatment for patients with rheumatoid arthritis. Rheumatol Int 2016; 36:295–300.
9. Quax RaM, Koper JW, Huisman AM, *et al.* Polymorphisms in the glucocorticoid receptor gene and in the glucocorticoid-induced transcript 1 gene are associated with disease activity and response to glucocorticoid bridging therapy in rheumatoid arthritis. Rheumatol Int 2015; 35:1325–1333.
10. Szostak B, Machaj F, Rosik J, Pawlik A. Using pharmacogenetics to predict methotrexate response in rheumatoid arthritis patients. Expert Opin Drug Metab Toxicol 2020; 16:617–626.
11. Taylor JC, Bongartz T, Massey J, *et al.* Genome-wide association study of response to methotrexate in early rheumatoid arthritis patients. Pharmacogenomics J 2018; 18:528–538.
12. Mirkov MU, Cui J, Vermeulen SH, *et al.* Genome-wide association analysis of anti-TNF drug response in rheumatoid arthritis patients. Ann Rheum Dis 2013; 72:1375–1381.
13. Massey J, Plant D, Hyrich K, *et al.,* BRAGGSS, MATURA Consortium. Genome-wide association study of response to tumour necrosis factor inhibitor therapy in rheumatoid arthritis. Pharmacogenomics J 2018; 18:657–664.
14. Luxembourger C, Ruyssen-Witrand A, Ladhari C, *et al.* A single nucleotide polymorphism of IL6-receptor is associated with response to tocilizumab in rheumatoid arthritis patients. Pharmacogenomics J 2019; 19:368–374.
15. Mikhaylenko DS, Nemtsova MV, Bure IV, *et al.* Genetic polymorphisms associated with rheumatoid arthritis development and antirheumatic therapy response. Int J Mol Sci 2020; 21:4911.
16. Wu X, Sheng X, Sheng R, *et al.* Genetic and clinical markers for predicting treatment responsiveness in rheumatoid arthritis. Front Med 2019; 13:411–419.
17. Acosta-Herrera M, González-Serna D, Martín J. The potential role of genomic medicine in the therapeutic management of rheumatoid arthritis. J Clin Med 2019; 8:826.
18. Nair N, Plant D, Verstappen SM, *et al.* Differential DNA methylation correlates with response to methotrexate in rheumatoid arthritis. Rheumatology (Oxford) 2020; 59:1364–1371.
19. Spiliopoulou A, Colombo M, Plant D, *et al.* Association of response to TNF inhibitors in rheumatoid arthritis with quantitative trait loci for CD40 and CD39. Ann Rheum Dis 2019; 78:1055–1061.
20. Scher JU, Nayak RR, Ubeda C, *et al.* Pharmacomicrobiomics in inflammatory arthritis: gut microbiome as modulator of therapeutic response. Nat Rev Rheumatol 2020; 16:282–292.
21. Crowson CS, Liao KP, Davis JM, *et al.* Rheumatoid arthritis and cardiovascular disease. Am Heart J 2013; 166:622–628.
22. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011; 365:2205–2219.
23. Holers VM, Demoruelle MK, Kuhn KA, *et al.* Rheumatoid arthritis and the mucosal origins hypothesis: protection turns to destruction. Nat Rev Rheumatol 2018; 14:542–557.
24. van Boxel JA, Paget SA. Predominantly T-cell infiltrate in rheumatoid synovial membranes. N Engl J Med 1975; 293:517–520.
25. ■ Zhang F, Wei K, Slowikowski K, *et al.* Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol 2019; 20:928–942.

This study isolated several cell types from rheumatoid and osteoarthritis synovium and subjected them to a variety of assays including mass cytometry, RNA-seq (bulk and single-cell), and flow cytometry. The combined data revealed subpopulations of fibroblasts, monocytes, B cells, and T cells expanded in the rheumatoid synovium, and connected secretion of inflammatory cytokines to some of these subsets.

26. Stephenson W, Donlin LT, Butler A, *et al.* Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. Nat Commun 2018; 9:791.
27. Orange DE, Agius P, DiCarlo EF, *et al.* Identification of three rheumatoid arthritis disease subtypes by machine learning integration of synovial histologic features and RNA sequencing data. Arthritis Rheumatol 2018; 70:690–701.
28. ■■ Alivernini S, MacDonald L, Elmesmari A, *et al.* Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020; 26:1295–1306.

This study used single-cell RNAseq to characterize the synovial tissue macrophages in biopsies of rheumatoid arthritis patients in different stages of disease, and then performed spatial and functional analyses to further focus on MerTK^{pos} macrophages that may be protective in maintaining a state of remission in RA.

29. Croft AP, Campos J, Jansen K, *et al.* Distinct fibroblast subsets drive inflammation and damage in arthritis. Nature 2019; 570:246–251.
30. Wei K, Korsunsky I, Marshall JL, *et al.* Notch signalling drives synovial fibroblast identity and arthritis pathology. Nature 2020; 582:259–264.
31. Mizoguchi F, Slowikowski K, Wei K, *et al.* Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. Nat Comm 2018; 9:789.
32. Fonseka CY, Rao DA, Teslovich NC, *et al.* Mixed-effects association of single cells identifies an expanded effector CD4+ T cell subset in rheumatoid arthritis. Sci Transl Med 2018; 10:.
33. Filkova M, Cope A, Mant T, *et al.* Is there a role of synovial biopsy in drug development? BMC Musculoskelet Disord 2016; 17:172.
34. ■ Humby F, Lewis M, Ramamoorthi N, *et al.* Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis 2019; 78:761–772.

See Ref. [36■].

35. ■ Lewis MJ, Barnes MR, Blighe K, *et al.* Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. Cell Rep 2019; 28:2455.e5–2470.e5.

See Ref. [36■].

36. ■ Lliso-Ribera G, Humby F, Lewis M, *et al.* Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). Ann Rheum Dis 2019; 78:1642–1652.

These three studies reported histology and expression data from synovial biopsies, combining those data with clinical features to improve prediction of response to DMARDs.

37. Nerviani A, Di Cicco M, Mahto A, *et al.* A Pauci-immune synovial pathotype predicts inadequate response to TNFα-blockade in rheumatoid arthritis patients. Front Immunol 2020; 11:845.
38. Rivellese F, Humby F, Bugatti S, *et al.,* PEAC-R4RA Investigators. B cell synovitis and clinical phenotypes in rheumatoid arthritis: relationship to disease stages and drug exposure. Arthritis Rheumatol 2020; 72:714–725.
39. Rao DA, Gurish MF, Marshall JL, *et al.* Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature 2017; 542:110–114.
40. Julià A, Àvila G, Celis R, *et al.* Lower peripheral helper T cell levels in the synovium are associated with a better response to anti-TNF therapy in rheumatoid arthritis. Arthritis Res Ther 2020; 22:196.
41. Aterido A, Cañete JD, Tornero J, *et al.* A combined transcriptomic and genomic analysis identifies a gene signature associated with the response to anti-TNF therapy in rheumatoid arthritis. Front Immunol 2019; 10:1459.
42. Bergenstråhle J, Larsson L, Lundeberg J. Seamless integration of image and molecular analysis for spatial transcriptomics workflows. BMC Genomics 2020; 21:482.
43. Carlberg K, Korotkova M, Larsson L, *et al.* Exploring inflammatory signatures in arthritic joint biopsies with spatial transcriptomics. Sci Rep 2019; 9:18975.
44. Cepika A-M, Banchereau R, Segura E, *et al.* A multidimensional blood stimulation assay reveals immune alterations underlying systemic juvenile idiopathic arthritis. J Exp Med 2017; 214:3449–3466.
45. ■■ Poubelle PE, Pagé N, Longchamps M-P, *et al.* The use of leukocytes' secretome to individually target biological therapy in autoimmune arthritis: a case report. Clin Transl Med 2019; 8:19.

This case report describes a patient with inflammatory arthritis resistant to multiple therapies, for whom the ultimately correct choice of IL-6 inhibition was made based on the observation that the patient's blood secreted more IL-6 in response to immunogenic stimulation than that of controls.

46. Ma J-D, Jing J, Wang J-W, *et al.* A novel function of artesunate on inhibiting migration and invasion of fibroblast-like synoviocytes from rheumatoid arthritis patients. Arthritis Res Ther 2019; 21:153.
47. Kuo D, Ding J, Cohn IS, *et al.* HBEGF+ macrophages in rheumatoid arthritis ■ induce fibroblast invasiveness. Sci Transl Med 2019; 11:eaau8587.
This study used single-cell RNAseq to identify macrophage subsets in rheumatoid synovium, including an HBEGF+ population that induces destructive behavior in fibroblasts. It also reports an ex-vivo drug assay that distinguished different drugs' effects on this macrophage subpopulation.
48. Ma H-P, Deng X, Chen D-Y, *et al.* A microfluidic chip-based co-culture of fibroblast-like synoviocytes with osteoblasts and osteoclasts to test bone erosion and drug evaluation. R Soc Open Sci 2018; 5:180528.
49. Rothbauer M, Höll G, Eilenberger C, *et al.* Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with noninvasive light scattering biosensing. Lab Chip 2020; 20:1461–1471.
50. McGarry T, Orr C, Wade S, *et al.* JAK/STAT blockade alters synovial bioenergetics, mitochondrial function, and proinflammatory mediators in rheumatoid arthritis. Arthritis Rheumatol 2018; 70:1959–1970.
51. Ma A, McDermaid A, Xu J, *et al.* Integrative methods and practical challenges for single-cell multiomics. Trends Biotechnol 2020; 38: 1007–1022.
52. Korsunsky I, Millard N, Fan J, *et al.* Fast, sensitive and accurate integration of single-cell data with Harmony. Nat Methods 2019; 16:1289–1296.
53. Stuart T, Butler A, Hoffman P, *et al.* Comprehensive integration of single-cell data. Cell 2019; 177:1888–1902.
54. Pitzalis C, Choy EHS, Buch MH. Transforming clinical trials in rheumatology: ■ towards patient-centric precision medicine. Nat Rev Rheumatol 2020; 16:590–599.
This article describes the advantages of alternative potential 'patient-centric' trial designs that incorporate personalized disease biomarkers.

The transition from enthesis physiological responses in health to aberrant responses that underpin spondyloarthritis mechanisms

Sibel Zehra Aydin[a], *Charles Bridgewood*[b], *Alen Zabotti*[c], *Nicolò Girolimetto*[d], *and Dennis McGonagle*[b]

Purpose of review
Despite immunology and translational therapeutics advances in inflammatory arthritis over the past two decades, the enthesis, which is the epicentric of the spondyloarthritis family pathological process, retains many mysteries because of tissue inaccessibility that hampers direct immune study. As entheses are subject to almost continuous mechanical stress and spondyloarthritis is linked to microdamage or injury and joint stress, it is cardinal to understand the physiological changes occurring within the entheses not only to be able to differentiate disease from health but also to understand the transition normal physiology break down and its merges into spondyloarthritis-related disease.

Recent findings
Imaging has played a major role in understanding the enthesis in human. Remarkable insights from enthesis functioning and microdamage in normal and with ageing including those linked to body mass index is emerging. The impact of mechanical stress and degenerative conditions on the development of the secondary entheseal vascular changes is not understood. Of note, ultrasound studies in psoriasis have shown higher power Doppler changes compared to controls pointing towards a role for vascular changes in the development of enthesitis in psoriatic arthritis.

Summary
The literature pertaining to normal entheses changes with age, microdamage and vascular changes in health is providing a roadmap for understanding of the enthesis and its potential role in evolution of spondyloarthritis including psoriatic arthritis.

Keywords
enthesis, psoriatic arthritis, spondyloarthritis, ultrasound

INTRODUCTION

The enthesis is the transitional zone between ligament/tendon or capsule and bone anchorage and includes the classically described four regions of dense fibrous connective tissue, uncalcified fibrocartilage, calcified fibrocartilage and underlying bone [1,2]. Beyond the anchorage point, fibrocartilage tissue may also line adjacent bone and tendon forming what is termed the synovio-entheseal complex that facilitates smooth locomotion and stress dissipation over a wide area [3,4]. There is also the functional integration with the adjacent bone and the entire structure is termed as the enthesis organ. There are two types of enthesis histologically: fibrocartilaginous and fibrous entheses with the latter being uncommon, e.g. the deltoid muscle insertion, which is not a common site of disease in inflammatory arthritis [5].

In addition to these, despite not being an actual insertion, the tendons that wrap around the bones are exposed to similar biomechanical stress as the insertion and share histological similarities including the presence of fibrocartilage, and similar

[a]Department of Medicine, Division of Rheumatology and the Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Ontario, Canada, [b]NIHR Leeds Musculoskeletal Biomedical Research Unit, Leeds Teaching Hospitals Trust & The University of Leeds, Leeds, United Kingdom, [c]Department of Medical and Biological Sciences, Rheumatology Clinic, University of Udine and [d]Department of Rheumatology, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

Correspondence to Dennis McGonagle, NIHR Leeds Musculoskeletal Biomedical Research Unit, Leeds Teaching Hospitals Trust & The University of Leeds, Leeds, United Kingdom. Tel: (0113) 206 6071; e-mail: D.G.McGonagle@leeds.ac.uk

Curr Opin Rheumatol 2021, 33:64–73
DOI:10.1097/BOR.0000000000000768

KEY POINTS

- In the absence of histological data for the entheses because of tissue inaccessibility, imaging modalities such as ultrasound, provide valuable information on tissue characteristics in the health and spondyloarthritis.
- The healthy entheses may have signs of inflammation and damage on ultrasound, in response to factors, such as increased age and BMI, male sex and high physical activity, without any clinical symptoms, mirroring pathological changes occurring in spondyloarthritis.
- The observed changes within the entheses on ultrasound may improve the understanding of how mechanical loading activates the entheseal immune system towards arthritis development.

patterns of osteitis disorder and have been called 'functional enthesis' [6]. Extensor tendons over small joints that fuse with the extensor capsules also form a type of functional enthesis, the relevance of which leads to disorder localization in psoriatic arthritis (PsA), but not rheumatoid arthritis [7]. Another type of functional enthesis occurs in the digital flexor tendons' accessory pulleys, which appear to be a major target for the dactylitis. That is distinct from the wrap around tendons of the ankle that compress bone for being soft tissue based without an osseous component [8]. Both the classically described and functional entheses are very difficult to study histologically because of the major issue of tissue inaccessibility. In this review, we further discuss entheseal defence mechanisms against injury in relationship to imaging findings and also discuss the transition from the health to disease state. We look further at both the osseous and soft tissue changes that occur in normal and diseased entheses. We also focus on emerging data from perturbations of functional entheses and how this may impact on the transition from normality to disease.

ENTHESEAL BIOMECHANICAL STRESS IN HEALTH

There are three major patterns of biomechanical stressing applied to the enthesis in the course of normal activity: compression (superficial tissue pressing down on deeper layer tissue on locomotion), tension (direct tractional forces on tendon/ligament) and shear forces (because of impact of gliding movements of adjacent tissue layers) [9▪▪]. To protect against the ravages of complex and large lifelong force application, several mechanisms exist and allow maintenance of enthesis integrity and function to minimize risk of injury including rupture or avulsion. The key factors are the dissipation of force over a wide area by the enthesis organ, intrinsic repair mechanisms and lubrication of the enthesis organ by the synovio-entheseal complex [4,10].

The human entheses including the Achilles and many others that are the target of spondyloarthritis are very difficult to study at the cellular and immune level *in vivo*, unlike studies into the readily accessible synovium in either rheumatoid arthritis or PsA. Furthermore, small animal models including mice may be poor surrogates for the much larger entheseal structure involvement in man. Fortunately, modern imaging, and ultrasound in particular, are well suited to understanding entheseal microanatomy in man, given their resolution and ability to dynamically visualise tissue inflammation, degenerative changes, osseous remodelling and erosion and vascularity. Indeed, there had been many ultrasound studies to date focusing on entheseal findings in patients with diseases, mostly spondyloarthritis including PsA and ankylosing spondylitis [11–15]. Importantly, there is emerging ultrasound-derived data on entheseal changes in healthy subjects including the impact of age, BMI and exercise on entheseal structure. Given the link between PsA with the three factors of age, BMI and injury, the role of ultrasound imaging in deciphering potential disease-related factors is both novel and noninvasive [16–18].

The fact that ultrasound features of spondyloarthritis, degenerative or mechanical enthesopathies overlapping is also interesting and likely relevant for a better understanding of enthesis disease mechanisms. Three studies have recently been published specifically investigating the ultrasound findings of the healthy entheses and factors leading to asymptomatic imaging abnormalities. Guldberg-Moller *et al.* [19] focussed on large entheses of the lower extremity and demonstrated abnormalities in 23% of all insertions examined with 73% (47/64) persons having at least one ultrasound imaging abnormality (including inflammatory features and damage). A second study of the same anatomical sites by Di Matteo *et al.* [20] reported one or more inflammatory features (hypoechogenicity, thickening and/or Doppler signals) in at least one enthesis in 30 out of 82 healthy participants (34%) and in 69/820 evaluated entheses (8.4%). Finally, our group recently published the ultrasound findings in 80 healthy participants, showing that older age, male sex, higher BMI, and high physical activity were independent predictors of enthesitis scores on ultrasound with some major differences among the younger (<50) and older (≥50) [21▪]. Collectively, the aforementioned studies improve our understanding of how changes associated with healthy entheses

homeostasis may imperceptibly merge with changes that are commonly seen in diseases. Of course, histological validation of what changes in different settings represents is lacking.

Soft tissue changes in health

Development of enthesis fibrocartilage in health

In the murine setting, entheseal fibrocartilage is absent at birth with only the presence of insertional fibroblasts. These fibroblasts undergo metaplasia and convert to fibrocartilage tissue with time, between 2 weeks and 2 months. However, fibrocartilage development is not spontaneous but is linked to muscular tissue biomechanical stressing, which was deduced from observations that botulinum toxin injection with muscle movement neutralization prevented the development of fibrocartilage [22]. Thus, with elimination of the limb biomechanical forces, fibrocartilage tissue did not develop, emphasizing the key role of movement and stress. With the repeated loading, the fibroblasts proliferate with ensuing type II collagen and proteoglycan production as part of a chondrocytic cellular differentiation programme in the peripheral enthesis [23]. Due to this chondrogenic differentiation process with the concomitant absence of insertional blood vessels, the insertion is rendered more resistant to trauma. The avascular nature of the insertion is a key feature, which both prevents microvascular-induced damage, because of vascular exclusion, and acts as a physical barrier for immune cell migration. By implication, the ability of fibrocartilage to both withstand physical damage and vascular microinvasion may also be critical to homeostasis and health with normal ageing.

Neovascularization of the entheses

As stated above, a key feature of the fibrocartilage tissue is its avascular nature. Fibrocartilage oxygenation is sustained by diffusion from the surrounding vascular tissues including the adjacent bone, perligamentous or tendinous vascular supply and the adjacent synovio-entheseal complex, wherever present [24]. With age, nondiseased joint fibrocartilage shows microdamage and infiltration with microvessels, which may be part of the normal physiological repair process, which was also supported by ultrasound studies in human [25,26]. These microvascular changes are associated with immune cells ingression or presence at these sites (Fig. 1). The presence of immune cells including both myeloid and lymphoid cells may contribute to enchondral bone formation. Healthy people may have evident entheseal vascularity as shown by ultrasound determined Doppler signal, although not frequently. In a

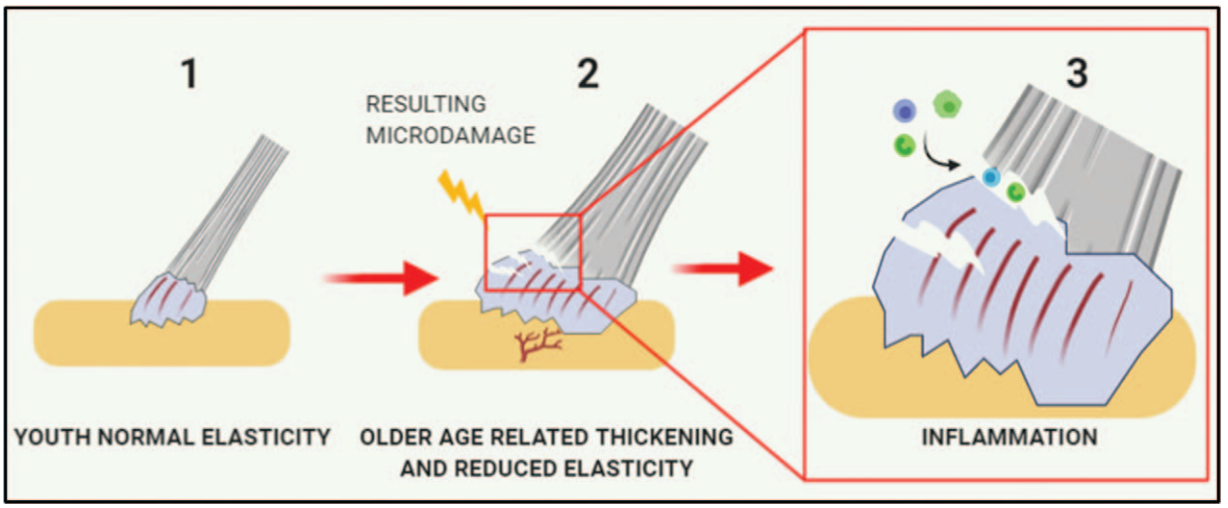

FIGURE 1. (a) In younger subjects, entheses are thin with very good elasticity, which protects against injury and microdamage. (b) With age, entheses show thickening because of changes in the collagen compositions. Higher BMI also contributes to thickening. The known age-related loss of musculoskeletal tissue flexibility likely makes the tissue more prone to microdamage and injury. This is associated with hypoechoic thickening of enthesis and occasional Doppler changes in older subjects, which has been confirmed in cadaveric studies where vascular abnormalities at the enthesis have been reported. Also, microscopic inflammatory changes have been reported in normal aged enthesis. (c) Age-related and BMI-related changes at the normal enthesis suggest a scenario whereby the enthesis 'fertile soil' parameters develop over time in PsA and lag psoriasis but the immunogenetic 'seed' then eventually manifests as joint disease. Therefore, It is proposed that changes in the biomechanical environment is associated with clinically demonstrable enthesitis in genetically susceptible people.

recent study on healthy people, our group has shown that only 1.1% (11/960) of the scanned entheses had Doppler signals, although it corresponded to 10% of the participants [21▪]. It is important to note that the majority of the Doppler positivity were only mild in severity and/or seen limited to one isolated site. These numbers are similar to what has been observed by Di Matteo *et al.* [20] and slightly higher than Guldberg-Muller *et al.* [19]. Given that vascular lesions are commoner in psoriasis patients without PsA compared with healthy participants then they may be a harbinger of enthesis homeostasis dysregulation that predispose to chronic immune activation with inflammation [27]. Such entheseal lesions may also regress under biological therapy for psoriasis, which potentially points to the ability to prevent PsA evolution [28]. More work is needed to understand the topography of these vascular lesions as being peri-entheseal in vascular tissue or linked to actual fibrocartilage vascular ingression.

Thickening of the entheses

Hypoechogenic entheseal thickening is certainly a feature of both inflammatory enthesitis and degenerative enthesopathies [29]. However, increased entheseal and tendon thickness has also frequently been reported in ultrasound studies in healthy people. The thickening may be because of two different processes: a) increased intra-tendinous collagen synthesis and b) due to immune cells infiltration or local proliferation of fibroblasts, although there is no histology data to prove that mechanism. The first mechanism, increased collagen synthesis, has been shown to occur as a response to strenuous exercise in human tendon and muscle in preexercise and postexercise biopsies [30]. Interestingly imaging studies that investigated the thickening as a response to short-term intense exercise have conflicting results. The increase in tendon thickness as a response to exercise have only been observed in younger participants (<25 years old) and not in the elderly population that is over 60 years old [31–33]. However, the imaging studies with cross sectional analysis, instead of pre-exercise and postexercise, usually suggest an increased thickening with age including the elderly population. The adaptation may be different during the skeletal development in the younger population, being more responsive by inducing collagen synthesis whereas the thickening in the elderly may be more multifactorial with loss of elasticity.

The two mechanisms of thickening would be expected to be visualized differently on ultrasound. If the thickening of the enthesis is because of an inflammatory process, it would be expected to see the irregular changes in the echogenicity of the structure, for example, the inflammatory deposition that separates the entheseal fibers from each other leading to thickening, whereas increased collagen synthesis would be expected to lead to homogenous changes, keeping the fibrillary echotexture of the tendon intact as shown in Fig. 2. Notwithstanding lack of histological data, the general perception among sonographers is to avoid measuring entheseal thickness as a sole surrogate in clinical practice to define a disorder but rather seek evidence for other additional key features of enthesitis, such as Doppler signals and/or erosions.

In addition to age, sex and BMI has been found as factors contributing to entheseal thickening in healthy people [21▪]. A high BMI leads commensurate and sustained higher mechanical stress on the entheses, especially in the lower extremities. Sex differences may be because of properties of the muscle tissue, including architectural characteristics, fibre types, biomechanical characteristics and neural activity [34–36]. Therefore, the thickness of the entheses needs to be taken into account in the context of these variables and associated findings/features that lead to thickening to differentiate a healthy adaptive response to biomechanical stress from abnormal responses at the entheseal level.

Bone changes in health

New bone formation at normal peripheral entheses

Entheseal bony spurs (enthesophytes) have long been recognized as incidental findings on radiographs and in cadaveric tissue studies of nondiseased people in addition to being recognized also in inflammatory, degenerative or metabolic enthesopathies [37]. The advent of ultrasound studies in healthy entheses in recent years has brought a new dimension to the study of entheseal new bone in normal (Fig. 3). The frequency of enthesophytes on ultrasound are highly variable depending on the anatomical site, usually being more frequent at the Achilles tendon insertion and as high as 78% of the healthy Achilles enthesis [21▪]. At the Achilles, it is clear that enthesophytes generally develop on the outer aspect of the insertion where tension is maximal during normal functioning whereas erosion occur more proximally. This likely reflects the differences in forces application between the bone in between the proximal and distal entheseal fibres, whereby bone forms along sites of tension being linked to enthesophytes mostly occurring at the distal insertion in accordance with Wolfs law (Fig. 3). This type of new bone formation may occur

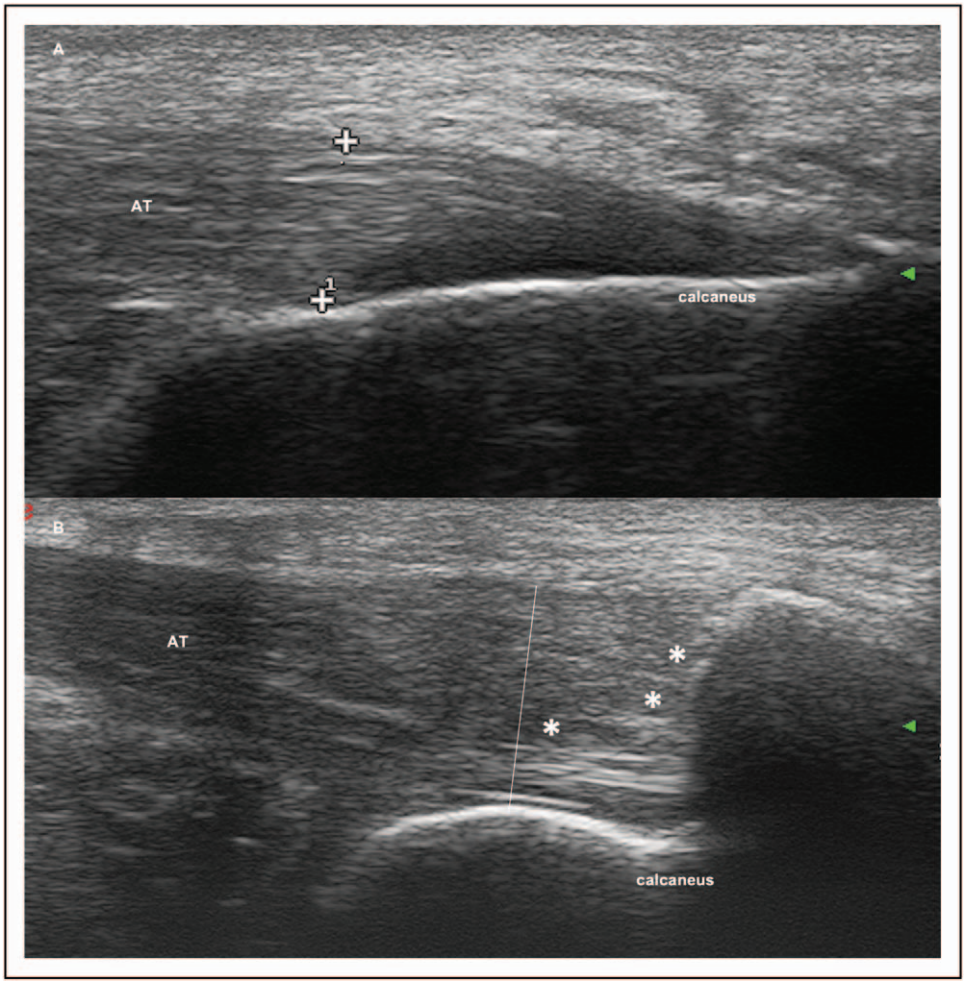

FIGURE 2. Entheseal thickening on longitudinal scans of the Achilles enthesis on ultrasound. The upper limit for the Achilles enthesis thickness is 5.29 mms. (a) Thickening of the Achilles enthesis with normal fibrillary echotexture (distance between the + signs; thickness 5.8 mm); (b) Thickening (line) because of infiltrates (*) separating the entheseal fibers. AT, Achilles tendon.

via an endochondral like bone formation with calcification of the entheseal fibrocartilage, uncoupled from an inflammatory process [37]. The typical distal enthesophytes do not always reflect disorders, especially when they are small in size, and these are more common with age, BMI and healthy men.

One mechanism for enthesophyte development is bony microtrabecular stress fractures and subsequent repair processes. Transverse microtears at the fibrocartilage-bone interface are subsequently filled with adipose tissue and the longitudinal microtears repair with proliferation of fibrocartilage cells and filled with amorphous material [38]. Benjamin *et al.* showed that this amorphous material can eventually become calcified and may mimic enthesophytes on the radiographs. We hypothesize that these calcifications that occur as a reaction to microcracks mimicking enthesophytes may be seen more proximally to the traditional enthesophytes. Our observations that lead to this hypothesis originates from the typical locations of the entheseal erosions. The erosions almost always occur more proximal to the enthesophytes, possibly because of the differences in biomechanical forces proximally and distally with the proximal operation of compression and shear leading to erosion (Fig. 4). Due to the type of these forces, microcracks or erosions may eventually lead to calcification as a repair and may lead to new bone formation at more atypical locations, such as the proximal entheseal insertion, unlike the typical enthesophytes that are because of the tractional forces, leading to increased bony surface. Interestingly one study from Spain demonstrated the change in entheseal erosions in spondyloarthritis using 2D and 3D ultrasound and showed that erosions can disappear over time [39]. This is powerful evidence indicating how microdamage with repair and tissue homeostasis restoration is a key

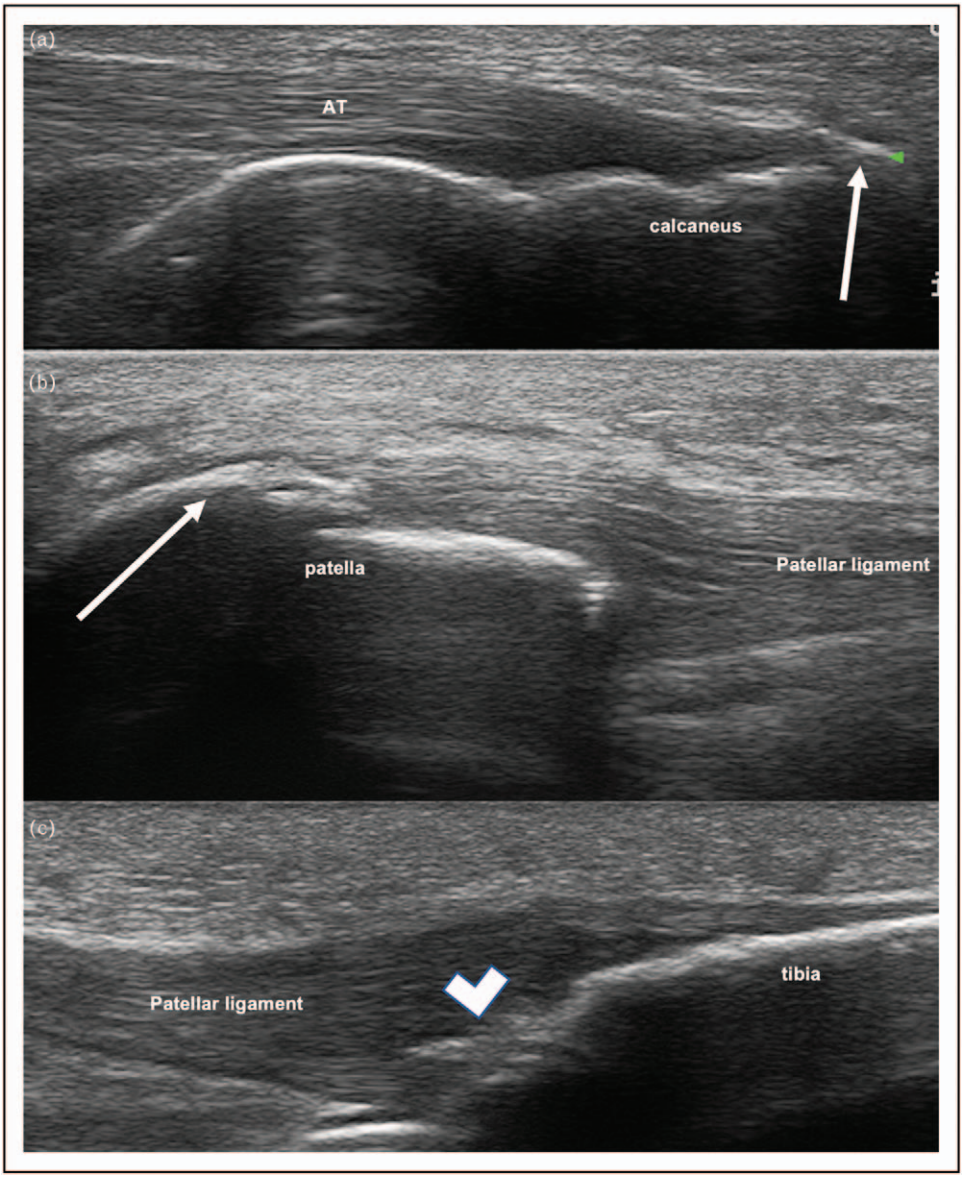

FIGURE 3. Enthesophytes in health and types of enthesophytes on ultrasound. (a) Longitudinal scan of the Achilles enthesis of a healthy person on ultrasound. A small enthesophyte is seen at the distal insertion (arrow) with no other disorders of the Achilles enthesis. AT, Achilles tendon. (b) Longitudinal scan of the Patellar ligament on ultrasound. Enthesophyte seen in the typical location at the border with increased tension at the origin of the patellar ligament (arrow). (c) Longitudinal scan of the Patellar ligament on ultrasound. Enthesophyte in the atypical location (arrow head), being in the middle of the entheseal insertion into the tibial tuberosity.

feature of the normal enthesis and that this repair response may become exaggerated during disease.

Entheseal response in spondyloarthritis patients and subtypes

Unlike the physical examination, which classifies the enthesitis as a binary finding, the ultrasound imaging allows to further characterize the entheseal changes and to potentially define and understand different phenotypes of enthesitis [40]. For example, our group has shown that comparing PsA and ankylosing spondylitis, the PsA enthesis shows approximately four times more damage – mostly being enthesophytes. This was despite having slightly less entheseal inflammation as determined by hypoechogenicity, thickening and Doppler signals on ultrasound [41]. In another patient population, in axial spondyloarthritis, psoriasis was again shown to be a risk factor for entheseal damage, mostly enthesophytes, but not for entheseal inflammation [42]. In PsA, the Koebner phenomenon that is observed as

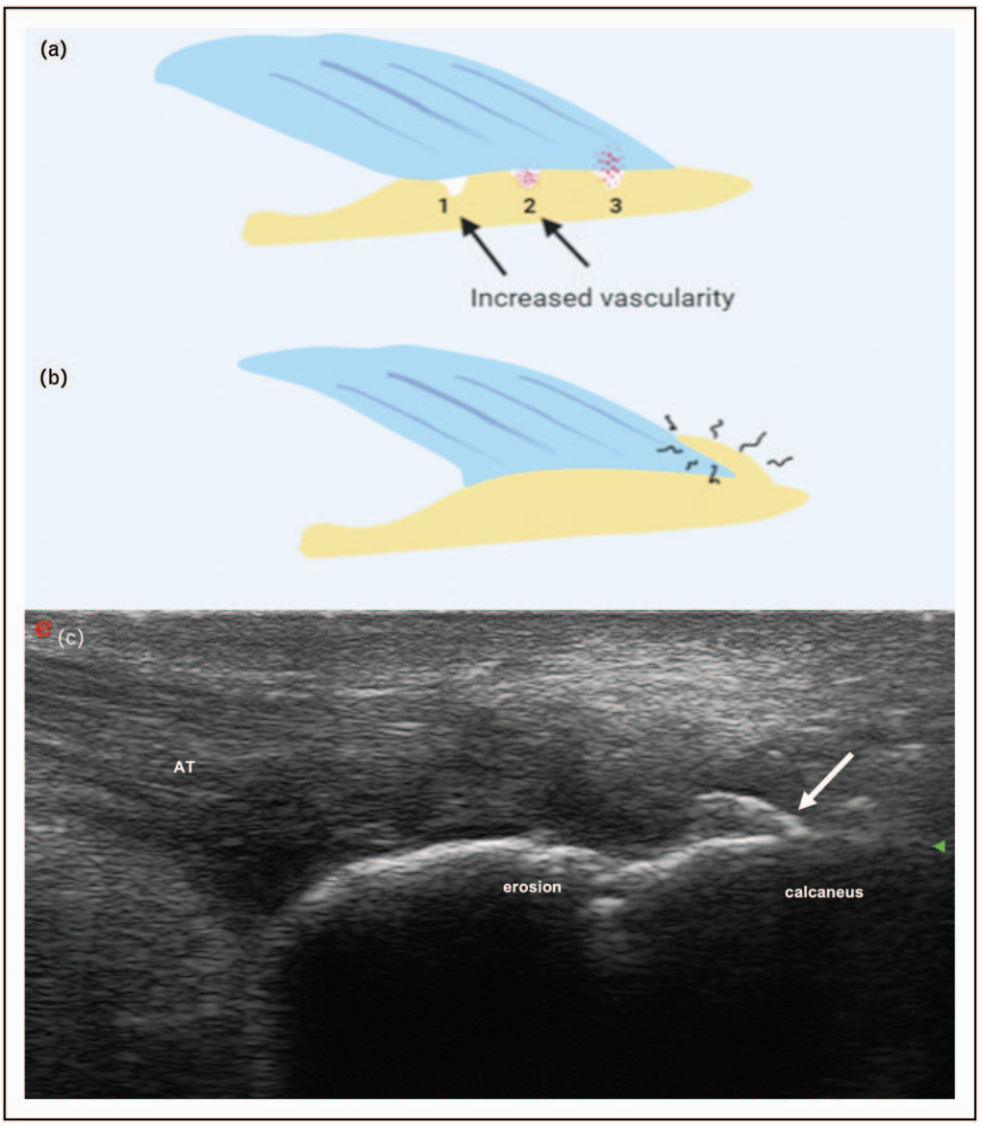

FIGURE 4. Entheseal erosions. (a) Entheseal erosions and repair process: 1: depicts normal microdamage and microfracture at enthesis and increased vascularity (arrows), 2: erosion filled with amorphous material as a repair process, 3: the repair process mimicking enthesophytes in atypical locations. (b) Tissue repair response with new bone on outside aspect of normal enthesis, which can also be a normal age-related finding. (c) Longitudinal scans of the Achilles enthesis on ultrasound in a patient with PsA. Typical erosions (arrow) are seen more proximal to the enthesophyte (*).

an exacerbated response at the level of the skin, is likely to be occurring at the deeper tissues as well, where larger and bulkier enthesophytes are detected that may be preceding an increased vascularity [27,41,42]. In parallel to this, the bulkier syndesmophytes in PsA have well been defined suggesting a similar mechanism affecting the spine and the peripheral enthesis [43]. Regardless of the underlying mechanism of the new bone formation, the ossification is preceded by vascular invasion in histology [37]. This is supported by ultrasound with the increased vascularity around the new bone formation that may or may not be proportional to the degree of other soft tissue changes. These observations may point to a vascular and/or new bone forming phenotype in spondyloarthritis. The preliminary assessment of the entheseal features in spondyloarthritis phenotypes may reflect the different mechanisms being involved, which may also partially explain the different treatment outcomes in axial disease in response to the treatments of the same modes of actions [44].

Functional enthesis responses

It has recently emerged that the mini-entheses that constrain bowstringing of the extensor and flexor tendons are an important target of inflammation in

the PsA. In disease, this is associated with Doppler signals as well as thickening and the epicentre of disease appears to be the A1 accessory pulley – a key microenthesis structure to minimize bowstringing [8,45]. Furthermore, imaging studies confirms that PsA presents inflammation affecting soft tissue, tendons and synovium. The sonographic detection of synovio-entheseal complex inflammation at metacarpophalangeal joints (i.e. extensor peritendinitis with or without synovitis) and at proximal interphalangeal joints (i.e. central slip enthesitis with or without synovitis) were common sonographic features in early PsA and useful for the differentiation with rheumatoid arthritis [7,46,47]. A common noninflammatory condition that arises in the A1 pulley is trigger finger that typically occurs in older subjects where presumed age-related changes in the pulley are a major contributory factor to the disorder.

Subcutaneous changes of the digit in healthy

In the context of dactylitis or peripheral arthritis in PsA, the link between tendons and accessory pulley functional entheses and pathological changes in the subcutaneous tissues is well described [48,49]. Subcutaneous inflammation of the digit is visualized by the loss of normal B-mode pattern with diffuse or localized hypoechoic areas on ultrasound, usually associated with Doppler signals. These changes could be interpreted as edema subsequent to vasodilation and neo-angiogenesis and/or as inflammatory involvement of the function entheseal skeleton of the digit that link skin to pulleys and flexor tendon. There is only one study that focuses on soft tissue of the digit in healthy. Rebollo-Giménez *et al.* found a positive correlation between ultrasound thickness of the subcutaneous tissue with age, male sex, BMI and the dominant hand compared with the nondominant hands [50]. These results are reminiscent of the ultrasound of enthesis in healthy and corroborate the need to study the skeleton of the digit during dactylitis and hands arthritis in PsA.

A NOVEL IMAGING INSIGHT INTO ACUTE AND CHRONIC DACTYLITIS

The pivotal role of the accessory pulleys as part of the functional enthesis network driving disease in PsA has also emerged. Recent cross sectional studies reported significant extracapsular inflammation (flexor tenosynovitis and soft tissue oedema) in early phases of dactylitis and a higher prevalence of joint synovitis in later stages [51]. Moreover, flexor tenosynovitis and soft tissue oedema have been reported strongly associated with local symptoms in course of dactylitis [52]. Painless dactylitis in established PsA appears related to synovial disease. Given that the accessory pulleys are thicker in subjects with a history of dactylitis and are the epicentre of dactylitis – an enthesitis-associated disorder linked to very high regional stressing of functional entheses may be leading to a chronic synovial pathology. Although preliminary, these data suggest a microanatomical link between extrasynovial inflammatory changes, dactylitis duration and symptoms, which could be important to understand the pathogenesis of dactylitis. Further work is needed to evaluate this concept whereby an enthesis organ-centric disorder may eventually drive a chronic synovitis.

THE IMMUNE SYSTEM OF THE ENTHESIS

What is the basis for imaging changes at the normal enthesis that share features of disease in spondyloarthritis in terms of imaging localization and thickening and new bone formation? We believe that tissue homeostatic mechanism including immune repair mechanisms are operational in the normal enthesis. The concept that the enthesis organ has its own immune system has gained traction over the last decade with seminal experiments in murine models [53]. In the last decade, it has emerged that the tissues immediately adjacent to the fibrocartilage have resident populations of immune cells including myeloid cells, ILCs, gamma delta T cells and conventional $CD4^+$ and $CD8^+$ T cells [54–56]. Therefore, the changes that we describe in health are occurring in a territory where both innate and adaptive immune cell populations are universally present in health. How the immune system may sculpt these changes that lead to new bone formation, entheseal thickening and erosion and how age impacts on this has not hitherto being considered.

Despite the growing acceptance that the enthesis has its own immune system, little is known about how this is interlinked with the concepts of Immunosenescence and Inflamm-Aging [57]. Immunosenescence is the age-associated decline of the immune system, and is theorized to contribute to increased incidence of disease in the elderly but very little is known about age-related immune changes in subjects in the fourth and fifth decades. Adaptive immune B and T-cell responses decline with older age which may be relevant for understanding why extremely old subjects wiht lots of enthesis microdamage have little new onset PsA [57]. Inflamm-Aging refers to a chronic low-grade inflammation that develops with age. As adaptive immune responses decline with age, this may be counteracted or compensated by increased innate immune

responses in older subjects but the lack of triggered adaptive immune response against joint antigens may account for the less severe inflammatory reactions [58].

CONCLUSION

Herein we described how enthesis biomechanical stressing in health may play a key role, reflected with age and BMI-related subclinical changes that mirror pathological changes occuring during disease. Experimental entheseal unloading in mice have unequivocally shown that mechanical activation is required for arthritis development, most notably from stromal cell cytokine production. How mechanical stress activates the entheseal immune system is an active area of research. It looks like factors that impact on entheseal loading and age-related microdamage in normal may be big determinants of disease development. Further studies exploring mechanisms behind the physiological changes within the entheses are essential to understand the pathogenesis of spondyloarthritis and differentiate disease from health.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Benjamin M, Toumi H, Ralphs JR, *et al.* Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. J Anat 2006; 208:471–490.
2. Benjamin M, Ralphs JR. Entheses–the bony attachments of tendons and ligaments. Ital J Anat Embryol 2001; 106(2 Suppl 1):151–157.
3. McGonagle D, Aydin SZ, Tan AL. The synovio-entheseal complex and its role in tendon and capsular associated inflammation. J Rheumatol Suppl 2012; 89:11–14.
4. McGonagle D, Lories RJ, Tan AL, Benjamin M. The concept of a 'synovio-entheseal complex' and its implications for understanding joint inflammation and damage in psoriatic arthritis and beyond. Arthritis Rheum 2007; 56:2482–2491.
5. Francois RJ, Braun J, Khan MA. Entheses and enthesitis: a histopathologic review and relevance to spondyloarthritides. Curr Opin Rheumatol 2001; 13:255–264.
6. Benjamin M, McGonagle D. The anatomical basis for disease localisation in seronegative spondyloarthropathy at entheses and related sites. J Anat 2001; 199(Pt 5):503–526.
7. Zabotti A, Salvin S, Quartuccio L, De Vita S. Differentiation between early rheumatoid and early psoriatic arthritis by the ultrasonographic study of the synovio-entheseal complex of the small joints of the hands. Clin Exp Rheumatol 2016; 34:459–465.
8. Tinazzi I, McGonagle D, Aydin SZ, *et al.* 'Deep Koebner' phenomenon of the flexor tendon-associated accessory pulleys as a novel factor in tenosynovitis and dactylitis in psoriatic arthritis. Ann Rheum Dis 2018; 77:922–925.
9. ■■ Gracey E, Burssens A, Cambre I, *et al.* Tendon and ligament mechanical loading in the pathogenesis of inflammatory arthritis. Nat Rev Rheumatol 2020; 16:193–207.

In this outstanding review, the authors introduce the cellular and molecular mechanisms in the tendons and ligaments in response to mechanical forcing.

10. Benjamin M, McGonagle D. Entheses: tendon and ligament attachment sites. Scand J Med Sci Sports 2009; 19:520–527.
11. Bakewell C, Aydin SZ, Ranganath VK, *et al.* Imaging techniques: options for the diagnosis and monitoring of treatment of enthesitis in psoriatic arthritis. J Rheumatol 2020; 47:973–982.
12. Eder L, Barzilai M, Peled N, *et al.* The use of ultrasound for the assessment of enthesitis in patients with spondyloarthritis. Clin Radiol 2013; 68:219–223.
13. Kaeley GS. Visualization of enthesitis by ultrasound: a key diagnostic tool in spondyloarthropathy diagnosis and management. Curr Rheumatol Rep 2020; 22:48.
14. Molina Collada J, Macia-Villa C, Plasencia C, *et al.* Doppler enthesitis: a potential useful outcome in the assessment of axial spondyloarthritis and psoriatic arthritis. Clin Rheumatol 2020. doi: 10.1007/s10067-020-05450-4.
15. Poulain C, D'Agostino MA, Thibault S, *et al.* Can power Doppler ultrasound of the entheses help in classifying recent axial spondyloarthritis? Data from the DESIR cohort. RMD Open 2018; 4:e000686.
16. Love TJ, Zhu Y, Zhang Y, *et al.* Obesity and the risk of psoriatic arthritis: a population-based study. Ann Rheum Dis 2012; 71:1273–1277.
17. Klingberg E, Bilberg A, Bjorkman S, *et al.* Weight loss improves disease activity in patients with psoriatic arthritis and obesity: an interventional study. Arthritis Res Ther 2019; 21:17.
18. Thorarensen SM, Lu N, Ogdie A, *et al.* Physical trauma recorded in primary care is associated with the onset of psoriatic arthritis among patients with psoriasis. Ann Rheum Dis 2017; 76:521–525.
19. Guldberg-Moller J, Terslev L, Nielsen SM, *et al.* Ultrasound pathology of the entheses in an age and gender stratified sample of healthy adult subjects: a prospective cross-sectional frequency study. Clin Exp Rheumatol 2019; 37:408–413.
20. Di Matteo A, Filippucci E, Cipolletta E, *et al.* How normal is the enthesis by ultrasound in healthy subjects? Clin Exp Rheumatol 2020; 38:472–478.
21. ■ Bakirci S, Solmaz D, Stephenson W, *et al.* Entheseal changes in response to age, body mass index, and physical activity: an ultrasound study in healthy people. J Rheumatol 2020; 47:968–972.

This study describes the prevalence of entheseal lesions on ultrasound on healthy enthesis, investigating the impact of age, BMI, sex and physical activity on inflammatory and damage-related features.

22. Thomopoulos S, Kim HM, Rothermich SY, *et al.* Decreased muscle loading delays maturation of the tendon enthesis during postnatal development. J Orthop Res 2007; 25:1154–1163.
23. Kuntz LA, Rossetti L, Kunold E, *et al.* Biomarkers for tissue engineering of the tendon-bone interface. PLoS One 2018; 13:e0189668.
24. Morel M, Boutry N, Demondion X, *et al.* Normal anatomy of the heel entheses: anatomical and ultrasonographic study of their blood supply. Surg Radiol Anat 2005; 27:176–183.
25. Binks DA, Gravallese EM, Bergin D, *et al.* Role of vascular channels as a novel mechanism for subchondral bone damage at cruciate ligament entheses in osteoarthritis and inflammatory arthritis. Ann Rheum Dis 2015; 74:196–203.
26. Aydin SZ, Bas E, Basci O, *et al.* Validation of ultrasound imaging for Achilles entheseal fibrocartilage in bovines and description of changes in humans with spondyloarthritis. Ann Rheum Dis 2010; 69:2165–2168.
27. Aydin SZ, Ash ZR, Tinazzi I, *et al.* The link between enthesitis and arthritis in psoriatic arthritis: a switch to a vascular phenotype at insertions may play a role in arthritis development. Ann Rheum Dis 2013; 72:992–995.
28. Savage L, Goodfield M, Horton L, *et al.* Regression of peripheral subclinical enthesopathy in therapy-naive patients treated with ustekinumab for moderate-to-severe chronic plaque psoriasis: a fifty-two-week, prospective, open-label feasibility study. Arthritis Rheumatol 2019; 71:626–631.
29. Yumusakhuylu Y, Kasapoglu-Gunal E, Murat S, *et al.* A preliminary study showing that ultrasonography cannot differentiate between psoriatic arthritis and nodal osteoarthritis based on enthesopathy scores. Rheumatology (Oxford) 2016; 55:1703–1704.
30. Miller BF, Olesen JL, Hansen M, *et al.* Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. J Physiol 2005; 567(Pt 3):1021–1033.
31. Docking SI, Cook J. How do tendons adapt? Going beyond tissue responses to understand positive adaptation and pathology development: A narrative review. J Musculoskelet Neuronal Interact 2019; 19:300–310.
32. Seynnes OR, Erskine RM, Maganaris CN, *et al.* Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. J Appl Physiol (1985) 2009; 107:523–530.
33. Standley RA, Harber MP, Lee JD, *et al.* Influence of aerobic cycle exercise training on patellar tendon cross-sectional area in older women. Scand J Med Sci Sports 2013; 23:367–373.

34. Blackburn JT, Padua DA, Weinhold PS, Guskiewicz KM. Comparison of triceps surae structural stiffness and material modulus across sex. Clin Biomech (Bristol, Avon) 2006; 21:159–167.
35. Flaxman TE, Smith AJ, Benoit DL. Sex-related differences in neuromuscular control: implications for injury mechanisms or healthy stabilisation strategies? J Orthop Res 2014; 32:310–317.
36. Wust RC, Morse CI, de Haan A, *et al.* Sex differences in contractile properties and fatigue resistance of human skeletal muscle. Exp Physiol 2008; 93:843–850.
37. Benjamin M, Rufai A, Ralphs JR. The mechanism of formation of bony spurs (enthesophytes) in the achilles tendon. Arthritis Rheum 2000; 43:576–583.
38. Rufai A, Ralphs JR, Benjamin M. Structure and histopathology of the insertional region of the human Achilles tendon. J Orthop Res 1995; 13:585–593.
39. de Miguel E, Falcao S, Castillo C, *et al.* Enthesis erosion in spondyloarthritis is not a persistent structural lesion. Ann Rheum Dis 2011; 70:2008–2010.
40. Aydin SZ, Bakirci S, Kasapoglu E, *et al.* The relationship between physical examination and ultrasonography of large entheses of the Achilles tendon and patellar tendon origin. J Rheumatol 2020; 47:1026–1030.
41. Arslan Alhussain F, Kasapoglu Gunal E, Kurum E, *et al.* Greater magnitude of entheseal microdamage and repair in psoriatic arthritis compared with ankylosing spondylitis on ultrasound. Rheumatology (Oxford) 2019; 58:299–303.
42. Solmaz D, Bakirci S, Jibri Z, *et al.* Psoriasis is an independent risk factor for entheseal damage in axial spondyloarthritis. Semin Arthritis Rheum 2020; 50:42–47.
43. Helliwell PS, Hickling P, Wright V. Do the radiological changes of classic ankylosing spondylitis differ from the changes found in the spondylitis associated with inflammatory bowel disease, psoriasis, and reactive arthritis? Ann Rheum Dis 1998; 57:135–140.
44. Deodhar A, Gensler LS, Sieper J, *et al.* Three multicenter, randomized, double-blind, placebo-controlled studies evaluating the efficacy and safety of ustekinumab in axial spondyloarthritis. Arthritis Rheumatol 2019; 71:258–270.
45. Tinazzi I, McGonagle D, Macchioni P, Aydin SZ. Power Doppler enhancement of accessory pulleys confirming disease localization in psoriatic dactylitis. Rheumatology (Oxford) 2020; 59:2030–2034.
46. Gutierrez M, Filippucci E, Salaffi F, *et al.* Differential diagnosis between rheumatoid arthritis and psoriatic arthritis: the value of ultrasound findings at metacarpophalangeal joints level. Ann Rheum Dis 2011; 70:1111–1114.
47. Zabotti A, Errichetti E, Zuliani F, *et al.* Early psoriatic arthritis versus early seronegative rheumatoid arthritis: role of dermoscopy combined with ultrasonography for differential diagnosis. J Rheumatol 2018; 45:648–654.
48. Zabotti A, Sakellariou G, Tinazzi I, *et al.* Novel and reliable DACTylitis glObal Sonographic (DACTOS) score in psoriatic arthritis. Ann Rheum Dis 2020; 79:1037–1043.
49. Fournie B, Margarit-Coll N, Champetier de Ribes TL, *et al.* Extrasynovial ultrasound abnormalities in the psoriatic finger. Prospective comparative power-doppler study versus rheumatoid arthritis. Joint Bone Spine 2006; 73:527–531.
50. Rebollo-Gimenez A, Martinez-Estupinan L, Olivas-Vergara O, *et al.* How variable is the volar subcutaneous tissue of the digits on b-mode and color Doppler ultrasound in non-psoriatic individuals and could it be included in a dactylitis score? Ultraschall Med 2020.
51. Girolimetto N, Macchioni P, Tinazzi I, *et al.* Ultrasonographic evidence of predominance of acute extracapsular and chronic intrasynovial patterns in 100 cases of psoriatic hand dactylitis. J Rheumatol 2020; 47:227–233.
52. Girolimetto N, Macchioni P, Tinazzi I, *et al.* Predominant ultrasonographic extracapsular changes in symptomatic psoriatic dactylitis: results from a multicenter cross-sectional study comparing symptomatic and asymptomatic hand dactylitis. Clin Rheumatol 2020; 39:1157–1165.
53. Watad A, Cuthbert RJ, Amital H, McGonagle D. Enthesitis: much more than focal insertion point inflammation. Curr Rheumatol Rep 2018; 20:41.
54. Bridgewood C, Sharif K, Sherlock J, *et al.* Interleukin-23 pathway at the enthesis: the emerging story of enthesitis in spondyloarthropathy. Immunol Rev 2020; 294:27–47.
55. Sherlock JP, Joyce-Shaikh B, Turner SP, *et al.* IL-23 induces spondyloarthropathy by acting on ROR-(t+ CD3+ CD4− CD8− entheseal resident T cells. Nat Med 2012; 18:1069–1076.
56. Watad A, Rowe H, Russell T, *et al.* Normal human enthesis harbours conventional CD4+ and CD8+ T cells with regulatory features and inducible IL-17A and TNF expression. Ann Rheum Dis 2020; 79:1044–1054.
57. Fulop T, Larbi A, Dupuis G, *et al.* Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? Front Immunol 2018; 8:1960.
58. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 2014; 69(Suppl_1):S4–S9.

Posttraumatic osteoarthritis: what have we learned to advance osteoarthritis?

Fiona E. Watt

Purpose of review
Current thinking in the study of posttraumatic osteoarthritis (PTOA) is overviewed: the osteoarthritis which follows acute joint injury. The review particularly highlights important publications in the last 18 months, also reflecting on key older literature, in terms of what have we have we learned and have yet to learn from PTOA, which can advance the osteoarthritis field as a whole.

Recent findings
PTOA is a mechanically driven disease, giving insight into mechanical drivers for osteoarthritis. A mechanosensitive molecular tissue injury response (which includes activation of pain, degradative and also repair pathways) is triggered by acute joint injury and seen in osteoarthritis. Imaging features of PTOA are highly similar to osteoarthritis, arguing against it being a different phenotype. The inflammatory pathways activated by injury contribute to early joint symptoms. However, later structural changes appear to be dissociated from traditional measures of synovial inflammation.

Summary
PTOA remains an important niche in which to understand processes underlying osteoarthritis and seek interventional targets. Whether PTOA has true molecular or clinical differences to osteoarthritis as a whole remains to be understood. This knowledge is important for a field where animal modelling of the disease relies heavily on the link between injury and osteoarthritis.

Keywords
inflammation, injury, mechanical, osteoarthritis, posttraumatic

INTRODUCTION

Musculoskeletal disorders are the second largest cause of years lived with disability worldwide: this is mostly because of the high prevalence of osteoarthritis, the most common form of arthritis, affecting ~21 million people in the United States alone [1]. Representing a major societal challenge, osteoarthritis has arguably received less focus than it deserves. Perhaps in part this is because of a misconception that osteoarthritis is an inevitable part of ageing. Although ageing is an important risk factor, only ~50% of people develop disease in their lifetime. Much of our modern understanding of disease pathogenesis has arisen from work interrogating the link between tissue damage or joint injury and osteoarthritis, in the laboratory, in preclinical models and in humans. The osteoarthritis which follows significant joint injury is so-called 'posttraumatic osteoarthritis' (PTOA) [2].

Significant knee joint injury, such as anterior cruciate ligament (ACL) rupture and acute meniscal tear is one of the single biggest risk factors for knee osteoarthritis and increasing in incidence [3,4]. For any given knee injury, ~50% of individuals will develop subsequent symptomatic disease, irrespective of whether surgical intervention occurs [5,6▪]. PTOA in its purest form is thought to account for ~12% of all cases of osteoarthritis [7]. Some believe that PTOA represents a disease 'subgroup' or phenotype, that is, a different manifestation of the disease.

Often silent at onset, insidious and intensively variable in its progression, with molecular and structural change often predating symptoms by many years, interrogating the earliest processes of osteoarthritis has often felt intractable. Studying and

Centre for Osteoarthritis Pathogenesis Versus Arthritis, Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences, University of Oxford, Oxford, UK

Correspondence to Fiona E. Watt, MD, PhD, Centre for Osteoarthritis Pathogenesis Versus Arthritis, Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences, University of Oxford, Roosevelt Drive, Oxford OX3 7FY, UK. Tel: +44 7702 864411; e-mail: fiona.watt@kennedy.ox.ac.uk

Curr Opin Rheumatol 2021, 33:74–83
DOI:10.1097/BOR.0000000000000760

KEY POINTS

- PTOA is a mechanically driven disease, which has provided insight into mechanically driven processes underlying osteoarthritis as a whole.
- A cellular, mechanosensitive, inflammatory injury response in joint tissues is triggered by acute joint injury and this response is also seen in osteoarthritis.
- Imaging features of PTOA are highly similar to osteoarthritis, arguing against it being a different phenotype but true similarities and differences need to be better understood.
- PTOA may allow us to approach the development of targeted novel therapeutics, which seek to prevent osteoarthritis.
- PTOA remains an important and tractable niche in which to better understand processes underlying osteoarthritis.

intervening in advanced human disease requires consideration of many different factors, for example, the individual experience of pain and its influences (on which many patient-reported outcomes depend) and the influence of comorbidities on the onset and course of disease [8,9]. The advantage of following those with joint trauma is that they are typically younger, with less comorbidity (at least at the outset) and the exact timing of the risk exposure is typically known, so initiating processes can be more easily studied. Animal models of PTOA also exist, which are well established, tractable platforms for studying osteoarthritis, in which translation of findings to human cohorts and the clinic is possible [10,11].

This review overviews current thinking, particularly seeking to not only highlight important publications in the last 18 months but also reflecting on key older literature, in terms of what have we learned, and have yet to learn from PTOA, which can advance the osteoarthritis field as a whole.

MECHANICAL LOAD, INJURIOUS OR OTHERWISE, IS CENTRAL TO OSTEOARTHRITIS PATHOGENESIS

There has been an increasing understanding that osteoarthritis is a mechanically driven, active cellular process with potential for intervention and cartilage regeneration, which is associated with not only substantial genetic but also other individual identifiable risk. Much, but not all of this knowledge has relied on investigating the relationship between injurious load and osteoarthritis in different settings.

Epidemiology

Identifying major aetiological factors for osteoarthritis which are likely to predict risk at an individual level has been exemplified by injury. Joint injury increases the risk of osteoarthritis 4–7-fold, being more common in professional sports people [12▪,13,14]. This is a major public health problem, affecting people during their young, working lives [3]. Just considering ACL rupture, one of a host of clinically significant soft tissue injuries at the knee alone, in the United States, there are an estimated 252 000 ACL injuries per year, for example, and this incidence appears to be increasing [4,7,15,16]. It is thought that approximately 50% of people with significant knee joint injuries, such as ACL rupture and/or acute meniscal tear subsequently develop symptomatic radiographic osteoarthritis within 10 years [5]; at least 33% of those with acute ACL rupture will have MRI-defined whole joint osteoarthritis after 5 years [17▪,18], with higher prevalence in the longer term [19▪]. The presence of meniscal tear or chondral lesions have been reproducibly shown to be independent predictors of adverse outcome after ACL rupture [20–22,23▪]. This reaffirms the importance of the integrity of the meniscus in knee osteoarthritis as a whole. Extrusion or defunctioning of the medial meniscus, acutely or chronically, appears to be a central step for many developing medial knee osteoarthritis [24,25]. It may be that these groups of individuals with either acute traumatic meniscal tear or acute symptomatic degenerative meniscal tear and associated osteoarthritis are interesting 'bridging' groups with translational potential for our understanding of osteoarthritis as a whole [26,27].

Laboratory and animal studies

Modelling tissue injury in the laboratory is a translational approach which seeks to exploit the association of joint injury with osteoarthritis. It has arguably taught us about processes in normal joint tissue physiology as well as responses in tissues which might lead to degradation [28]. It has been known for some time that connective tissues, such as articular cartilage respond directly to experimental injury by activation of intracellular signalling pathways leading to degradation and tissue repair [29–31]. Activation of these same pathways is also seen in osteoarthritis , suggesting that studies of injured tissues may shed light on processes relevant to osteoarthritis as a whole.

This connective tissue injury response is a discrete cellular response characterised by a rapid wave (within seconds) of inflammatory signalling and

subsequent inflammatory gene transcription [31,32]. This is similar to, but distinct from that induced by interleukin-(IL)-1. IL-1 has been frequently used to activate similar pathways to study degradation in articular cartilage *in vitro*. However, there is a lack of definitive evidence that classical inflammatory cytokines, such as IL-1 or TNFα are induced or secreted at biologically significant levels during either injury or osteoarthritis , or that they are pathological drivers of this process in the way they are in, for example, rheumatoid arthritis (RA) [33]. FGF-2 release from the matrix on tissue injury mediates some of the inflammatory pathway activation and appears to be an important physiological regulator in cartilage [31,34]. It was first isolated because of its large-scale release from articular cartilage on experimental sharp injury [35]. However, a pro-inflammatory factor secreted following tissue injury, which is responsible for the remaining inflammatory signalling activation has never been identified, suggesting that this may in fact be a 'hard wired' tissue response, directly responding to mechanical injury *per se*. The JNK-2 pathway would appear important for much IL-1-induced aggrecan degradation *in vitro* [36] and also after experimental acute meniscal destabilisation [37]; it is not clear if this is true of injury-induced pathways in humans.

Several injury-induced models of osteoarthritis , primarily in rodents, are now in wide use with differing levels of validation. These rely on various forms of experimental joint injury reliably leading to osteoarthritic features, particularly in genetically homogeneous models. Some models acutely destabilize the joint by surgical transection, by destabilisation or removal of the medial meniscus, or anterior cruciate ligament transection (ACLT). Others use some form of controlled external loading to cause injuries, such as acute ACL rupture (ACLr) or osteochondral fracture [38–41]. There are considerations for use of each particular model [38]. ACLT/r models will reliably lead to blood in the joint (haemarthrosis), likely to be an important clinical factor in some injuries, which benefits from its inclusion [42]. On the other hand, removing the effects of blood and other less well controlled tissue trauma may lead to a more reliable signal and easier interrogation of the connective tissue response to the mechanical destabilisation itself. One particularly utilised model is destabilisation of the medial meniscus (DMM). Here, the medial meniscus of the mouse knee is surgically destabilised by transecting the meniscotibial ligament leading to acute extrusion of the meniscus, similar to an acute peripheral detachment of medial meniscus seen in humans [10,27,30].

Several experimental observations come from this model, which are arguably highly relevant to our approach to investigation of human disease: the joint injury response and subsequent osteoarthritis development is variable rather than inevitable, dependent on a number of individual factors (for example, sex and genetic strain modify rates of disease substantially) [43]; some reparative elements of the mechanosensitive molecular response to initial injury and destabilisation are protective of later osteoarthritis [44,45]; subsequent relevant signalling and disease in an acutely unstable joint is largely dependent on mechanical joint loading [30,46]; and this is a process in which we can successfully intervene (e.g. joint offloading or modifying certain signalling pathways reduces subsequent osteoarthritis) [30,47,48] (Fig. 1).

Clinical studies and trials

The immediate tissue injury response is also detectable after joint injury at a protein level in synovial fluid (SF) in humans *in vivo*, with raised levels of most proteins falling over time [49–52]. This initial molecular response has been associated with measures of joint degradation and later structural outcomes [53,54]. Although this response is detectable

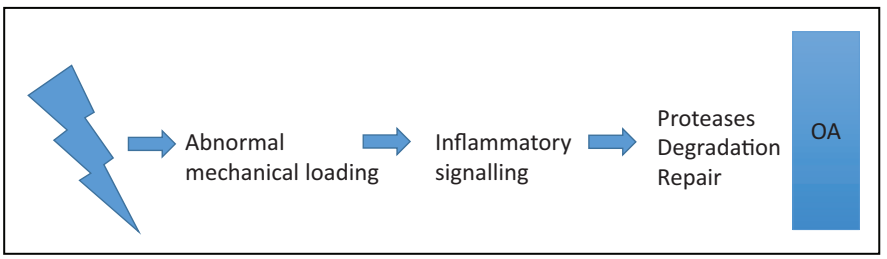

FIGURE 1. The pathway from injury to osteoarthritis: what have we learned from animal models? Destabilising injuries bring about abnormal mechanical load by surgical or nonsurgical surrogate injury. The subsequent abnormal loading across the joint appears necessary to initiate signalling pathways, which bring about joint damage and later osteoarthritis. Although this appears a linear process, it would appear both a modifiable and potentially reversible one, influenced by many individual and exogenous factors in both humans and mice (see Fig. 4).

systemically, there are often far lower protein levels in serum/plasma with poor correlation with SF [51,52]. SF is an ultrafiltrate of plasma but bathes the connective tissues in the joint, so more closely reflects molecular changes in the secretome of these joint-facing tissues. Serum and plasma proteins are in addition also influenced by nonjoint sources. This appears to be an important consideration when searching for biomarkers of the joint injury response or indeed for osteoarthritis in general, where reliability and clinical utility are sought.

One hypothesis is that many of the pathways responsible for PTOA progression might also be common to nontraumatic osteoarthritis , through common pathways relating to mechanical overload or micro-injury. Such theories are best tested in cohort or experimental medicine studies. Cohort studies of individuals with degenerative meniscal tear find that SF biomarker findings often recapitulate the findings in joint injury, and may also be associated with outcomes, such as knee pain [51,52,55].

Does re-stabilisation of the knee joint protect from osteoarthritis after injury? Surgical reconstructive surgery after ACL remains controversial in terms of whether it protects from osteoarthritis in the shorter, or longer term [6▪,18,19▪]. But studies are usually in comparison to physiotherapy, which also seeks to restabilise the joint, with both likely having an effect. If mechanical overload is detrimental or even causative in osteoarthritis , is there other evidence that offloading may be positive for the joint? In a relatively experimental surgical intervention for osteoarthritis, joint distraction, an external fixator causes joint surfaces to be pulled apart ('distracted') by ~5 mm. Groups in the Netherlands and in Japan have reported apparent clinical benefits for several years following this 6-week intervention [56,57]. This 'offloading' of the joint would intriguingly appear to lead to cartilage regeneration in the most affected compartment by MRI [58]. As the biological effects appear to be mechanically induced, can markers of the injury response give us any insight into the molecular processes underlying this apparent successful cartilage repair? A panel of 10 markers from the DMM mouse model, validated in a cohort of those with acute human knee joint injury was measured in the SF of just 20 individuals at three time points during the distraction period and their change related to patient-reported outcome [59▪]. Interestingly 6/10 markers modulated by injury were also influenced by this offloading. An increase in two molecules over the course of distraction, FGF-2 and TGFβ was associated with clinical response over a 6-month period. This study suggests that mechanoresponsive genes and markers identified in a joint injury setting may be informative translational markers for osteoarthritis as a whole.

INFLAMMATORY PATHWAYS ARE MECHANICALLY ACTIVATED TARGETS IN JOINT INJURY AND IN OSTEOARTHRITIS

It is clear then that when we refer to inflammatory pathways in osteoarthritis that this is largely tissue-based inflammation, perhaps associated with innate immune pathway activation [60,61] (rather than a humoral response as is seen in RA). It is impossible to discuss this in isolation from mechanosensitive pathways as these appear one and the same. However, it is worthwhile examining some of the inflammatory targets that have come out of injury/PTOA studies.

It is worth reminding ourselves of the striking molecular similarities particularly relating to the outcome of inflammatory pathway activation, matrix-olysis in the injured knee and the osteoarthritic knee, albeit with difference in the magnitude of elevations of fragments of COMP, collagen II and aggrecan, suggesting a similar process, acute versus chronic [62–66].

Laboratory and animal studies

Recent studies in DMM have identified molecules, which if blocked modify disease-relevant readouts and would thus appear to be potential therapeutic targets; for example, molecules driving pain, such as NGF (for which blockade by monoclonal antibodies has completed late phase human trials) or CCL-2 (MCP-1), a molecule, which has been variously targeted in oncological and inflammatory disease [67–69]. Other pathways if enhanced may lead to regeneration, such as TGFβ, CTGF and FGF-2, albeit with safety considerations in humans [45].

IL-6 or its signalling pathway have also been implicated in the pathogenesis of murine PTOA [48]. However, experiments in animal models can give conflicting findings [70]. IL-6 has been associated with osteoarthritis progression in the disease as a whole, having been targeted in recent clinical trials of established osteoarthritis [71,72]. Translational studies in humans are needed to deconvolute the role of IL-6 and its signalling pathway and whether or not it truly represents a treatment target in either PTOA, established disease or both [2].

Clinical studies and trials

There is currently no accepted model for delivering prevention of osteoarthritis studies after joint injury (conventional outcomes, such as radiography would

require these trials to be many years long). However, short-term studies, primarily focussing on knee-based symptomatic outcomes or molecular outcomes have been carried out for two agents in this space. An initial proof-of-concept randomised controlled trial (RCT) delivering (a recombinant interleukin-1 (IL-1) receptor antagonist anakinra) or placebo to just 11 participants [73] sought to shift this paradigm, reporting possible effects over a fairly short time frame. The second RCT was in young people with ACL injury, with patient-reported outcomes and biomarkers collected after randomisation to intra-articular dexamethasone or isotonic saline [74]. This showed a substantial effect of dexamethasone on measures such as the collagen degradation maker in SF, CTX-II. Injury, and its resolution or otherwise, would appear to be a critical time for the joint. What relative effects corticosteroids have on simultaneous repair pathways and how they influence longer term clinical outcomes are yet to be understood. This is of interest, given the reports of negative effects on cartilage of repeated steroid treatments in knee osteoarthritis progression [75].

There have been a number of disappointments for inflammatory targets in osteoarthritis clinical trials; it seemed an obvious hypothesis that traditional antirheumatic drugs and biologics used in the treatment of RA might suppress the inflammatory processes and associated symptoms in osteoarthritis. But their outcome is less than resounding, and has left many questioning whether this rules out rather than rules in 'classical inflammation' as a disease target in human osteoarthritis [76–78]. Part of this assumption was that synovial inflammation present in osteoarthritis was a treatment target as it is in RA. But insight from the PTOA setting is that synovial inflammation may be dislinked from other processes in the disease or simply a bystander or secondary phenomenon. Its presence by MRI or biomarker measurement at 2 years after knee injury did not correlate with later outcomes, either structural [17▪] or patient-reported [79]. More work is needed to understand whether measurement of processes nearer to the time of injury may have more bearing on these outcomes.

Not all inflammation is the same. Modulating a single potentially adverse but often short-lived process near to an injury may be very different to attempting to modulate such pathways in a chronic way in perhaps irreversibly damaged joint tissue or an irreversibly mechanically challenged joint environment, as may be true in established radiographic disease. Joint injury may be quite different to osteoarthritis in this respect but we have done little so far to test this.

INSIGHTS FROM IMAGING POSTTRAUMATIC OSTEOARTHRITIS

Imaging outcomes have been challenging in osteoarthritis research, with many agreeing that issues of sensitivity and specificity of X-ray and MRI outcomes, respectively, have held back progress in the field generally, reducing our ability to take potentially viable targets through clinical trials [80]. Assessment of joint injury by these two modalities has thrown a spotlight on some particular issues and unanswered questions for the field as a whole. How can cartilage swell (get thicker) on MRI after injury but yet be associated with joint space narrowing on X-ray? [81] Why should we focus on articular cartilage change when the bone changes in response to joint destabilisation arguably occur far earlier [82,83▪]. It is also intriguing that the pattern of flattening and condylar osteophytosis, whether in an ACL-deficient knee or primary osteoarthritic knee appears the same [83▪] and mirrors that seen in DMM in the mouse [84]. This suggests that, whatever the cause, the effects of mechanical forces in the disease may be fairly universal, and the shared aspects of PTOA and non-PTOA are perhaps greater than the perceived differences.

POSTTRAUMATIC OSTEOARTHRITIS AS A PLATFORM FOR DESIGNING EXPERIMENTAL MEDICINE STUDIES AND TRIALS

Interventional studies at or near the time of joint injury, which seek to prevent an adverse process in the joint in the longer term are a form of secondary prevention (where preventing the injury in the first place would be primary prevention) [85]. Such an approach is really quite different to what we seek to do in drug trials in established osteoarthritis (Fig. 2).

In recent years, considerations about the definition of early osteoarthritis have followed guidelines around the conduct of trials in established osteoarthritis. These are important if we are to successfully intervene earlier in the disease [86,87]. However, there is no international consensus on the design and conduct of trials testing interventions seeking to prevent osteoarthritis after joint injury and a number of challenges and opportunities have been identified by triallists in this area [88,89]. As such, there is no Food and Drug Administration (FDA) 'label' for osteoarthritis prevention, creating a barrier to clinical translation.

In 2017, an international consensus workshop of international experts and stakeholders produced the first considerations in this therapeutic area [90▪]. One of the identified key unmet research needs was for valid biomarkers, which can stratify patients for trials (selecting those at highest risk to weight trials

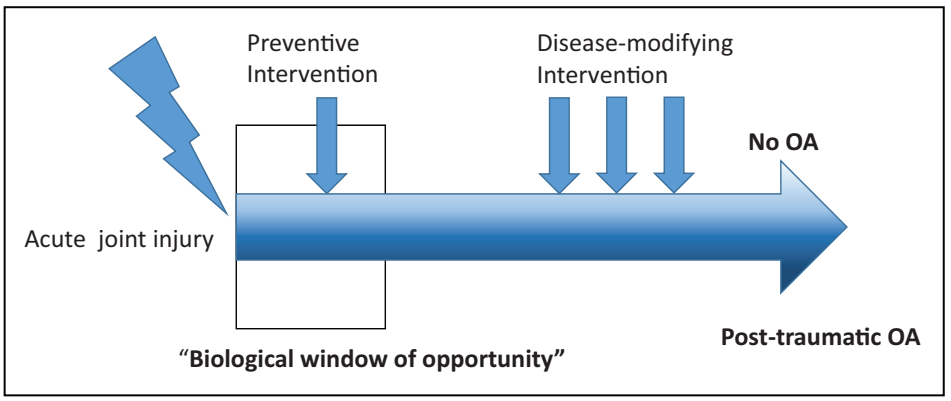

FIGURE 2. Intervening to prevent posttraumatic osteoarthritis. A preventive intervention in the context of injury can be delivered in a 'window of opportunity', near to the time of the injury. This is a different approach to delivering disease–modifying interventions in early or established disease and is a therapeutic opportunity which is unique to PTOA, but could inform our approach to treatment of OA. OA, osteoarthritis; PTOA, posttraumatic osteoarthritis.

towards 'success' in identifying a signal) or act as surrogate endpoints (to shorten trials and make them financially viable). It is interesting to consider that these have been recognised as arguably two of the biggest priorities for osteoarthritis research overall [91,92].

If we are to develop and translate our findings to the clinic, continued validation work in cohorts to support guidelines development including all aspects of design and delivery is necessary (Fig. 3). Involving Pharma and regulators would seem essential in accelerating drug development and potentially opening up a fresh approach for osteoarthritis therapeutics.

Changing the paradigm: stratification and intervening to prevent

Not all osteoarthritis progresses: there is evidence that it may reverse or stabilise in a substantial number [93]. Whilst risk factors for osteoarthritis progression are fairly well established, there is no clinically accepted way of predicting outcome at an individual level or even in populations with osteoarthritis. Recently a large FNIH-OARSI consortium was created to identify biomarkers of progression. To date, most work has been performed in serum or plasma, primarily measuring cartilage matrix proteins or their fragments, which are lost upon degeneration. Although effects in prognostic models were seen, for CTX-II and urinary NTX-1, for example [94] and certain imaging biomarkers [95], their effects once other factors are accounted for appear relatively weak. It is difficult to see how this will map to an algorithm fit for clinical trials or the clinic. Clinical factors, such as age, sex and obesity and the radiographic stage of the disease are still the most reliable predictors of progression in osteoarthritis.

So can we do better in PTOA? If we can identify molecular pathways or their markers which predict

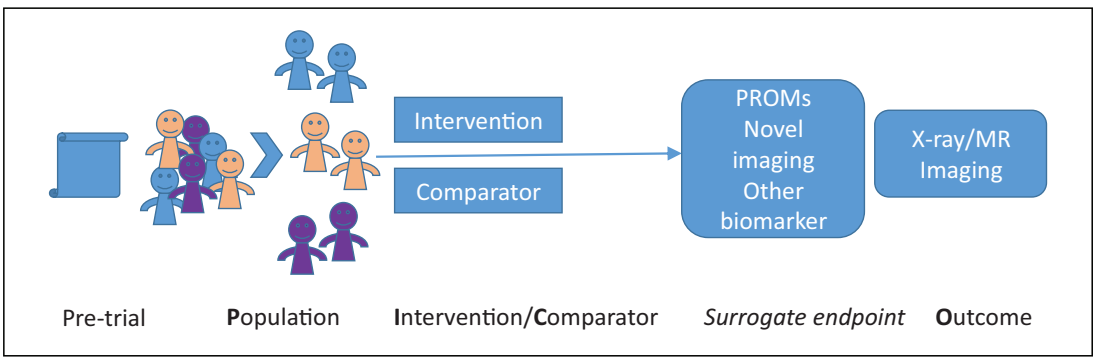

FIGURE 3. Considerations for the design and delivery of interventional studies at the time of injury. In designing studies at the time of joint injury, aspects such as governance and approvals, who to include (and exclude) in such trials using approaches including stratification, when interventions need to occur (the 'window of opportunity') and how they should be delivered (mode, challenges of intra-articular delivery) should be considered. An additional consideration is what molecular, clinical and imaging outcomes are informative, how they relate to each other and whether true 'surrogate endpoints' can be established, which shorten studies.

at the time of their joint injury those individuals at high risk of later PTOA, we can make progress in intervening in this process to prevent osteoarthritis. There are a number of positives here. There is a strong clinical argument for stratification in this setting. Patients want to know their risk; those at highest risk would likely benefit from an effective therapeutic, but safety and cost would likely argue against treating all (given the proportion who do well). There is less comorbidity and other pharmacology to confound outcomes than are seen in established osteoarthritis cohorts seeking valid biomarkers, and the ability to make measurements at the time of injury and sequentially from this point theoretically makes any biomarker easier to follow in a population.

HOW SIMILAR IS POSTTRAUMATIC OSTEOARTHRITIS TO NONTRAUMATIC OSTEOARTHRITIS ?

One of the biggest questions for this field is how similar (or different) PTOA and other posttraumatic osteoarthritis truly are. Whether there are any subgroups that are actually definable in osteoarthritis remains uncertain [96]. There have been reports that PTOA is different as it occurs in younger people (because of when these injuries occur) or is faster progressing (still uncertain given the risk of ascertainment bias here). However, there remains little compelling radiological, clinical or molecular evidence that truly delineates PTOA as a different disease or even a true subgroup or different phenotype of osteoarthritis. The clinical endpoint looks remarkably similar, albeit in an often younger person ('young people with old knees'-L.S. Lohmander). The structural appearances look the same [83▪] and the molecular changes within the joint look similar at the point of developing disease, as far as has been described at a candidate protein level [62,97]. Is a person with PTOA someone who has brought forward the osteoarthritis that they would have developed anyway, because of other risk factors? Or are there different processes at play, which are governed by different genetic risk and predisposing factors? We know that obesity, ageing, malalignment and a defunctioning meniscus are important in predicting accelerated knee osteoarthritis , whether you have been exposed to joint trauma or not [98,99] (Fig. 4). Those developing accelerated knee osteoarthritis which is nontraumatic were twice as likely to have degeneration of ACL, again suggesting a common pathway may be likely [100].

Are PTOA and more 'usual' osteoarthritis genetically the same, or different? Knowing what genes confer risk of PTOA would be invaluable in answering this. About 60% of all knee osteoarthritis risk is estimated to be heritable. Despite large-scale genome-wide association studies (GWAS) in osteoarthritis, which often exclude cases of PTOA, there are no validated genetic predictive markers for knee osteoarthritis , although this approach has reproducibly identified a number of loci and relevant biological pathways. Now a total of ~90 loci have been associated with osteoarthritis , with a growing number as case–control studies get ever larger [101,102]. It is interesting to note that several 'hits' are in genes known to be mechanosensitive, or in repair pathways. However, an individual's genetic risk of osteoarthritis after an acute joint injury is unknown, as is the extent of any such risk being

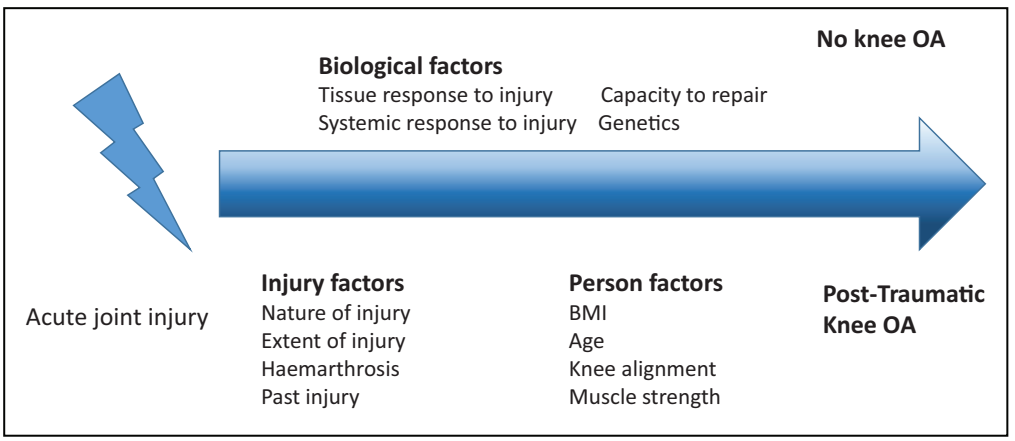

FIGURE 4. Factors influencing development of posttraumatic osteoarthritis are similar to osteoarthritis. A number of factors influence or predict the development of PTOA, although more work is needed to fully understand their relative influence. These can be divided into related groups of injury factors, person factors and biological factors. It can be seen that there is likely to be much overlap with OA as a whole. OA, osteoarthritis; PTOA, posttraumatic osteoarthritis.

shared with those at risk of nontraumatic osteoarthritis. Interestingly, some osteoarthritis risk variants conferred higher risk in those giving a retrospective history of knee injury than that seen in nontraumatic cases [103]. However, retrospective injury ascertainment likely lacks specificity, including those with early osteoarthritis presentation. No genomics studies have been performed in prospective acute knee injury cohorts or examined more recently identified loci. Testing whether some of these genes might mediate their effects via mechanically induced pathways might be amenable to a PTOA setting.

CONCLUSION

Clinically significant injury to a joint can be thought of as a 'joint attack', akin to a heart attack: a dangerous situation for some with an opportunity for prevention and outcome modification, which is time urgent. Whether considered as a subgroup of osteoarthritis or the same disease, studies in PTOA to date have been an achievable way of making traction in our understanding of osteoarthritis. We need to discover more about individual responses to tissue injury, whether these are governed by genetic variants and indeed whether there are true osteoarthritis 'subgroups'. This will allow us to stratify and move to treat those at risk. Careful analysis, testing unproven hypotheses around disease phenotyping is much needed. PTOA may be a minority sport, but researching a well defined niche will likely allow us a much-needed foothold on the insurmountable problem of osteoarthritis.

Acknowledgements

Fiona Watt acknowledges the expertise, discussion and support of colleagues and collaborators in this important and developing area, particularly Tonia Vincent, Andy Williams, Andrew Judge, Luke Jostins-Dean, Ele Zeggini, Debbie Mason, Virginia Kraus and Stefan Lohmander. Whilst this manuscript seeks to express my summary of current opinion and sometimes personal viewpoints, it is likely to also reflect some views shared and expressed by others and I wish to fully acknowledge them.

Financial support and sponsorship

This work was supported by the Centre for OA Pathogenesis Versus Arthritis (grant 21621). F.W. is supported by a UKRI Future Leaders Fellowship (S016538) and the NIHR Oxford Biomedical Research Centre. She is also a member of Centre for Sport, Exercise and Osteoarthritis Research Versus Arthritis (Grant 21595).

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Conflicts of interest

F.W. has received previous clinical study grants from Pfizer and Astellas Pharma in relation to studies in osteoarthritis not related to this work.

REFERENCES AND RECOMMENDED READING

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

- ■ of special interest
- ■■ of outstanding interest

1. Furman BD, Olson SA, Guilak F. The development of posttraumatic arthritis after articular fracture. J Orthop Trauma 2006; 20:719–725.
2. Wang LJ, Zeng N, Yan ZP, *et al.* Posttraumatic osteoarthritis following ACL injury. Arthritis Res Ther 2020; 22:57.
3. Roos EM. Joint injury causes knee osteoarthritis in young adults. Curr Opin Rheumatol 2005; 17:195–200.
4. Showery JE, Kusnezov NA, Dunn JC, *et al.* The rising incidence of degenerative and posttraumatic osteoarthritis of the knee in the United States Military. J Arthroplasty 2016; 31:2108–2114.
5. Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. Am J Sports Med 2007; 35:1756–1769.
6. ■ Cinque ME, Dornan GJ, Chahla J, *et al.* High rates of osteoarthritis develop after anterior cruciate ligament surgery: an analysis of 4108 patients. Am J Sports Med 2018; 46:2011–2019.

A large longitudinal observational study examining clinical outcomes after ACL reconstruction.

7. Brown TD, Johnston RC, Saltzman CL, *et al.* Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease. J Orthop Trauma 2006; 20:739–744.
8. Teirlinck CH, Dorleijn DMJ, Bos PK, *et al.* Prognostic factors for progression of osteoarthritis of the hip: a systematic review. Arthritis Res Ther 2019; 21:192.
9. Swain S, Sarmanova A, Coupland C, *et al.* Comorbidities in osteoarthritis: a systematic review and meta-analysis of observational studies. Arthritis Care Res (Hoboken) 2020; 72:991–1000.
10. Glasson SS. In vivo osteoarthritis target validation utilizing genetically-modified mice. Curr Drug Targets 2007; 8:367–376.
11. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. Osteoarthritis Cartilage 2010; 18(Suppl 3):S17–23.
12. ■ Khan T, Alvand A, Prieto-Alhambra D, *et al.* ACL and meniscal injuries increase the risk of primary total knee replacement for osteoarthritis: a matched case-control study using the Clinical Practice Research Datalink (CPRD). Br J Sports Med 2019; 53:965–968.

UK-based analysis of usual clinical care data seeking to quantify the true increase in relative risk of osteoarthritis after joint injury.

13. Gelber AC, Hochberg MC, Mead LA, *et al.* Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. Ann Intern Med 2000; 133:321–328.
14. Fernandes GS, Parekh SM, Moses J, *et al.* Prevalence of knee pain, radiographic osteoarthritis and arthroplasty in retired professional footballers compared with men in the general population: a cross-sectional study. Br J Sports Med 2018; 52:678–683.
15. Frobell RB, Lohmander LS, Roos HP. Acute rotational trauma to the knee: poor agreement between clinical assessment and magnetic resonance imaging findings. Scand J Med Sci Sports 2007; 17:109–114.
16. Griffin LY, Albohm MJ, Arendt EA, *et al.* Understanding and preventing noncontact anterior cruciate ligament injuries: a review of the Hunt Valley II meeting, January 2005. Am J Sports Med 2006; 34:1512–1532.
17. ■ Roemer FW, Englund M, Turkiewicz A, *et al.* Molecular and structural biomarkers of inflammation at two years after acute anterior cruciate ligament injury do not predict structural knee osteoarthritis at five years. Arthritis Rheumatol 2019; 71:238–243.

Further analysis from the KANON data set examining both synovial fluid biomarkers and imaging-based measures of synovial inflammation and their relationship to imaging-based outcomes.

18. Frobell RB, Roos HP, Roos EM, *et al.* Treatment for acute anterior cruciate ligament tear: five year outcome of randomised trial. BMJ 2013; 346:f232.
19. ■ Kvist J, Filbay S, Andersson C, *et al.* Radiographic and symptomatic knee osteoarthritis 32 to 37 years after acute anterior cruciate ligament rupture. Am J Sports Med 2020; 48:2387–2394.

Very long-term follow-up of cases after ACL rupture, demonstrating its prevalence at this late time.

20. Van Ginckel A, Verdonk P, Witvrouw E. Cartilage adaptation after anterior cruciate ligament injury and reconstruction: implications for clinical management and research? A systematic review of longitudinal MRI studies. Osteoarthritis Cartilage 2013; 21:1009–1024.
21. van Meer BL, Oei EH, Meuffels DE, *et al.* Degenerative changes in the knee 2 years after anterior cruciate ligament rupture and related risk factors: a prospective observational follow-up study. Am J Sports Med 2016; 44:1524–1533.
22. Jones MH, Spindler KP. Risk factors for radiographic joint space narrowing and patient reported outcomes of posttraumatic osteoarthritis after ACL reconstruction: data from the MOON cohort. J Orthop Res 2017; 35:1366–1374.
23. ■ Pedersen M, Johnson JL, Grindem H, *et al.* Meniscus or cartilage injury at the time of anterior cruciate ligament (ACL) tear are associated with worse prognosis for patient-reported outcome 2 to 10 years after ACL injury: a systematic review. J Orthop Sports Phys Ther 2020; 50:490–502.

Important systematic review in this area, bringing together data, which suggests that meniscal and chondral injury are both important independent risk factors, regardless of the nature of other injury.

24. Englund M, Lohmander LS. Risk factors for symptomatic knee osteoarthritis fifteen to twenty-two years after meniscectomy. Arthritis Rheum 2004; 50:2811–2819.
25. Englund M, Guermazi A, Roemer FW, *et al.* Meniscal tear in knees without surgery and the development of radiographic osteoarthritis among middle-aged and elderly persons: the Multicenter Osteoarthritis Study. Arthritis Rheum 2009; 60:831–839.
26. Englund M, Guermazi A, Lohmander LS. The meniscus in knee osteoarthritis. Rheum Dis Clin North Am 2009; 35:579–590.
27. Rai MF, Brophy RH, Rosen V. Molecular biology of meniscus pathology: lessons learned from translational studies and mouse models. J Orthop Res 2020; 38:1895–1904.
28. Lee JH, Fitzgerald JB, Dimicco MA, Grodzinsky AJ. Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. Arthritis Rheum 2005; 52:2386–2395.
29. Alexander S, Watt F, Sawaji Y, *et al.* Activin A is an anticatabolic autocrine cytokine in articular cartilage whose production is controlled by fibroblast growth factor 2 and NF-kappaB. Arthritis Rheum 2007; 56:3715–3725.
30. Burleigh A, Chanalaris A, Gardiner MD, *et al.* Joint immobilization prevents murine osteoarthritis and reveals the highly mechanosensitive nature of protease expression in vivo. Arthritis Rheum 2012; 64:2278–2288.
31. Watt FE, Ismail HM, Didangelos A, *et al.* Src and fibroblast growth factor 2 independently regulate signaling and gene expression induced by experimental injury to intact articular cartilage. Arthritis Rheum 2013; 65:397–407.
32. Gruber J, Vincent TL, Hermansson M, *et al.* Induction of interleukin-1 in articular cartilage by explantation and cutting. Arthritis Rheum 2004; 50:2539–2546.
33. Vincent TL. IL-1 in osteoarthritis: time for a critical review of the literature. F1000Res 2019; 8.
34. Vincent T, Saklatvala J. Basic fibroblast growth factor: an extracellular mechanotransducer in articular cartilage? Biochem Soc Trans 2006; 34(Pt 3):456–457.
35. Vincent T, Hermansson M, Bolton M, *et al.* Basic FGF mediates an immediate response of articular cartilage to mechanical injury. Proc Natl Acad Sci U S A 2002; 99:8259–8264.
36. Ismail HM, Yamamoto K, Vincent TL, *et al.* Interleukin-1 acts via the JNK-2 signaling pathway to induce aggrecan degradation by human chondrocytes. Arthritis Rheumatol 2015; 67:1826–1836.
37. Ismail HM, Miotla-Zarebska J, Troeberg L, *et al.* JNK2 controls aggrecan degradation in murine articular cartilage and the development of experimental osteoarthritis. Arthritis Rheumatol 2015; 68:1165–1171.
38. Christiansen BA, Guilak F, Lockwood KA, *et al.* Noninvasive mouse models of posttraumatic osteoarthritis. Osteoarthritis Cartilage 2015; 23:1627–1638.
39. Gilbert SJ, Bonnet CS, Stadnik P, *et al.* Inflammatory and degenerative phases resulting from anterior cruciate rupture in a noninvasive murine model of posttraumatic osteoarthritis. J Orthop Res 2018; 36:2118–2127.
40. Poulet B, Hamilton RW, Shefelbine S, Pitsillides AA. Characterizing a novel and adjustable noninvasive murine joint loading model. Arthritis Rheum 2011; 63:137–147.
41. Furman BD, Strand J, Hembree WC, *et al.* Joint degeneration following closed intraarticular fracture in the mouse knee: a model of posttraumatic arthritis. J Orthop Res 2007; 25:578–592.
42. Olsson O, Isacsson A, Englund M, Frobell RB. Epidemiology of intra- and peri-articular structural injuries in traumatic knee joint hemarthrosis - data from 1145 consecutive knees with subacute MRI. Osteoarthritis Cartilage 2016; 24:1890–1897.
43. Ma HL, Blanchet TJ, Peluso D, *et al.* Osteoarthritis severity is sex dependent in a surgical mouse model. Osteoarthritis Cartilage 2007; 15:695–700.
44. Chia SL, Sawaji Y, Burleigh A, *et al.* Fibroblast growth factor 2 is an intrinsic chondroprotective agent that suppresses ADAMTS-5 and delays cartilage degradation in murine osteoarthritis. Arthritis Rheum 2009; 60:2019–2027.
45. Tang X, Muhammad H, McLean C, *et al.* Connective tissue growth factor contributes to joint homeostasis and osteoarthritis severity by controlling the matrix sequestration and activation of latent TGFbeta. Ann Rheum Dis 2018; 77:1372–1380.
46. Chong K, Chanalaris A, Burleigh A, *et al.* FGF2 drives changes in gene expression following cartilage injury in vitro and in vivo. Arthritis Rheum 2013; 5:2346–2355.
47. Chong KW, Chanalaris A, Burleigh A, *et al.* Fibroblast growth factor 2 drives changes in gene expression following injury to murine cartilage in vitro and in vivo. Arthritis Rheum 2013; 65:2346–2355.
48. Latourte A, Cherifi C, Maillet J, *et al.* Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. Ann Rheum Dis 2017; 76:748–755.
49. Cuellar VG, Cuellar JM, Golish SR, *et al.* Cytokine profiling in acute anterior cruciate ligament injury. Arthroscopy 2010; 26:1296–1301.
50. Bigoni M, Sacerdote P, Turati M, *et al.* Acute and late changes in intraarticular cytokine levels following anterior cruciate ligament injury. J Orthop Res 2013; 31:315–321.
51. Struglics A, Larsson S, Kumahashi N, *et al.* Changes in cytokines and aggrecan ARGS neoepitope in synovial fluid and serum and in C-terminal crosslinking telopeptide of type II Collagen and N-terminal crosslinking telopeptide of type i collagen in urine over five years after anterior cruciate ligament rupture: an exploratory analysis in the knee anterior cruciate ligament, nonsurgical versus surgical treatment trial. Arthritis Rheumatol 2015; 67:1816–1825.
52. Watt FE, Paterson E, Freidin A, *et al.* Acute molecular changes in synovial fluid following human knee injury: association with early clinical outcomes. Arthritis Rheumatol 2016; 68:2129–2140.
53. Tourville TW, Poynter ME, DeSarno MJ, *et al.* Relationship between synovial fluid ARGS-aggrecan fragments, cytokines, MMPs, and TIMPs following acute ACL injury: a cross-sectional study. J Orthop Res 2015; 33:1796–803.
54. Amano K, Huebner JL, Stabler TV, *et al.* Synovial fluid profile at the time of anterior cruciate ligament reconstruction and its association with cartilage matrix composition 3 years after surgery. Am J Sports Med 2018; 46:890–899.
55. Cuellar JM, Scuderi GJ, Cuellar VG, *et al.* Diagnostic utility of cytokine biomarkers in the evaluation of acute knee pain. J Bone Joint Surg Am 2009; 91:2313–2320.
56. van der Woude JA, van Heerwaarden RJ, Spruijt S, *et al.* Six weeks of continuous joint distraction appears sufficient for clinical benefit and cartilaginous tissue repair in the treatment of knee osteoarthritis. Knee 2016; 23:785–791.
57. Wiegant K, Intema F, van Roermund PM, *et al.* Evidence of cartilage repair by joint distraction in a canine model of osteoarthritis. Arthritis Rheumatol 2015; 67:465–474.
58. Intema F, Van Roermund PM, Marijnissen AC, *et al.* Tissue structure modification in knee osteoarthritis by use of joint distraction: an open 1-year pilot study. Ann Rheum Dis 2011; 70:1441–1446.
59. ■ Watt FE, Hamid B, Garriga C, *et al.* The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis. Osteoarthritis Cartilage 2020; 28:324–333.

Syovial fluid biomarkers of joint injury are tested in another mechanodependent situation, knee joint distraction with evidence of clinically relevant translation to this OA setting of selected markers.

60. Wang Q, Rozelle AL, Lepus CM, *et al.* Identification of a central role for complement in osteoarthritis. Nat Med 2011; 17:1674–1679.
61. Struglics A, Okroj M, Sward P, *et al.* The complement system is activated in synovial fluid from subjects with knee injury and from patients with osteoarthritis. Arthritis Res Ther 2016; 18:223.
62. Dahlberg L, Roos H, Saxne T, *et al.* Cartilage metabolism in the injured and uninjured knee of the same patient. Ann Rheum Dis 1994; 53:823–827.
63. Lohmander LS, Saxne T, Heinegard DK. Release of cartilage oligomeric matrix protein (COMP) into joint fluid after knee injury and in osteoarthritis. Ann Rheum Dis 1994; 53:8–13.
64. Lohmander LS, Ionescu M, Jugessur H, Poole AR. Changes in joint cartilage aggrecan after knee injury and in osteoarthritis. Arthritis Rheum 1999; 42:534–544.
65. Lohmander LS, Atley LM, Pietka TA, Eyre DR. The release of crosslinked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. Arthritis Rheum 2003; 48:3130–3139.
66. Larsson S, Lohmander LS, Struglics A. Synovial fluid level of aggrecan ARGS fragments is a more sensitive marker of joint disease than glycosaminoglycan or aggrecan levels: a cross-sectional study. Arthritis Res Ther 2009; 11:R92.
67. von Loga IS, El-Turabi A, Jostins L, *et al.* Active immunisation targeting nerve growth factor attenuates chronic pain behaviour in murine osteoarthritis. Ann Rheum Dis 2019; 78:672–675.
68. Miotla Zarebska J, Chanalaris A, Driscoll C, *et al.* CCL2 and CCR2 regulate pain-related behaviour and early gene expression in posttraumatic murine osteoarthritis but contribute little to chondropathy. Osteoarthritis Cartilage 2017; 25:406–412.
69. Raghu H, Lepus CM, Wang Q, *et al.* CCL2/CCR2, but not CCL5/CCR5, mediates monocyte recruitment, inflammation and cartilage destruction in osteoarthritis. Ann Rheum Dis 2017; 76:914–922.
70. de Hooge AS, van de Loo FA, Bennink MB, *et al.* Male IL-6 gene knock out mice developed more advanced osteoarthritis upon aging. Osteoarthritis Cartilage 2005; 13:66–73.

71. Livshits G, Zhai G, Hart DJ, *et al.* Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The Chingford Study. Arthritis Rheum 2009; 60:2037–2045.

72. Stannus O, Jones G, Cicuttini F, *et al.* Circulating levels of IL-6 and TNF-alpha are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage 2010; 18:1441–1447.

73. Kraus VB, Birmingham J, Stabler TV, *et al.* Effects of intraarticular IL1-Ra for acute anterior cruciate ligament knee injury: a randomized controlled pilot trial (NCT00332254). Osteoarthritis Cartilage 2012; 20:271–278.

74. Lattermann C, Jacobs CA, Proffitt Bunnell M, *et al.* A multicenter study of early anti-inflammatory treatment in patients with acute anterior cruciate ligament tear. Am J Sports Med 2017; 45:325–333.

75. McAlindon TE, LaValley MP, Harvey WF, *et al.* Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: a randomized clinical trial. JAMA 2017; 317:1967–1975.

76. Kloppenburg M, Ramonda R, Bobacz K, *et al.* Etanercept in patients with inflammatory hand osteoarthritis (EHOA): a multicentre, randomised, double-blind, placebo-controlled trial. Ann Rheum Dis 2018; 77:1757–1764.

77. Kloppenburg M, Peterfy C, Haugen IK, *et al.* Phase IIa, placebo-controlled, randomised study of lutikizumab, an antiinterleukin-1alpha and antiinterleukin-1beta dual variable domain immunoglobulin, in patients with erosive hand osteoarthritis. Ann Rheum Dis 2019; 78:413–420.

78. Kingsbury SR, Tharmanathan P, Keding A, Corbacho B, Watt FE, Scott DL, Roddy E, Birrell F, Arden NK, Arundel C, Ronaldson S, Vernon L, Hewitt C, Doherty M, Torgerson D, Conaghan PG. Significant Pain Reduction with Oral Methotrexate in Knee Osteoarthritis; Results from a Randomised Controlled Phase III Trial of Treatment Effectiveness [abstract]. Arthritis Rheumatol. 2018; 70 (suppl 10). https://acrabstracts.org/abstract/significant-pain-reduction-with-oral-methotrexate-in-knee-osteoarthritis-results-from-a-randomised-controlled-phase-iii-trial-of-treatment-effectiveness/.

79. Struglics A, Turkiewicz A, Larsson S, *et al.* Molecular and imaging biomarkers of local inflammation at 2 years after anterior cruciate ligament injury do not associate with patient reported outcomes at 5 years. Osteoarthritis Cartilage 2020; 28:356–362.

80. Reichmann WM, Maillefert JF, Hunter DJ, *et al.* Responsiveness to change and reliability of measurement of radiographic joint space width in osteoarthritis of the knee: a systematic review. Osteoarthritis Cartilage 2011; 19:550–556.

81. Eckstein F, Wirth W, Lohmander LS, *et al.* Five-year followup of knee joint cartilage thickness changes after acute rupture of the anterior cruciate ligament. Arthritis Rheumatol 2015; 67:152–161.

82. Hunter DJ, Lohmander LS, Makovey J, *et al.* The effect of anterior cruciate ligament injury on bone curvature: exploratory analysis in the KANON trial. Osteoarthritis Cartilage 2014; 22:959–968.

83. ■ Bowes MA, Lohmander LS, Wolstenholme CBH, *et al.* Marked and rapid change of bone shape in acutely ACL injured knees - an exploratory analysis of the Kanon trial. Osteoarthritis Cartilage 2019; 27:638–645.

3D exploration showing rapid bone shape changes after joint injury in a particular pattern, highlighting the similarities with bone remodelling patterns in osteoarthritis overall.

84. Das Neves Borges P, Vincent TL, Marenzana M. Automated assessment of bone changes in cross-sectional micro-CT studies of murine experimental osteoarthritis. PLoS One 2017; 12:e0174294.

85. Emery CA, Roos EM, Verhagen E, *et al.* OARSI Clinical Trials Recommendations: design and conduct of clinical trials for primary prevention of osteoarthritis by joint injury prevention in sport and recreation. Osteoarthritis Cartilage 2015; 23:815–825.

86. Luyten FP, Bierma-Zeinstra S, Dell'Accio F, *et al.* Toward classification criteria for early osteoarthritis of the knee. Semin Arthritis Rheum 2018; 47:457–463.

87. McAlindon TE, Driban JB, Henrotin Y, *et al.* OARSI Clinical Trials Recommendations: design, conduct, and reporting of clinical trials for knee osteoarthritis. Osteoarthritis Cartilage 2015; 23:747–760.

88. Lattermann C, Jacobs CA, Bunnell MP, *et al.* Logistical challenges and design considerations for studies using acute anterior cruciate ligament injury as a potential model for early posttraumatic osteoarthritis. J Orthop Res 2017; 35:641–650.

89. Olson SA, Furman BD, Kraus VB, *et al.* Therapeutic opportunities to prevent posttraumatic arthritis: lessons from the natural history of arthritis after articular fracture. J Orthop Res 2015; 33:1266–1277.

90. ■ Watt FE, Corp N, Kingsbury SR, *et al.* Towards prevention of posttraumatic osteoarthritis: report from an international expert working group on considerations for the design and conduct of interventional studies following acute knee injury. Osteoarthritis Cartilage 2019; 27:23–33.

First clinical trial considerations article in this area around the design and conduction of studies seeking to prevent osteoarthritis after injury, based on a consensus exercise and highlighting key areas and research needs.

91. Bay-Jensen AC, Henrotin Y, Karsdal M, Mobasheri A. The need for predictive, prognostic, objective and complementary blood-based biomarkers in osteoarthritis (OA). EBioMedicine 2016; 7:4–6.

92. Kraus VB, Burnett B, Coindreau J, *et al.,* OARSI FDA Osteoarthritis Biomarkers Working Group. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthritis Cartilage 2011; 19:515–542.

93. Nicholls E, Thomas E, van der Windt DA, *et al.* Pain trajectory groups in persons with, or at high risk of, knee osteoarthritis: findings from the Knee Clinical Assessment Study and the Osteoarthritis Initiative. Osteoarthritis Cartilage 2014; 22:2041–2050.

94. Kraus VB, Collins JE, Hargrove D, *et al.,* OA Biomarkers Consortium. Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH OA Biomarkers Consortium. Ann Rheum Dis 2017; 76:186–195.

95. Deveza LA, Kraus VB, Collins JE, *et al.* Association between biochemical markers of bone turnover and bone changes on imaging: data from the osteoarthritis initiative. Arthritis Care Res (Hoboken) 2017; 69:1179–1191.

96. Deveza LA, Melo L, Yamato TP, *et al.* Knee osteoarthritis phenotypes and their relevance for outcomes: a systematic review. Osteoarthritis Cartilage 2017; 25:1926–1941.

97. Scanzello CR, McKeon B, Swaim BH, *et al.* Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. Arthritis Rheum 2011; 63:391–400.

98. Driban JB, Harkey MS, Barbe MF, *et al.* Risk factors and the natural history of accelerated knee osteoarthritis: a narrative review. BMC Musculoskelet Disord 2020; 21:332.

99. Driban JB, Bannuru RR, Eaton CB, *et al.* The incidence and characteristics of accelerated knee osteoarthritis among women: the Chingford cohort. BMC Musculoskelet Disord 2020; 21:60.

100. Davis JE, Harkey MS, Ward RJ, *et al.* Accelerated knee osteoarthritis is associated with preradiographic degeneration of the extensor mechanism and cruciate ligaments: data from the Osteoarthritis Initiative. BMC Musculoskelet Disord 2019; 20:308.

101. Zengini E, Hatzikotoulas K, Tachmazidou I, *et al.* Genome-wide analyses using UK Biobank data provide insights into the genetic architecture of osteoarthritis. Nat Genet 2018; 50:549–558.

102. Tachmazidou I, Hatzikotoulas K, Southam L, *et al.* Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. Nat Genet 2019; 51:230–236.

103. Valdes AM, Doherty SA, Muir KR, *et al.* The genetic contribution to severe posttraumatic osteoarthritis. Ann Rheum Dis 2013; 72:1687–1690.

Role of adipose tissues in osteoarthritis

Natalia Zapata-Linares[a], *Florent Eymard*[b,c], *Francis Berenbaum*[a,d], *and Xavier Houard*[a]

Purpose of review
Epidemiologic studies reveal that the link between obesity and osteoarthritis cannot be uniquely explained by overweight-associated mechanical overload. For this reason, much attention focuses on the endocrine activity of adipose tissues. In addition to the systemic role of visceral and subcutaneous adipose tissues, many arguments highlight the involvement of local adipose tissues in osteoarthritis.

Recent findings
Alteration in MRI signal intensity of the infrapatellar fat pad may predict both accelerated knee osteoarthritis and joint replacement. In this context, recent studies show that mesenchymal stromal cells could play a pivotal role in the pathological remodelling of intra-articular adipose tissues (IAATs) in osteoarthritis. In parallel, recent findings underline bone marrow adipose tissue as a major player in the control of the bone microenvironment, suggesting its possible role in osteoarthritis.

Summary
The recent description of adipose tissues of various phenotypes within an osteoarthritic joint allows us to evoke their direct involvement in the initiation and progression of the osteoarthritic process. We can expect in the near future the discovery of novel molecules targeting these tissues.

Keywords
adipokines, adipose tissue, bone marrow adipose tissue, intra-articular adipose tissues, osteoarthritis

INTRODUCTION

Osteoarthritis is the most common musculoskeletal disease and is one of the leading causes of disability worldwide. The disability-adjusted life years index for osteoarthritis rose by 34.8% between 2005 and 2015 [1]. The increase in the number of osteoarthritis patients cannot be explained solely by the ageing of the world population, highlighting the importance of other risk factors. Obesity is the main modifiable risk factor for osteoarthritis [2]. The WHO estimates that the worldwide prevalence of obesity nearly tripled since 1975 with more than 1.9 billion adults overweight in 2016, among them 650 million were obese.

The role of overweight-associated mechanical overload has long been pointed out to explain the link between osteoarthritis and obesity. Clinical studies indeed described positive correlations between BMI and both the incidence and the progression of knee osteoarthritis [3,4]. However, obesity also impacts nonweight bearing joints [5], suggesting that factors other than mechanical overload also contribute to joint damage in obese patients.

In addition to their role in energetic metabolism, adipose tissues are endocrine organs releasing factors acting on distant organs. These factors, of which the prototype and the better known is leptin, are defined as adipokines [6]. Blood levels of leptin increase with BMI as they are in osteoarthritis patients [7,8]. Evidence argue for a role of leptin in osteoarthritis [9]. Numerous other adipokines are produced by adipose tissues and their secretion pattern is also affected by obesity [10]. This altered secretion pattern of adipose tissues related to obesity reflects modifications in their tissue composition as well as modifications in the phenotype of cells present within adipose tissues.

Adipose tissues do not constitute a unique entity. White and brown adipose tissues have been described, differing by their developmental origin,

[a]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine (CRSA), [b]Department of Rheumatology, AP-HP Henri Mondor Hospital, [c]Gly-CRRET Research Unit 4397, Université Paris-Est Créteil and [d]Sorbonne Université, INSERM CRSA, AP-HP Hopital Saint Antoine, Paris, France

Correspondence to Francis Berenbaum, MD, PhD, Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine (CRSA), 184 Rue du Faubourg Saint-Antoine, F-75012 Paris, France.
E-mail: francis.berenbaum@aphp.fr

Curr Opin Rheumatol 2021, 33:84–93
DOI:10.1097/BOR.0000000000000763

KEY POINTS

- Visceral and subcutaneous adipose tissues secrete adipokines, which differentially affect joint tissue homeostasis.
- IAAT fibrosis and inflammation are early events in osteoarthritis and alteration in MRI signal intensity of infrapatellar fat pad may predict both accelerated knee osteoarthritis and replacement.
- Inflammatory and remodelling factors secreted by IAAT may be responsible for cell and tissue damages of both IAAT and synovium, as components of a same functional unit.
- Bone marrow adipose tissue is a newly studied adipose tissue and a known regulator of bone microenvironment. Its volume changes in pathophysiological conditions associated with osteoarthritis and its composition is enriched in $n-6$ fatty acids, especially arachidonic acid, in osteoarthritic patients, suggesting that it may be a new adipose tissue playing role in osteoarthritis.

the phenotype of their adipocytes and their function in energetic metabolism and thermogenesis. Moreover, multiple white adipose tissues (WAT) exist, present in the whole body as separate fat pads with specific features. In this review, we will describe the known features of different adipose tissues, including subcutaneous, visceral, intra-articular and bone marrow adipose tissues (BMATs), and will focus on their potential roles in osteoarthritis.

METHODOLOGY

A search for original articles published between January 2017 and October 2020 was performed on PubMed. The search terms used were 'Adipose tissue AND Osteoarthritis' for reviews, 'Adipokines AND Osteoarthritis', 'Lipodistrophy AND joint health', 'Leptin', 'Adiponectin', 'Visfatin', 'Resistin', 'Chemerin-1', 'Progranulin', 'Omentin', 'Lipocalin-2', 'infapatellar fat pad', 'intra-articular fat pad' and 'Bone marrow adipose tissue AND lipids'. All articles identified were English-language articles. In addition relevant references from selected publications and relevant references were identified.

ROLE OF SYSTEMIC ADIPOSE TISSUES

Description and physiology

Adipose tissues can be related to osteoarthritis progression by biomechanical and metabolic mechanisms (Fig. 1). The biomechanical ones refer to an increase in body weight due to adipose tissues gain leading to abnormal loading on the joints. The metabolic ones include abnormal lipid profile and secretion of adipokines by adipocytes. Herein, we summarize the implication of subcutaneous adipose tissues (SCAT) and visceral adipose tissues (VAT) on those mechanisms.

SCAT is situated beneath the skin whereas VAT fills the peritoneal cavity and the space between internal organs. Augmentation on either of them implies an increase on body weight and on joint loading. Mechanical stress is an important factor on osteoarthritis initiation and development [11–13]. Exercise produces a loss of adipose tissues weight which alleviates pain symptoms in osteoarthritis patients. Regarding the metabolic component, SCAT explants from osteoarthritis patients stimulated with IL1β have been reported to increase proinflammatory and anti-inflammatory signals [14]. Visceral adipocytes seem to be more active in terms of lipolysis and lipogenesis and a major source of adipokines and cytokines in comparison with other types of adipocytes. Adipocytes are also found in the middle of skeletal muscles and their accumulation on females is correlated with osteoarthritis progression [11,15]. Below we mention some of the most studied adipokines secreted by these different tissues and how they are related to osteoarthritis.

Systemic adipokines and osteoarthritis

Adipokines may play a role in early diagnosis and management of osteoarthritis symptoms due to their role on cartilage degradation, synovial inflammation and bone remodelling (Table 1). The evaluation of adipokine content in clinical and experimental models is obtained from serum, plasma or synovial fluid. Asides of adipose tissues, joint tissues participate in adipokines secretion. A great amount of adipokines have been correlated to osteoarthritis onset, development and progression, being leptin the most studied one, followed by adiponectin, resistin and visfatin. The table summarizes recently published data on adipokines, whereas the text below focuses on the best described adipokines. These adipokines in osteoarthritis drive pathways directly related to inflammation, cartilage degradation, infiltration of joint tissues by immune cells, mesenchymal stem cells (MSCs) differentiation, chondrocytes dedifferentiation or osteoclast activation [16–18]. In addition, resistin and visfatin have been described as markers of knee function while leptin and adiponectin as pain markers in osteoarthritis [19,20], but further studies need to be performed.

Omentin-1 and vaspin have been reported to be secreted exclusively by VAT but their role seems to

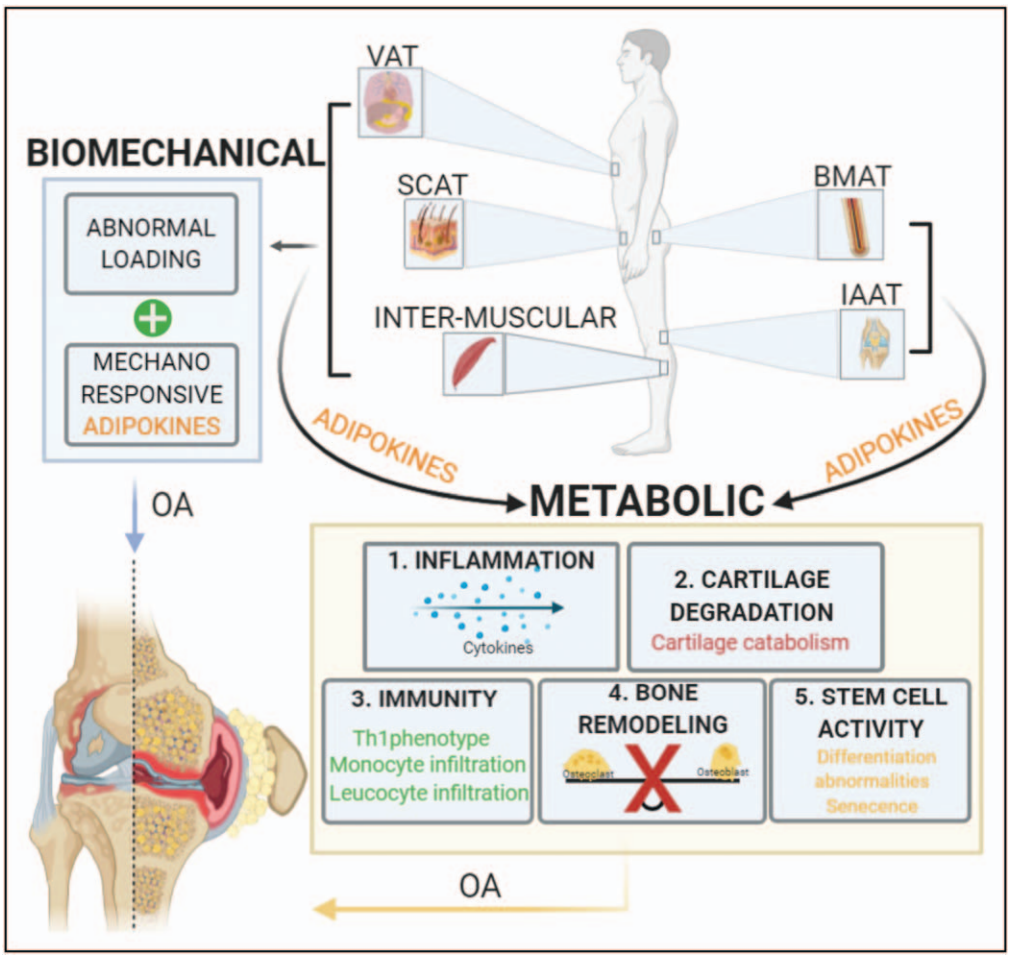

FIGURE 1. Roles of the different adipose tissues on osteoarthritis progression by biomechanical and metabolic mechanisms. Increases on systemic adipose tissues like subcutaneous adipose tissue, visceral adipose tissue and intra-muscular adipose tissue contribute to abnormal loading of the joint, this mechanical stress have been shown to be part of osteoarthritis onset and progression. Lipocalin adipokine family has emerged as sensors of mechanical load, inflammatory status and catabolic stimuli of the joint, suggesting its involvement in osteoarthritis pathophysiology. On the other hand, the paracrine role of subcutaneous adipose tissue, visceral adipose tissues, intra-muscular adipose tissues and local adipose tissues bone marrow adipose tissue and intra-articular adipose tissue affect joint health. The adipokines secreted by all those tissues have proven to promote directly: 1. Secretion of inflammatory cytokines like IL-1β and TNF-α which are well documented for their active involvement in the pathophysiology of osteoarthritis, 2. Cartilage catabolism, including inhibition of proliferation in chondrocytes and degradation of the matrix components, collagen type 2 and agrecan, 3. Immune response by the infiltration of joint tissues by monocytes and leucocytes which increases even more the inflmmatory signals present on the joint affected, 4. Loss of balance between osteoclast and osteoblast affecting directly bone remodelling, changes on bone constitution are part of osteoarthritis pathophysiology and 5. Changes on stem-cell principal characteristics like prolifereation and differentiation capacity.

be opposed to the rest of other adipokines. *In vitro*, they display chondro-protective activity and are negatively related to osteoarthritis severity [17▪]. Leptin, adiponectin and visfatin could also act under specific conditions as anti-inflammatory and anticatabolic agents, avoiding tissue degradation. Chemerin for instance could be a marker for obesity-associated osteoarthritis and with a possible role on innate immune system-associated inflammation on those patients, while lipocalin-2 has been suggested to be a mechano-responsive adipokine [17▪,18]. Significantly, apelin is the only adipokine described so far to be directly involved with synovium angiogenesis, a known marker of severity in osteoarthritis [35]. Many other adipokines have been shown to have a possible role on osteoarthritis [17▪,40]. Researchers keep testing if those interesting molecules could serve on the early diagnosis of osteoarthritis as well as targets for future therapeutic strategies.

Table 1. Adipokines in osteoarthritis

Adipokine	Source of detection	Described action	References 2018–2020
Leptin	Plasma	Biomarker in synovial fluid for human knee OA	[21,22]
	Serum	Remarkable diagnostic value in the incidence of human knee OA	[23]
	Synovial fluid	Leptin and its receptor may be an emerging target for intervention in human metabolic-associated OA	[9,24]
Adiponectin	Serum	Promising biomarker on human OA pathogenesis	[25]
	Synovial fluid	Low levels observed in synovial fluids patients of lower OA grades	[22]
		Gene polymorphism intensifies the risk of human knee OA	[26]
Visfatin	Synovial fluid	Oxidative stress induction in human OA synoviocytes	[27,28]
		Human cartilage catabolic effects (apoptosis, matrix degradation, oxidative stress)	[29]
		Bone remodelling on pig OA model	[30]
Resistin	Plasma	Modulates OA miRs with visfatin	[27]
	Serum	Progression and pathogenesis of human knee OA	[31]
	Synovial fluid	Novel and reliable biomarker for human OA severity	
		Proinflammatory effects in human OA	[32]
Chemerin	Serum	Cartilage degradation	[33]
		Inflammation	
		Found on serums of patients with primary OA of the hand, knee or hip	
Omentin-1	Synovial fluid serum	Possible chondroprotective role in human cells	[16]
Vaspin	*In vitro*	Possible anticatabolic effect in human cartilage	[16]
		Possible anti-inflammatory effect	
Lipocalin-2	Synovial fluid	Proinflammatory effects in human OA	[34]
		Its downregulation reduces chondrocyte inflammation and cartilage degradation	
Apelin	In-vitro human cells	Synovium angiogenesis	[35]
		Catabolic effects	
Progranulin	In vitro human cells	Triggers anabolic markers	[36,37]
		Anti-inflammatory and anticatabolic effects	
Nesfatin-1	In-vitro human cells	Possible protective role in the development of OA	[38]
	Animal model	Upregulated in OA chondrocytes	[39]
RBP4	Synovial fluid	Matrix degradation in human cartilage	[40]
	Blood samples	Positive correlation with other OA adipokines	
New adipokines (SERPINE2, WISP2, GPBMB, ITIH5)	*In vitro*	Secreted by human OA chondrocytes, human OA sclerotic subchondral bone, human OA synovial tissues and human OA IAAT	[41–44]

IAAT, intra-articular adipose tissue; OA, osteoarthritis.

ROLE OF INTRA-ARTICULAR ADIPOSE TISSUES

Description and physiology

Intra-articular adipose tissues (IAAT) are fat pads found between the synovium and the joint capsule. The best characterized and the largest IAAT is the infrapatellar fat pad (IFP). IAAT are WAT as SCAT and VAT. Although their characteristics are close to those of VAT, IAAT share common features with SCAT that distinguish them from VAT [45]. There is no clear consequence of high fat diet on adipocyte size or inflammation of IFP in mice, with contradictory published results [46–48]. Recent data on human osteoarthritis patients reported an absence of link between obesity and IFP volume [49] or

between BMI of osteoarthritis patients and either adipocyte or inflammatory features of IFP [50], suggesting that IAAT may be different to SCAT and VAT and display specific functions.

The physiological roles of IAAT are still not well characterized. IFP was initially supposed by Clopton Harvers at the end of 17th Century to secrete the synovial fluid and latter, by Jean Cruveilhier in the 19th Century, to fill gaps in the joint. By increasing the synovial surface, IFP facilitates the distribution of the synovial fluid. It may protect the patellar tendon and the anterior horns of the menisci and may supply nutriments to the patellar ligament [51]. IFP is also supposed to act as a shock absorber during joint movement. More recently, it was shown that IFP secrete factors [52,53], especially prostaglandin $F_{2\alpha}$ and prostaglandin E_2, which induce a fibrotic and inflammatory response in fibroblast-like synoviocytes [54,55], suggesting that IAAT and synovium are partners of a same functional unit [45,56].

Intra-articular adipose tissue and osteoarthritis

A debate exists for several years on the protective or detrimental effect of IAAT on osteoarthritis. The role of IFP as a shock absorber has been pointed out to explain its possible protective effect, as recently reviewed [11,57]. A protective effect of IFP-secreted factors and IFP-derived MSCs have been also proposed [11,57]. Nevertheless, meta-analyses showed little if any detrimental effect of IFP resection on clinical outcomes after total knee arthroplasty [58–60]. On the other side, alteration in MRI signal intensity of IFP has been linked to osteoarthritis progression [61] and may predict both accelerated knee osteoarthritis [62,63] and knee replacement [64▪]. Significantly, with the aim of an early detection of osteoarthritis progressors, Bonakdari *et al.* [65] developed a method to predict the volume of IFP. Although the relationship between IFP volume and osteoarthritis remains unclear, IFP volume is related to patello–femoral joint osteoarthritis pain [66]. IFP contains numerous sensitive fibers [67] and is considered as a major source of knee pain [68,69]. Osteoarthritis IAAT are characterized by inflammatory cell infiltration, fibrosis and increased vascularization [45,70,71]. Fibrosis and inflammation of IFP are known features of anterior knee pain. They are associated with an increased vascularization and calcitonin-positive nerve fibers in the fibrotic areas of IFP [72]. Similar observations were obtained with the monoiodoacetic acid model of osteoarthritis, in which IFP changes occurred before cartilage degradation [73,74].

IAAT secrete factors with proinflammatory and tissue remodelling activities [45,52,53,55,71] (Fig. 1). Significantly, IFP from patients with osteoarthritis and rheumatoid arthritis display distinct fatty acid (FA) signatures [75], suggesting disease-specific phenotypes for IFP. The osteoarthritis-specific secretory phenotype of IAAT may be directly involved in synovial inflammation and fibrosis [45,54,55] since IFP remodelling precedes synovitis [74].

IAAT cellular composition comprises adipocytes, leukocytes, endothelial and mesenchymal cells, all participating in the osteoarthritis-specific secretory phenotype of IAAT [71,76,77]. Although the specific roles of IAAT macrophages remains unknown [78,79], those of MSCs are more understood. Initially, an anti-inflammatory activity of IFP-derived MSCs from osteoarthritis patients has been reported [80]. It has been recently proposed that IFP-derived MSCs may be deleterious in osteoarthritis via their secretion of inflammatory factors, their ability to recruit monocytes and their exacerbated response to an inflammatory stimulus [76,77]. In addition, cell lineage tracing experiments identified IFP perivascular MSCs as able to transdifferentiate into myofibroblasts and induce IFP fibrosis in posttraumatic osteoarthritis model [81,82▪]. Moreover, fibroblasts isolated from fibrotic IFP have been involved in inflammatory cell recruitment and pain [83▪].

ROLE OF BONE MARROW ADIPOSE TISSUE

Description and physiology

BMAT constitutes over 10% of total adipose mass and 70% of the bone marrow volume in young lean healthy human adults. The initial concept of BMAT as a passive fat storage depot has been challenged in the recent years although little is known about its physiological roles. It is now well accepted that BMAT has a unique development, molecular profile, regulation and modulation of the anatomical context that make it different from the other types of adipose tissues.

BMAT volume changes upon the pathophysiological conditions; it increases with ageing, obesity, type 2 diabetes, osteoporosis or skeletal unloading [84], whereas it decreases with exercise [85], mechanical loading and hormonal changes (Fig. 2). BMAT can be classified into constitutive BMAT (cBMAT) and regulated BMAT (rBMAT). Both of them differ by the time of their development, their localization in the skeleton, their gene expression pattern and their content in saturated/unsaturated lipids [86]. These differences could indicate different functions and even different progenitors. Nevertheless, rBMAT could also change to a cBMAT phenotype under specific conditions [84].

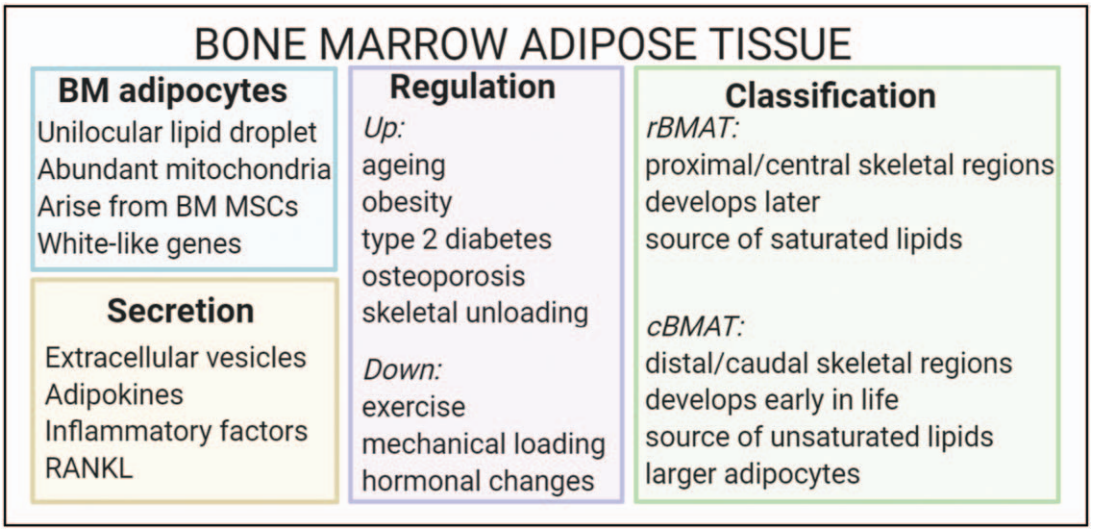

FIGURE 2. General characteristics of bone marrow adipose tissue. Bone marrow adipose tissue is currently considered as a tissue with significant paracrine and endocrine activities which make it a major player on different pathologies. Bone marrow adipocytes' gene expression pattern is similar to white-like adipocytes, they have one unilocular lipid droplet with abundant mitochondria and recent study has proved the progenitors to be more white-like. Their secretory profile includes extracellular vesicles and numerous molecules like inflammatory factors, adipokines or RANKL. Bone marrow adipose tissue is a unique adipose tissue which functions are still to be revelled. Bone marrow adipose tissue has a high intrinsic plasticity, increases with age as well as in other pathological contexts like: obesity, type 2 diabetes or osteoporosis. Bone marrow adipose tissue content can also decrease with exercise, mechanical loading or hormonal changes. In terms of development it can be classified into constitutive bone marrow adipose tissue or constitutive bone marrow adipose tissue and regulated bone marrow adipose tissue or regulated bone marrow adipose tissue. Constitutive bone marrow adipose tissue developed early in life, located in the distal skeleton, repository of unsaturated lipids and constituted by adipocytes larger in size with reduced expression of adipogenic markers. On the other hand, regulated bone marrow adipose tissue increases with age, is located in the proximal skeleton where the adipocytes contain saturated lipids and express high levels of known adipogenic markers.

Bone marrow adipocytes (BMAds) have one unilocular lipid droplet with abundant mitochondria [87] and their gene expression pattern is similar to white adipocytes [84]. It is believed that BMAds arise from bone marrow MSCs, probably the same progenitors as osteoblasts. A recent study has proved the progenitors to be more white-like [88] even though it is possible multiple populations within the BMAds could exist [89]. BMAds secrete extracellular vesicles and numerous soluble factors, which may control bone microenvironment [84,90▪▪]. Zou *et al.* [91▪▪] indeed recently showed that BMAds ablation provokes massive bone formation due to the activation of bone morphogenetic protein receptor signalling pathway in MSCs. In addition, lack of adipo-progenitors on mice produces bone loss and abnormal vasculature [92▪▪].

Aside of its paracrine role, BMAT could regulate systemic metabolism. Moreover, patients with BMAT alteration frequently develop ectopic storage of fat resulting on insulin resistance [93]. BMAT lipogenesis is triggered by short-term cold exposure and is less dependent on insulin than WAT [88]. Little is known about the lipolysis mechanisms on BMAT, but it could be either cytoplasmic lipase-mediated or by lipophagy [90▪▪,94▪]. Specifically, the uptake and esterification of FAs is greater in BMAT than in WAT and those FAs fuel hematopoietic tumours and their oxidation is crucial for hematopoietic stem-cell maintenance [95,96]. Suchacki *et al.* [88] have shown that BMAds have high basal glucose uptake that is greater in the axial skeleton than in long bones, suggesting that BMAT may influence systemic glucose homeostasis and that this characteristic is needed to support normal metabolic function and de-novo lipogenesis.

Bone marrow adipose tissue and osteoarthritis

Pathophysiological conditions where bone homeostasis is lost have been directly related to an increase in BMAT. Surprisingly, they all constitute osteoarthritis risk factors. In addition, osteoarthritis entails subchondral bone remodelling and bone marrow is the only tissue where adipocytes and bone cells are in close association. All of these argue for a possible role of BMAT on osteoarthritis (Fig. 1). Moreover,

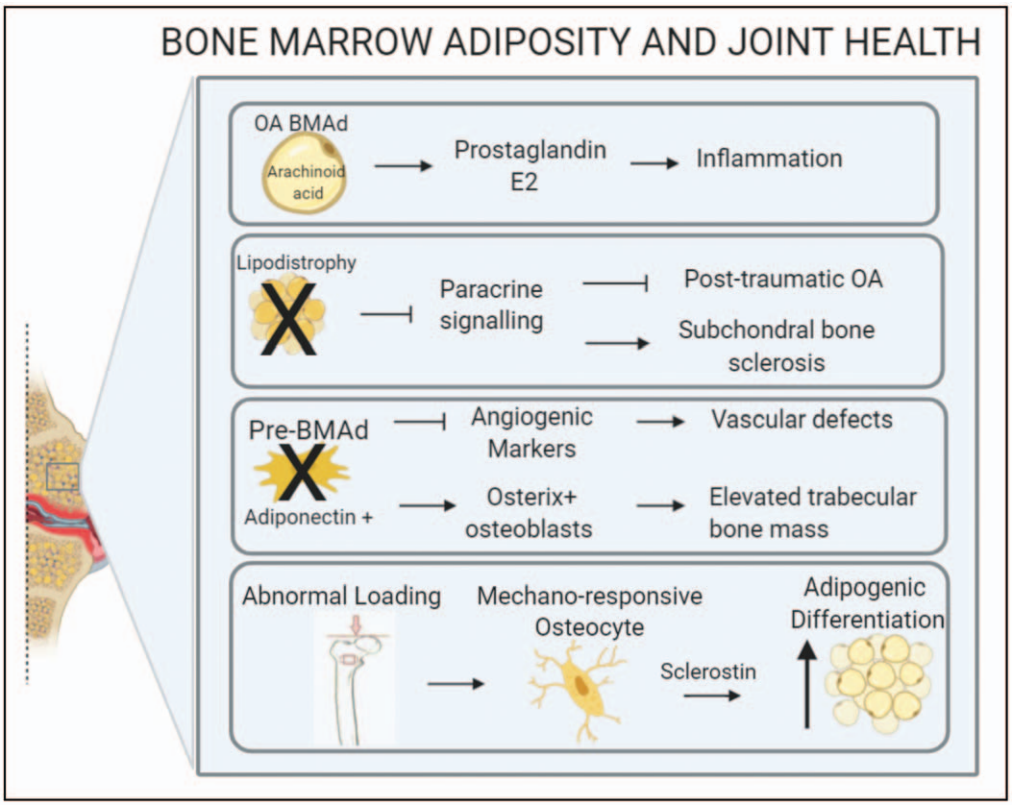

FIGURE 3. Possible role of bone marrow adiposity in joint health. Bone marrow adipose tissue may play a role on inflammation, subchondral bone sclerosis, aberrant angiogenesis, adipogenic differentiation and bone remodelling all of them involved on joint health and osteoarthritis development and progression. Femoral heads from osteoarthritis patients contain high amounts of fat, especially arachidonic acid precursor of prostaglandin E_2 a known participant on osteoarthritis inflammation [97]. Lipodystrophic mice were protected from spontaneous or posttraumatic osteoarthritis, this study proposes that adipose tissue is a critical antagonist of cartilage health and integrity due precisely to the paracrine signalling from fat [98]. Mice without adiponectin-positive progenitors had elevated trabecular bone mass and their vessels within the bone marrow were less in number and high in diameter; characteristics that were far from normal. Sclerostin produced by the bone-mechanosensing osteocytes inhibits Wnt signalling stimulated adipogenesis of mouse mesenchymal stem cells and human mesenchymal stem cells [100]. Nevertheless, the cross-talk between all joint tissues and bone marrow adipose tissue is far from being unveiled and more studies are needed to describe the mechanisms, adipokines, pathways and signalling involved on osteoarthritis pathogenesis. OA, osteoarthritis; OA BMAd, bone marrow adipocytes from osteoarthritis patients; Pre-BMAd, bone marrow adipocyte precursors.

the femoral heads from osteoarthritis patients contained high amounts of fat and of $n-6$ FAs, especially arachidonic acid [97] (Fig. 3). Early this year, Collins *et al.* [98] proposed that knee joints of lipodystrophic mice were protected from spontaneous or posttraumatic osteoarthritis, independently from diet. Susceptibility to posttraumatic osteoarthritis was reintroduced using implantation of adipose tissues derived from wild type animals, probably due to the paracrine signalling from fat [98]. Nevertheless, lipodystrophic patients have multiple bone abnormalities such as subchondral bone sclerosis, similar to osteoarthritis patients [99]. Significantly, osteoblasts and osteocytes can also accumulate lipids [90▪▪]. The cross-talk between BMAT and joint tissues is far from being unveiled and more studies are needed to describe the mechanisms involved on osteoarthritis pathogenesis.

Since all joint tissues are of mesenchymal origin and osteoarthritis is a whole joint disease, it is possible that osteoarthritis affects MSC features. Both the synthesis of a poorly mineralized matrix and high content of fat characterize osteoarthritis bone. This may result from a defect on the differentiation capacity of MSCs favouring preferentially adipogenic over osteogenic lineage. Moreover, a direct role of sclerostin in inducing bone marrow adipogenesis through inhibiting Wnt signalling has recently been reported [101]. The inhibition of Wnt signalling increased the expression of adipogenic transcription factors Pparγ and Cebpα and stimulated adipogenesis [100]. However, lack of

adiponectin-positive progenitors in mice leads to both bone and angiogenic defects [92▪▪].

The role of BMAT in osteoarthritis still remains speculative but numerous arguments indicate that it could be involved in the dysregulation of joint tissues in osteoarthritis. Future studies are needed to explore in detail the role of BMAT in osteoarthritis.

CONCLUSION

The discovery of the role of low-grade inflammation in certain phenotypes of osteoarthritis has opened up new physiopathological hypotheses involving adipose tissues. The recent description of adipose tissues of various phenotypes within an osteoarthritic joint allows us to evoke their direct involvement in the initiation and progression of the osteoarthritic process (Fig. 1). We can expect in the near future the discovery of novel molecules targeting these tissues.

Acknowledgements

Authors' contribution: Drafting of the article: N.Z.-L., F.E., F.B., X.H. Final approval of the article: N.Z.-L., F.E., F.B., X.H.

Financial support and sponsorship

The current work was supported by a grant from the Société Française de Rhumatologie. N.Z.-L. was supported by a grant from the Fondation pour la Recherche Médicale: SPF20160936284.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. GDaH Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study. Lancet 2016; 388:1603–1658.
2. Felson DT, Anderson JJ, Naimark A, *et al.* Obesity and knee osteoarthritis. The Framingham Study. Ann Intern Med 1988; 109:18–24.
3. Reijman M, Pols HA, Bergink AP, *et al.* Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: the Rotterdam Study. Ann Rheum Dis 2007; 66:158–162.
4. Lohmander LS, Gerhardsson de Verdier M, Rollof J, *et al.* Incidence of severe knee and hip osteoarthritis in relation to different measures of body mass: a population-based prospective cohort study. Ann Rheum Dis 2009; 68:490–496.
5. Yusuf E, Nelissen RG, Ioan-Facsinay A, *et al.* Association between weight or body mass index and hand osteoarthritis: a systematic review. Ann Rheum Dis 2010; 69:761–765.
6. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004; 92:347–355.
7. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol 2002; 147:173–180.
8. de Boer TN, van Spil WE, Huisman AM, *et al.* Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. Osteoarthritis Cartilage 2012; 20:846–853.
9. Gao YH, Zhao CW, Liu B, *et al.* An update on the association between metabolic syndrome and osteoarthritis and on the potential role of leptin in osteoarthritis. Cytokine 2020; 129:155043.
10. Iannone F, Lapadula G. Obesity and inflammation – targets for OA therapy. Curr Drug Targets 2010; 11:586–598.
11. Chang J, Liao Z, Lu M, *et al.* Systemic and local adipose tissue in knee osteoarthritis. Osteoarthritis Cartilage 2018; 26:864–871.
12. Ramage L, Nuki G, Salter DM. Signalling cascades in mechanotransduction: cell–matrix interactions and mechanical loading. Scand J Med Sci Sports 2009; 19:457–469.
13. Visser AW, de Mutsert R, le Cessie S, *et al.* The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. Ann Rheum Dis 2015; 74:1842–1847.
14. Kontny E, Zielinska A, Ksiezopolska-Orlowska K, Gluszko P. Secretory activity of subcutaneous abdominal adipose tissue in male patients with rheumatoid arthritis and osteoarthritis – association with clinical and laboratory data. Reumatologia 2016; 54:227–235.
15. Li S, Schwartz AV, LaValley MP, *et al.* Association of visceral adiposity with pain but not structural osteoarthritis. Arthritis Rheumatol 2020; 72:1103–1110.
16. Carrion M, Frommer KW, Perez-Garcia S, *et al.* The adipokine network in rheumatic joint diseases. Int J Mol Sci 2019; 20:4091.
17. Tu C, He J, Wu B, *et al.* An extensive review regarding the adipokines in the ▪ pathogenesis and progression of osteoarthritis. Cytokine 2019; 113:1–12.
A detailed review on the different cytokines with a role on osteoarthritis pathogenesis.
18. Xie C, Chen Q. Adipokines: new therapeutic target for osteoarthritis? Curr Rheumatol Rep 2019; 21:71.
19. Calvet J, Orellana C, Albinana Gimenez N, *et al.* Differential involvement of synovial adipokines in pain and physical function in female patients with knee osteoarthritis. A cross-sectional study. Osteoarthritis Cartilage 2018; 26:276–284.
20. Askari A, Arasteh P, Homayounfar R, *et al.* The role of adipose tissue secretion in the creation and pain level in osteoarthritis. Endocr Regul 2020; 54:6–13.
21. Boffa A, Merli G, Andriolo L, *et al.* Synovial fluid biomarkers in knee osteoarthritis: a systematic review and quantitative evaluation using BIPEDs criteria. Cartilage 2020; 1947603520942941. [Epub ahead of print]
22. Sachdeva M, Aggarwal A, Sharma R, *et al.* Chronic inflammation during osteoarthritis is associated with an increased expression of CD161 during advanced stage. Scand J Immunol 2019; 90:e12770.
23. Min S, Shi T, Han X, *et al.* Serum levels of leptin, osteopontin, and sclerostin in patients with and without knee osteoarthritis. Clin Rheumatol 2020. [Epub ahead of print]
24. Yan M, Zhang J, Yang H, Sun Y. The role of leptin in osteoarthritis. Medicine 2018; 97:e0257.
25. Xiao K, Yu L, Zhu L, *et al.* Urine proteomics profiling and functional characterization of knee osteoarthritis using iTRAQ technology. Horm Metab Res 2019; 51:735–740.
26. Shang H, Hao Y, Hu W, *et al.* Association between ADIPOQ gene variants and knee osteoarthritis in a Chinese population. Biosci Rep 2019; 39:BSR20182104.
27. Cheleschi S, Gallo I, Barbarino M, *et al.* MicroRNA mediate visfatin and resistin induction of oxidative stress in human osteoarthritic synovial fibroblasts via NF-kappaB pathway. Int J Mol Sci 2019; 20:5200.
28. Yapici Yavuz G, Simsek Kaya G, Kiziltunc A. Analysis of synovial fluid visfatin level in temporomandibular joint disorders. Cranio 2019; 37:296–303.
29. Cheleschi S, Tenti S, Mondanelli N, *et al.* MicroRNA-34a and microRNA-181a mediate visfatin-induced apoptosis and oxidative stress via NF-kappaB pathway in human osteoarthritic chondrocytes. Cells 2019; 8:874.
30. Macfadyen MA, Daniel Z, Kelly S, *et al.* The commercial pig as a model of spontaneously-occurring osteoarthritis. BMC Musculoskelet Disord 2019; 20:70.
31. Alissa EM, Alzughaibi LS, Marzouki ZM. Relationship between serum resistin, body fat and inflammatory markers in females with clinical knee osteoarthritis. Knee 2020; 27:45–50.
32. Chen WC, Lin CY, Kuo SJ, *et al.* Resistin enhances VCAM-1 expression and monocyte adhesion in human osteoarthritis synovial fibroblasts by inhibiting MiR-381 expression through the PKC, p38, and JNK signaling pathways. Cells 2020; 9:1369.
33. Cajas Santana LJ, Rondon Herrera F, Rojas AP, *et al.* Serum chemerin in a cohort of Colombian patients with primary osteoarthritis. Reumatol Clin 2020. [Epub ahead of print]
34. Pirozzi C, Francisco V, Guida FD, *et al.* Butyrate modulates inflammation in chondrocytes via GPR43 receptor. Cell Physiol Biochem 2018; 51:228–243.
35. Wang YH, Kuo SJ, Liu SC, *et al.* Apelin affects the progression of osteoarthritis by regulating VEGF-dependent angiogenesis and miR-150-5p expression in human synovial fibroblasts. Cells 2020; 9:594.
36. Feng D, Kang X, Wang R, *et al.* Progranulin modulates cartilage-specific gene expression via sirtuin 1-mediated deacetylation of the transcription factors SOX9 and P65. J Biol Chem 2020; 295:13640–13650.

37. Zhi L, Zhao J, Zhao H, *et al.* Downregulation of LncRNA OIP5-AS1 induced by IL-1beta aggravates osteoarthritis via regulating miR-29b-3p/PGRN. Cartilage 2020; 1947603519900801. [Epub ahead of print]
38. Jiang L, Xu K, Li J, *et al.* Nesfatin-1 suppresses interleukin-1beta-induced inflammation, apoptosis, and cartilage matrix destruction in chondrocytes and ameliorates osteoarthritis in rats. Aging 2020; 12:1760–1777.
39. Wang Q, Xu X, Kang Z, *et al.* Paeonol prevents IL-1beta-induced inflammatory response and degradation of type II collagen in human primary chondrocytes. Artif Cells Nanomed Biotechnol 2019; 47:2139–2145.
40. Scotece M, Koskinen-Kolasa A, Pemmari A, *et al.* Novel adipokine associated with OA: retinol binding protein 4 (RBP4) is produced by cartilage and is correlated with MMPs in osteoarthritis patients. Inflamm Res 2020; 69:415–421.
41. Conde J, Scotece M, Abella V, *et al.* Identification of novel adipokines in the joint. Differential expression in healthy and osteoarthritis tissues. PLoS One 2015; 10:e0123601.
42. Li H, Yang HH, Sun ZG, *et al.* Whole-transcriptome sequencing of knee joint cartilage from osteoarthritis patients. Bone Joint Res 2019; 8:288–301.
43. Sanchez C, Mazzucchelli G, Lambert C, *et al.* Comparison of secretome from osteoblasts derived from sclerotic versus nonsclerotic subchondral bone in OA: a pilot study. PLoS One 2018; 13:e0194591.
44. Tang S, Deng S, Guo J, *et al.* Deep coverage tissue and cellular proteomics revealed IL-1beta can independently induce the secretion of TNF-associated proteins from human synoviocytes. J Immunol 2018; 200:821–833.
45. Eymard F, Pigenet A, Citadelle D, *et al.* Knee and hip intra-articular adipose tissues (IAATs) compared with autologous subcutaneous adipose tissue: a specific phenotype for a central player in osteoarthritis. Ann Rheum Dis 2017; 76:1142–1148.
46. Iwata M, Ochi H, Hara Y, *et al.* Initial responses of articular tissues in a murine high-fat diet-induced osteoarthritis model: pivotal role of the IPFP as a cytokine fountain. PLoS One 2013; 8:e60706.
47. Barboza E, Hudson J, Chang WP, *et al.* Profibrotic infrapatellar fat pad remodeling without M1 macrophage polarization precedes knee osteoarthritis in mice with diet-induced obesity. Arthritis Rheumatol 2017; 69:1221–1232.
48. Warmink K, Kozijn AE, Bobeldijk I, *et al.* High-fat feeding primes the mouse knee joint to develop osteoarthritis and pathologic infrapatellar fat pad changes after surgically induced injury. Osteoarthritis Cartilage 2020; 28:593–602.
49. Masaki T, Takahashi K, Hashimoto S, *et al.* Volume change in infrapatellar fat pad is associated not with obesity but with cartilage degeneration. J Orthop Res 2019; 37:593–600.
50. de Jong AJ, Klein-Wieringa IR, Andersen SN, *et al.* Lack of high BMI-related features in adipocytes and inflammatory cells in the infrapatellar fat pad (IFP). Arthritis Res Ther 2017; 19:186.
51. Eymard F, Chevalier X. Inflammation of the infrapatellar fat pad. Joint Bone Spine 2016; 83:389–393.
52. Distel E, Cadoudal T, Durant S, *et al.* The infrapatellar fat pad in knee osteoarthritis: an important source of interleukin-6 and its soluble receptor. Arthritis Rheum 2009; 60:3374–3377.
53. Ushiyama T, Chano T, Inoue K, Matsusue Y. Cytokine production in the infrapatellar fat pad: another source of cytokines in knee synovial fluids. Ann Rheum Dis 2003; 62:108–112.
54. Bastiaansen-Jenniskens YM, Wei W, Feijt C, *et al.* Stimulation of fibrotic processes by the infrapatellar fat pad in cultured synoviocytes from patients with osteoarthritis: a possible role for prostaglandin f2alpha. Arthritis Rheum 2013; 65:2070–2080.
55. Eymard F, Pigenet A, Citadelle D, *et al.* Induction of an inflammatory and prodegradative phenotype in autologous fibroblast-like synoviocytes by the infrapatellar fat pad from patients with knee osteoarthritis. Arthritis Rheumatol 2014; 66:2165–2174.
56. Macchi V, Stocco E, Stecco C, *et al.* The infrapatellar fat pad and the synovial membrane: an anatomo-functional unit. J Anat 2018; 233:146–154.
57. Jiang LF, Fang JH, Wu LD. Role of infrapatellar fat pad in pathological process of knee osteoarthritis: future applications in treatment. World J Clin Cases 2019; 7:2134–2142.
58. Sun C, Zhang X, Lee WG, *et al.* Infrapatellar fat pad resection or preservation during total knee arthroplasty: a meta-analysis of randomized controlled trials. J Orthop Surg Res 2020; 15:297.
59. White L, Holyoak R, Sant J, *et al.* The effect of infrapatellar fat pad resection on outcomes posttotal knee arthroplasty: a systematic review. Arch Orthop Trauma Surg 2016; 136:701–708.
60. Ye C, Zhang W, Wu W, *et al.* Influence of the infrapatellar fat pad resection during total knee arthroplasty: a systematic review and meta-analysis. PLoS One 2016; 11:e0163515.
61. Ruhdorfer A, Haniel F, Petersohn T, *et al.* Between-group differences in infrapatellar fat pad size and signal in symptomatic and radiographic progression of knee osteoarthritis vs nonprogressive controls and healthy knees – data from the FNIH Biomarkers Consortium Study and the Osteoarthritis Initiative. Osteoarthritis Cartilage 2017; 25:1114–1121.
62. Davis JE, Ward RJ, MacKay JW, *et al.* Effusion-synovitis and infrapatellar fat pad signal intensity alteration differentiate accelerated knee osteoarthritis. Rheumatology 2019; 58:418–426.
63. Harkey MS, Davis JE, Lu B, *et al.* Early preradiographic structural pathology precedes the onset of accelerated knee osteoarthritis. BMC Musculoskelet Disord 2019; 20:241.
64. ■ Wang K, Ding C, Hannon MJ, *et al.* Signal intensity alteration within infrapatellar fat pad predicts knee replacement within 5 years: data from the osteoarthritis initiative. Osteoarthritis Cartilage 2018; 26:1345–1350.

A clinical study showing that infrapatellar fat pad (IFP) alterations revealed by MRI predicts knee replacement within 5 years.

65. Bonakdari H, Tardif G, Abram F, *et al.* Serum adipokines/related inflammatory factors and ratios as predictors of infrapatellar fat pad volume in osteoarthritis: applying comprehensive machine learning approaches. Sci Rep 2020; 10:9993.
66. Cowan SM, Hart HF, Warden SJ, Crossley KM. Infrapatellar fat pad volume is greater in individuals with patellofemoral joint osteoarthritis and associated with pain. Rheumatol Int 2015; 35:1439–1442.
67. Bohnsack M, Meier F, Walter GF, *et al.* Distribution of substance-P nerves inside the infrapatellar fat pad and the adjacent synovial tissue: a neurohistological approach to anterior knee pain syndrome. Arch Orthop Trauma Surg 2005; 125:592–597.
68. Belluzzi E, Stocco E, Pozzuoli A, *et al.* Contribution of infrapatellar fat pad and synovial membrane to knee osteoarthritis pain. Biomed Res Int 2019; 2019:6390182.
69. Draghi F, Ferrozzi G, Urciuoli L, *et al.* Hoffa's fat pad abnormalities, knee pain and magnetic resonance imaging in daily practice. Insights Imaging 2016; 7:373–383.
70. Favero M, El-Hadi H, Belluzzi E, *et al.* Infrapatellar fat pad features in osteoarthritis: a histopathological and molecular study. Rheumatology 2017; 56:1784–1793.
71. Klein-Wieringa IR, Kloppenburg M, Bastiaansen-Jenniskens YM, *et al.* The infrapatellar fat pad of patients with osteoarthritis has an inflammatory phenotype. Ann Rheum Dis 2011; 70:851–857.
72. Onuma H, Tsuji K, Hoshino T, *et al.* Fibrotic changes in the infrapatellar fat pad induce new vessel formation and sensory nerve fiber endings that associate prolonged pain. J Orthop Res 2020; 38:1296–1306.
73. Clements KM, Ball AD, Jones HB, *et al.* Cellular and histopathological changes in the infrapatellar fat pad in the monoiodoacetate model of osteoarthritis pain. Osteoarthritis Cartilage 2009; 17:805–812.
74. Inomata K, Tsuji K, Onuma H, *et al.* Time course analyses of structural changes in the infrapatellar fat pad and synovial membrane during inflammation-induced persistent pain development in rat knee joint. BMC Musculoskelet Disord 2019; 20:8.
75. Mustonen AM, Kakela R, Lehenkari P, *et al.* Distinct fatty acid signatures in infrapatellar fat pad and synovial fluid of patients with osteoarthritis versus rheumatoid arthritis. Arthritis Res Ther 2019; 21:124.
76. Bravo B, Guisasola MC, Vaquero J, *et al.* Gene expression, protein profiling, and chemotactic activity of infrapatellar fat pad mesenchymal stem cells in pathologies of the knee joint. J Cell Physiol 2019; 234:18917–18927.
77. Eymard F, Pigenet A, Rose C, *et al.* Contribution of adipocyte precursors in the phenotypic specificity of intra-articular adipose tissues in knee osteoarthritis patients. Arthritis Res Ther 2019; 21:252.
78. Wu CL, Harasymowicz NS, Klimak MA, *et al.* The role of macrophages in osteoarthritis and cartilage repair. Osteoarthritis Cartilage 2020; 28:544–554.
79. Xie J, Huang Z, Yu X, *et al.* Clinical implications of macrophage dysfunction in the development of osteoarthritis of the knee. Cytokine Growth Factor Rev 2019; 46:36–44.
80. Manferdini C, Maumus M, Gabusi E, *et al.* Adipose-derived mesenchymal stem cells exert antiinflammatory effects on chondrocytes and synoviocytes from osteoarthritis patients through prostaglandin E2. Arthritis Rheum 2013; 65:1271–1281.
81. Sono T, Hsu CY, Negri S, *et al.* Platelet-derived growth factor receptor-beta (PDGFRbeta) lineage tracing highlights perivascular cell to myofibroblast transdifferentiation during posttraumatic osteoarthritis. J Orthop Res 2020. [Epub ahead of print]
82. ■ Sono T, Hsu CY, Wang Y, *et al.* Perivascular fibro-adipogenic progenitor tracing during post-traumatic osteoarthritis. Am J Pathol 2020; 190:1909–1920.

Using cell lineage tracing, this study reveals the importance of perivascular mesenchymal stem cell (MSC) in IFP fibrosis.

83. ■ Paish HL, Kalson NS, Smith GR, *et al.* Fibroblasts promote inflammation and pain via IL-1alpha induction of the monocyte chemoattractant chemokine (C-C Motif) ligand 2. Am J Pathol 2018; 188:696–714.

An interesting study that highlights the role of fibrosis and fibroblasts in IFP inflammation and pain.

84. Li Y, Meng Y, Yu X. The unique metabolic characteristics of bone marrow adipose tissue. Front Endocrinol 2019; 10:69.
85. Patel VS, Ete Chan M, Rubin J, Rubin CT. Marrow adiposity and hematopoiesis in aging and obesity: exercise as an intervention. Curr Osteoporos Rep 2018; 16:105–115.
86. Scheller EL, Doucette CR, Learman BS, *et al.* Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. Nat Commun 2015; 6:7808.
87. Li Z, Hardij J, Bagchi DP, *et al.* Development, regulation, metabolism and function of bone marrow adipose tissues. Bone 2018; 110:134–140.

88. Suchacki KJ, Tavares AAS, Mattiucci D, *et al.* Bone marrow adipose tissue is a unique adipose subtype with distinct roles in glucose homeostasis. Nat Commun 2020; 11:3097.
89. Horowitz MC, Berry R, Holtrup B, *et al.* Bone marrow adipocytes. Adipocyte 2017; 6:193–204.
90. Rendina-Ruedy E, Rosen CJ. Lipids in the bone marrow: an evolving ■■ perspective. Cell Metab 2020; 31:219–231.
A detailed review specific on the lipid content of bone marrow adipose tissue that could help to bring some light on the still unknown functions of this tissue.
91. Zou W, Rohatgi N, Brestoff JR, *et al.* Ablation of fat cells in adult mice induces ■■ massive bone gain. Cell Metab 2020. [Epub ahead of print]
An interesting study showing that bone marrow adipocytes (BMAds) display a negative control on bone mass via the secretion of inhibitors of bone morphogenetic protein receptor signalling pathway in MSCs.
92. Zhong L, Yao L, Tower RJ, *et al.* Single cell transcriptomics identifies a unique ■■ adipose lineage cell population that regulates bone marrow environment. Elife 2020; 9:e54695.
An original article revealing a progenitor population for BMAds and showing finally the need to find a balance between adipogenesis and bone remodelling.
93. Yamamoto A, Kusakabe T, Sato K, *et al.* Seipin-linked congenital generalized lipodystrophy type 2: a rare case with multiple lytic and pseudo-osteopoikilosis lesions. Acta Radiol Open 2019; 8:2058460119892407.
94. Sebo ZL, Rendina-Ruedy E, Ables GP, *et al.* Bone marrow adiposity: basic ■ and clinical implications. Endocr Rev 2019; 40:1187–1206.
A Review on the BMAd characteristics in comparison with other adipose tissues.
95. Diedrich JD, Herroon MK, Rajagurubandara E, Podgorski I. The lipid side of bone marrow adipocytes: how tumor cells adapt and survive in bone. Curr Osteoporos Rep 2018; 16:443–457.
96. Zhang Z, Huang Z, Ong B, *et al.* Bone marrow adipose tissue-derived stem cell factor mediates metabolic regulation of hematopoiesis. Haematologica 2019; 104:1731–1743.
97. Plumb MS, Aspden RM. High levels of fat and ($n-6$) fatty acids in cancellous bone in osteoarthritis. Lipids Health Dis 2004; 3:12.
98. Collins KH, Lenz KL, Pollitt EN, *et al.* Adipose tissue is a critical regulator of osteoarthritis. bioRxiv 2020; 134601; doi: 10.1101/2020.06.04. [Epub ahead of print]
99. Teboul-Core S, Rey-Jouvin C, Miquel A, *et al.* Bone imaging findings in genetic and acquired lipodystrophic syndromes: an imaging study of 24 cases. Skeletal Radiol 2016; 45:1495–1506.
100. Fairfield H, Falank C, Harris E, *et al.* The skeletal cell-derived molecule sclerostin drives bone marrow adipogenesis. J Cell Physiol 2018; 233:1156–1167.
101. Lories RJ, Monteagudo S. Review article: is wnt signaling an attractive target for the treatment of osteoarthritis? Rheumatol Ther 2020; 7:259–270.

Recent advances in targeted drug delivery for treatment of osteoarthritis

Shikhar Mehta[a,*], *Tengfei He*[a,*], *and Ambika G. Bajpayee*[a,b]

Purpose of review
Osteoarthritis is associated with severe joint pain, inflammation, and cartilage degeneration. Drugs injected directly into intra-articular joint space clear out rapidly providing only short-term benefit. Their transport into cartilage to reach cellular targets is hindered by the tissue's dense, negatively charged extracellular matrix. This has limited, despite strong preclinical data, the clinical translation of osteoarthritis drugs. Recent work has focused on developing intra-joint and intra-cartilage targeting drug delivery systems (DDS) to enable long-term therapeutic response, which is presented here.

Recent findings
Synovial joint targeting hybrid systems utilizing combinations of hydrogels, liposomes, and particle-based carriers are in consideration for pain-inflammation relief. Cartilage penetrating DDS target intra-cartilage constituents like aggrecans, collagen II, and chondrocytes such that drugs can reach their cellular and intra-cellular targets, which can enable clinical translation of disease-modifying osteoarthritis drugs including gene therapy.

Summary
Recent years have witnessed significant increase in both fundamental and clinical studies evaluating DDS for osteoarthritis. Steroid encapsulating polymeric microparticles for longer lasting pain relief were recently approved for clinical use. Electrically charged biomaterials for intra-cartilage targeting have shown promising disease-modifying response in preclinical models. Clinical trials evaluating safety of viral vectors are ongoing whose success can pave the way for gene therapy as osteoarthritis treatment.

Keywords
cartilage targeting, drug delivery, nanoparticles, osteoarthritis, pain and function treatment

INTRODUCTION

Musculoskeletal diseases, such as osteoarthritis (OA), rheumatoid arthritis (RA), and low back pain represent the second leading cause of disability globally, imposing a significant physiologic and economic burden on society [1,2]. Such diseases are characterized by tissue degeneration and inflammatory activity that can cause chronic pain and severe joint damage [3]. Specifically, osteoarthritic joints are most affected by articular cartilage degradation and synovial inflammation because of their load-bearing nature, which over time result in loss of joint function and mobility. Overexpression of biological factors, such as inflammatory cytokines [e.g. interleukin (IL)-1, IL-6, tumor necrosis factor α (TNFα)] and degradative enzymes [e.g. matrix metalloproteinase (MMP)13, a disintegrin metalloproteinase with thrombospondin motifs 5 (ADAMTS5)] accelerate progression to osteoarthritis, especially in case of joint injury [4]. The avascular nature of cartilage limits its self-regenerative capacity; timely therapeutic intervention is thus needed to repair the tissue and inhibit further disease progression [5].

In the early stages of osteoarthritis, patients usually experience mild pain and stiffness after performing routine activities, which is typically treated by either topical or oral nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics [6]. As the disease progresses to its mid-stage, joint space begins to narrow and shows signs of osteophyte formation and cartilage damage whereas chondrocytes begin to experience a hypertrophic state in an effort to

[a]Department of Bioengineering, Northeastern University and [b]Department of Mechanical & Industrial Engineering, Northeastern University, Boston, Massachusetts, USA

Correspondence to Ambika G. Bajpayee, ISEC Room 216, 805 Columbus Avenue, Boston, MA 02115, USA. Tel: +1 617 373 7018; e-mail: a.bajpayee@northeastern.edu

*Shikhar Mehta and Tengfei He contributed equally.

Curr Opin Rheumatol 2021, 33:94–109
DOI:10.1097/BOR.0000000000000761

KEY POINTS

- Drug delivery for osteoarthritis therapy remains a challenge because of rapid joint clearance following intra-articular administration and the inability to penetrate through the dense cartilage matrix to reach target cells.
- To prolong joint residence times and provide sustained drug release intended for pain and inflammation relief, delivery systems like hydrogels, micelles, polymeric particles are in consideration owing to their large size or viscous nature.
- Steroid encapsulating polymeric micron sized particles for providing longer lasting pain relief were recently approved for clinical use.
- To restore joint structure and function, osteoarthritis drugs must penetrate through the full thickness of cartilage to reach their cellular and intra-cellular targets; electrically charged carriers targeting negatively charged aggrecans have shown promise in preclinical models.
- Current clinical trials are evaluating the safety of viral-vectors whose success can pave the way for gene therapy as osteoarthritis treatment.

restore tissue damage [6]. At this stage, interventions, such as intra-articular (IA) injections of high-dose corticosteroids or viscosupplements like hyaluronic acid are often recommended for relieving some of the pain and inflammation [7]. However, the aforementioned methods only provide temporary relief and fail to initiate any disease-modifying effect. As the disease progresses to end-stage osteoarthritis, surgical interventions using tissue engineering approaches [8], microfracture, and joint arthroplasty may be considered but eventually total joint replacement is required [7]. Early-stage intervention with disease-modifying osteoarthritis drugs (DMOADs) has the potential to slow down osteoarthritis progression and restore joint structure and function [9] but no such drugs have translated to clinical practice, in part because of a lack of effective delivery systems that can penetrate through the dense meshwork of cartilage to target chondrocytes and provide controlled low drug doses over a period of time with minimal off target effects [10,11▪].

Most small molecule drugs clear out rapidly from the synovial joint (with half-lives of 1–4 h) following their intra-articular administration because of fast exchange of synovial fluid requiring multiple injections of high drug doses that cause toxicity [12]. In order to prolong joint residence times and provide sustained drug release intended for pain and inflammation relief, delivery systems like hydrogels, micelles, polymeric particles are in consideration owing to their large size or viscous nature [13] (Fig. 1). These systems can only target the synovium or the synovial fluid and use high drug doses, thus are only useful for providing pain relief. To achieve cartilage protection – that is to inhibit catabolism and stimulate regeneration, DMOADs must penetrate through the full thickness of cartilage and reach chondrocytes and other matrix target sites, a majority of which lie within the tissue deep zone [10]. Therefore, nanosized carriers that can penetrate into the cartilage and bind within to provide sustained drug release are under consideration [11▪].

This review presents recent basic science and clinical developments in nanoparticle-based delivery systems for prolonging drug residence time within the joint space for pain-inflammation relief and targeting specific intra-cartilage components to restore joint structure and function for osteoarthritis therapy.

INTRA-JOINT DELIVERY

In the native knee, the primary source of pain arises from intra-joint components, such as the synovium, outer-third of meniscus, and osteochondral junction [14]. This is because the capillary network present in these regions begins to multiply (angiogenesis) in osteoarthritis, and contributes towards synovitis (hypertrophy of synovial macrophages and fibroblast-like synoviocytes), osteochondral damage, and osteophyte formation [14]. Thus, current efforts in the design of drug delivery systems (DDS) are focused on prolonging intra-joint residence time of intra-articular administered pain and inflammation relievers to enable efficacy over an extended period of time with a single low-dose administration. We have discussed recent advances in intra-joint DDS under three categories (Table 1): hybrid systems, smart environment responsive systems, and systems with specificity to intra-joint components, such as synoviocytes and vasculature.

Hybrid systems combine a variety of particle-based and hydrogel-based DDS to leverage their advantages. For example, micelles formed by antioxidant, eicosapentaenoic acid (EPA) encapsulated within a gelatin hydrogel enabled controlled drug release over 4 weeks in mouse joints [15▪]. Following intra-articular injection of EPA hydrogels into DMM (destabilization of medial meniscus) mice, significantly greater suppression of glycosaminoglycan (GAG) loss and IL-1β and MMP13 expression was observed at 8 weeks' time compared with EPA alone [15▪]. Another study tagged gold nanoparticles (possessing antioxidant activity) with fish oil protein

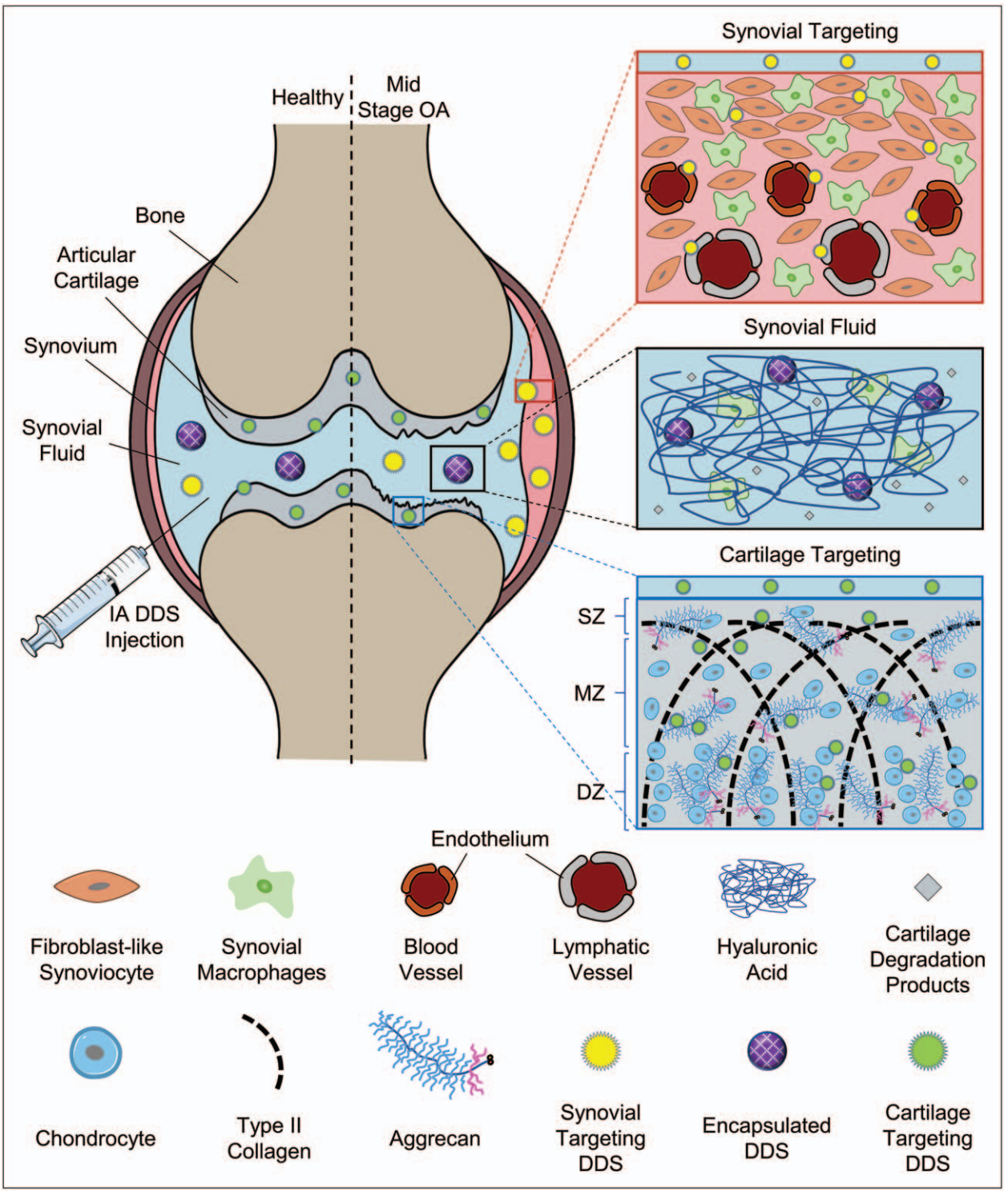

FIGURE 1. Schematic showing healthy and mid-stage osteoarthritis of the knee. In osteoarthritis, the synovium undergoes hypertrophy with an increase in synovial macrophages and fibroblast-like synoviocytes (FLS) accompanied by an outgrowth of blood and lymphatic vessels (angiogenesis), which contribute towards significant pain and inflammation. The environment of the synovial fluid becomes acidic and infiltrated by macrophages and cartilage degradation products. Cartilage and its matrix components (aggrecan and collagen II) begin to degrade, while chondrocytes enter a hypertrophic and apoptotic state. To prolong drug residence and provide long-term osteoarthritis therapy, drugs can be administered via intra-articular injection and modified in the form of drug delivery systems (DDS) to specifically target intra-joint components as shown in the synovium (top), synovial fluid (middle) and cartilage (bottom) insets. DDS can be designed for targeting the synovium (FLS, macrophages, microvasculature endothelium), prolonging synovial fluid residence or targeting the cartilage (aggrecan, collagen II, chondrocytes).

Table 1. Recent developments in intra-joint drug delivery systems

DDS	Drug	In-vivo model	Major outcomes		References
Hybrid systems					
Micelle and hydrogel	EPA	PTOA – mouse DMM	4-week sustained release; suppression of GAG loss, expression levels of IL-1β and MMP13 and NF-κB ÿignalling pathway for 8 weeks		[15▪]
Gold NP and liposomes	Fish oil protein	OA – Mouse bacterial collagenase injection	Release over 24 h; suppression of expression levels of TNFα and IL-6 and NF-κB ÿignalling pathway over 15 days		[16▪]
Liposome and hydrogel	Kartogenin	PTOA – Rat DMM	5-week joint retention; 75% sustained release over 25 days; reduction of osteophytes, lesser decrease in aggrecan and type II collagen expression		[17▪]
Smart joint environnent responsive systems					
HMS NP modified with chitosan	Celastrol	OA – Rat MIA injection	pH responsive	68.9% release at pH 6.0, 21.7% release at pH 7.0 over 24 h; improvements in paw withdrawal threshold, articular surface erosion and joint effusion	[18▪]
PLGA NP encapsulated with NH_4HCO_3	HA	PTOA – mouse DMM	pH responsive	Joint retention over 35 days, sustained drug release over 10 days at pH 5.0; reduced osteophyte formation, did not worsen OA progression over 35 days	[19▪▪]
MOF modified with HA	PCA	PTOA – rat ACLT	pH responsive	13% release at pH 7.4, 23% at pH 5.6 over 24 h; reduction in synovial inflammation, downregulation of inflammatory markers, promotion of cartilage-specific marker expression for 8 weeks	[20▪]
PBAE modified with Curcumin	Curcumin	OA – mouse MIA injection	pH responsive	Sustained release over 7 days at pH 6.0; suppression of IL-1 and TNF-α production, improvements in articular surface erosion at 28 days	[21▪]
MoS_2 nanosheet modified with chitosan	Dex	OA – mouse papain injection	NIR radiation triggered	Dex released on demand by controlling the NIR light source chitosan, prolonged residence time; attenuated cartilage erosion, reduced toxicity, suppression of MMP13, ADAMTS5 over 28 days	[22▪]
Hemoglobin and PLGA-PEG NP	NO, Notch1 siRNA	OA – mouse papain injection	NIR radiation triggered	24 h joint retention; photothermal-triggered NO release; inhibition of pro-inflammatory cytokine expression, prevention of cartilage erosion	[23▪▪]
N-isopropyl acrylamide	MK2-inhibiting peptide	PTOA – IL-1β chondrocytes; healthy – rat	Temperature responsive	Joint retention time over 7 days, sustained release over 5 days at 37°C; suppression of IL-6 production for 4 days	[24▪▪]
Targeted systems					
PDN surfaced with FA-modified HA ligand	CORM-401	OA – rat MIA injection	Macrophage targeting	Suppression of IL-1β, IL-6, TNFα secretion, inhibition of CO release, depletes ROS in OA joints for 23 days	[25▪]
ZIF-8 NP modified with anti-CD16/32 Ab	SMT, CAT	PTOA – mouse ACLT	Macrophage targeting	4-day joint retention; both drugs release rapidly at pH 5.4 and release steadily at pH 7.4 over 24 h; rescuing of mitochondrial function, inhibition of cartilage degradation for 4 weeks	[26▪▪]
Dextran sulfate-TCA	TCA	OA – mouse MIA injection	Macrophage targeting	24 h drug release; targeting specificity for SR-A on macrophages; alleviation of structural cartilage damage and pro-inflammatory cytokine expression for 3 weeks	[27▪]
Microgel with PLGA, modified with HAP-1	–	PTOA – rat MMT	FLS Targeting	Specific binding to rat and human synoviocytes; trapped withing synovial membrane, 3-week intra-joint retention with no degenerative changes	[30▪▪]
PLA-PCL-PEG NP modified with CKSTHDRLC	Methotrexate	RA – rat AIA and CIA	MVE targeting	Specific homing to MVE, accumulation in inflamed joints; prevented AIA and CIA at low dose and lower frequency, prevention of neo-angiogenesis and synovial inflammation	[31▪]
Liposome modified with CKPFDRALC	Dex	RA – rat MTB injection	MVE targeting	Enhanced endothelial cell binding, 6-day intra-joint retention; improvement in arthritis scores, no further adverse effects	[32]

Ab, Aantibody; ACLT, Anterior cruciate ligament transection; ADAMTS, a disintegrin and metalloproteinase with a thrombospondin motif; AIA, antigen-induced arthritis; CAT, catalase; CIA, collagen-induced arthritis; CORM, carbon monoxide release molecules; Dex, dexamethasone; DMM, destabilization of the medial meniscus; EPA, eicosapentanoic acid; FA, folic acid; FLS, fibroblast-like synoviocyte; GAG, glycosaminoglycan; HA, hyaluronic acid; HMS, hollow mesoporous silica; IL, interleukin; MIA, monosodium iodoacetate; MK2, mitogen-activated protein kinase-activated protein kinase 2; MMP, matrix metalloproteinase; MMT, medial meniscus transection; MOF, metal organic framework; MTB, *Mycobacterium tuberculosis*; MVE, microvasculature endothelium; NF-κB, nuclear factor κB; NIR, near infrared; NO, nitric oxide; OA, osteoarthritis; PBAE, poly-beta-amino-ester; PCA, protocatechuic acid; PDN, peptide dendrimer nanogel; PEG, polyethylene glycol; PLGA, poly-lactic co-glycolic acid; PTOA, posttraumatic OA; RA, rheumatoid arthritis; ROS, reactive oxygen species; SMT, S-methylisothiourea hemisulfate salt; SR-A, scavenger receptor class A; TCA, triamcinolone acetonide; TNFα, tumor necrosis factor α; ZIF, zeolitic imidazolate framework.

1040-8711

(antiarthritic and anti-inflammatory) to create a hydrophilic structure, which was then encapsulated within a hydrophobic dipalmitoyl phosphatidylcholine (DPPC) liposome (~295 nm diameter) to increase joint retention time and provide lubrication [16▪]. The liposomal encapsulation of nanoparticles led to further suppression of NF-κB and iNOS among other synovial fluid catabolic markers obtained from osteoarthritis mouse knees compared with fish oil-tagged gold nanoparticles alone at 15 days following treatment [16▪]. Another hybrid system incorporated Kartogenin (KGN), a chondrogenic drug, within a liposome loaded into a photocrosslinkable Gelatin methacryloyl (GelMA) matrix in order to improve the drug stability and release from liposomes [17▪]. The microinjectable hydrogel composite system (GelMa@Lipo@KGN, 100 μm diameter) was retained within rat DMM joints for over 5 weeks (compared with 2 weeks for liposomes), contributing to 75% KGN release over 25 days and resulting in reduction of osteophyte formation [17▪].

Smart DDS incorporate pH or thermoresponsive materials. For example, to utilize the acidic osteoarthritic environment within synovial fluid, a hydrophobic drug, Celastrol, was loaded into pH-responsive, chitosan-coated, hollow, 275 nm sized mesoporous silica nanoparticles (CSL@HMSNs-Cs) [18▪]. Greater than three-fold higher drug release was observed in acidic condition (pH 6) compared with a neutral pH 7 environment over 24 h *in vitro*. Following intra-articular administration, CSL@HMSNs-Cs led to greater improvements in paw withdrawal threshold and reduced cartilage erosion at 8 weeks following MIA (monosodium iodoacetate) induction in rats compared with free Celastrol and drug-free nanoparticles [18▪]. Similarly, polylactic co-glycolic acid (PLGA) nanoparticles were encapsulated with ammonium bicarbonate for pH sensitivity and hyaluronic acid, yielding sustained hyaluronic acid release over 10 days at pH 5. They were retained within the knee joints of DMM mice for 35 days and suppressed the incidence of osteophyte formation significantly greater than non-pH-responsive nanoparticles [19▪▪]. Metal-organic frameworks (MOF) possessing pH sensitivity (23% drug release at pH 5.6 compared with 13% at pH 7.4) were modified with hyaluronic acid and loaded with an anti-inflammatory drug, leading to enhanced reductions in synovial inflammation and inflammatory marker expression measured at 8 weeks in ACLT (anterior cruciate ligament transection) rats compared with free drug [20▪]. Acid-activatable poly-beta-amino-ester (PBAE) curcumin nanoparticles, because of protonable tertiary amine groups present on its backbone, were shown to enhance drug release over 7 days in acidic conditions and decreased inflammatory cytokine production greater than unmodified curcumin in osteoarthritis mice joints over 28 days [21▪]. Thermoresponsive DDS, chitosan-modified MoS_2 (molybdenum disulfide), was shown to release a small molecule drug, dexamethasone (Dex) inside the mouse knee joint cavity, when near-infrared (NIR) light was applied on the joint from outside the body [22▪]. NIR triggered photothermal conversion of MoS_2 to provide controlled site-specific drug delivery and resulted in greater suppression of joint TNFα, IL-1β and IL-8 expression levels for 28 days following treatment compared with free Dex or NIR-free Dex-loaded nanoparticles [22▪]. Hemoglobin, a molecule possessing photothermal properties, was recently conjugated with nitric oxide (NO) and Notch1 siRNA prior to encapsulation within a PLGA-polyethylene glycol (PEG) vesicle (NHsPP) [23▪▪]. Application of NIR light at 650 nm wavelength triggered a 24 h NO release *in vitro* and was able to further inhibit inflammatory cytokine expression in osteoarthritis mice for 36 days compared with nanoparticles without drug [23▪▪]. Deloney *et al.* [24▪▪] utilized the thermosensitive property of N-isopropyl acrylamide (NIPAm) to generate 'hollow' core nanoparticles; preparation of these particles at 4°C (below the lower critical solution temperature) allows for the structure to swell, facilitating removal of noncrosslinked cores to increase the drug-loading capacity. These 'hollow' core nanoparticles were capable of loading and releasing significantly higher amounts of MK2 (mitogen-activated protein kinase-activated protein kinase 2) inhibiting peptides compared with solid nanoparticles, contributing to enhanced suppression of IL-1β-stimulated IL-6 production in chondrocytes [24▪▪]. Additionally, these particles possessed the ability to reduce their size to 200 nm in diameter at 37 °C because of deswelling, preventing any inflammatory response often seen with larger-sized particles [24▪▪]. These nanoparticle were shown to be retained within the rat knee joints for 7 days following their intra-articular administration [24▪▪].

DDS design has also focused on targeting macrophages, fibroblast-like synoviocytes (FLS), microvasculature endothelium (MVE) and angiogenesis, all of which are overexpressed in an inflamed joint. For example, a positively charged peptide dendrimer nanogel (PDN, constituted of crosslinked polyhedral oligomeric silsesquioxane core-based generation 3 poly (L-lysine) dendrimers), was constructed by physically encapsulating carbon monoxide (CO) release molecules and tagging their surface with folic acid-modified hyaluronic acid to target macrophages [25▪]. A macrophage-targeted and pH-responsive zeolitic imidazolate framework (ZIF)-8

was modified with anti-CD16/32 antibody, resulting in a prolonged synovial macrophage and intra-joint retention [26▪▪]. Another study reported the design and preparation of dextran sulfate–triamcinolone acetonide conjugate (DS-TCA) nanoparticles for treating osteoarthritis by specifically targeting scavenger receptor class A (SR-A) on activated macrophages leading to alleviation of cartilage damage for 3 weeks [27▪]. Surface modification of polylactic acid (PLA)-PEG nanoparticles with adenosine, via binding to A2A adenosine receptor to stimulate cAMP production to prevent or treat osteoarthritis, was designed to target both the macrophages and chondrocytes to exhibit an anti-inflammatory effect in both *in vitro* and *in vivo* [28].

FLS targeting has previously been achieved using peptide SFHQFARATLAS (HAP-1) [29]. In a recent study, HAP-1-modified microgels containing PLGA nanoparticles were found to be bound to rat and human synoviocytes *in vitro* and were retained within the synovial membrane and joint space of rats for 3 weeks without inducing degenerative activity [30▪▪]. Peptide sequence CKSTHDRLC coated on a PLA, polycaprolactone and PEG nanoparticle, specifically homed to the synovial MVE over 7 days following intravenous injection and suppressed arthritic activity in rats upon delivery of peptide-nanoparticle encapsulated methotrexate [31▪]. Another MVE-targeting peptide, CKPFDRALC was coated onto Dex-encapsulated liposomes, leading to effective inhibition of arthritis progression in rats over a period of 3 weeks [32].

Hybrid systems, smart, environmentally sensitive and synovium-targeting methods, thus have the potential to increase intra-joint residence time of drugs, providing controlled drug release and enabling long-term therapeutic benefit. However, these strategies require complex formulation processes that can hinder their clinical translatability. Additionally, as these carriers cannot penetrate into the cartilage deep zones where most of the target sites reside, their use is limited to pain and inflammation relief.

INTRA-CARTILAGE DELIVERY

Articular cartilage is a dense, avascular tissue constituting of a meshwork of a high density of negatively charges aggrecans (35% dry weight), collagen II (50–60% dry weight) and a low density of chondrocytes (<5% dry weight), which together contribute to the tissue's structure and function [11▪]. Aggrecans contain several highly sulfated GAG side chains conferring high negative fixed charge density (FCD) to the tissue that provides hydration, swelling pressures and compressive stiffness [11▪,33]. As joints are loaded, increased electrostatic repulsion between the intra-cartilage negatively charged groups helps resist deformation enabling the tissue to re-swell and regain back its original shape [33]. Although these negatively charged aggrecans are critical for tissue function, they make penetration and drug delivery into cartilage extremely difficult; it is imperative that drugs and drug carriers reach the tissue deep zones as a majority of cells and matrix target sites reside there [10,34▪]. Below, we present a variety of intra-cartilage nano-carrier-based drug delivery systems designed to target either the aggrecans, collagen II or chondrocytes enabling multi-stage drug delivery at tissue, cellular and intracellular levels (Table 2).

Aggrecan targeting

Bajpayee *et al.* [35] showed that particles have to be smaller than 10 nm in hydrodynamic diameter to be able to penetrate through the full thickness of normal cartilage; larger sized particles are sterically hindered and get trapped within the tissue's superficial zones. They showed that the high negative FCD of tissues can be converted from a barrier to drug entry into a drug depot by modifying drugs with optimally charged cationic domains such that the weak-reversible nature of electrostatic interactions can enhance their intra-cartilage transport, uptake and retention [35–38]. Avidin, a cationic glycoprotein, because of its optimal size (<10 nm in hydrodynamic diameter) and net charge (between +6 and +20) penetrated through the full thickness of rabbit cartilage in high concentrations and was present within the tissue even after 2 weeks of its intra-articular administration in a rabbit ACLT model of posttraumatic osteoarthritis (PTOA) [38]. Avidin was conjugated to four moles of a broad spectrum glucocorticoid, Dexamethasone (Dex), using hydrolysable ester linkers and administered in a single low-dose intra-articular injection 1 week following ACLT in a rabbit model [39]. Avidin-Dex suppressed injury-induced joint inflammation, synovitis and reduced incidence and severity of osteophyte formation significantly greater than free Dex [39]. To increase the drug-loading content of this delivery system, recently multiarm Avidin (mAv) constituting of branched PEG chains was developed providing 28 sites for covalent conjugation of small molecule drugs [Fig. 2a(i)]) [40▪▪]. Similar to Avidin, mAv also penetrated through the full thickness of healthy and osteoarthritis cartilage explants [Fig. 2a(ii)] [40▪▪]. mAv was conjugated to Dex (mAv-Dex) using a combination of hydrolysable ester linkers enabling controlled Dex release over a period of 2 weeks [41]; its single low-

Table 2. Recent developments in intra-cartilage nanoparticle-based drug delivery systems targeting aggrecans, collagen type II, chondrocytes for applications in gene delivery

Targeted carrier	Drug	Model	Major outcomes	References
Aggrecan				
Multiarm Avidin	Dexamethasone	PTOA – bovine cartilage explants	Full depth cartilage penetration, 2-week sustained release; suppression of GAG loss, cell death, inflammation	[40▪▪]
Avidin grafted dextran	–	LBP – bovine nucleus pulposus explants	Month long intra-tissue retention through combined effects of size and charge	[42▪]
Cationic peptide carriers (CPC)	–	Healthy and OA – bovine cartilage explants	Rapid full thickness penetration, high uptake and 7-day retention with CPC +14; weak and reversible binding required for full depth penetration	[43▪▪]
Cysteine dense peptides (CDP)	Dexamethasone, TCA	RA – rat collagen-induced arthritis	Cartilage accumulation with CDP-11R following systemic IV injection; reduction of joint inflammation and off-target toxicities	[44▪▪]
Supercharged – green fluorescent proteins (S-GFP)	–	Healthy – human, bovine cartilage explants	High-uptake and fast transport through full thickness with S-GFP +9 and S-GFP +15	[45▪]
MnO_2 NP	–	PTOA – bovine cartilage explants; healthy rats	Full depth cartilage and chondrocyte penetration, suppression of IL-1-induced GAG loss and NO release; less than 1-week intra-joint residence, accumulation on chondral surfaces	[48▪]
Poly-beta-amino-esters (PBAE)	Dexamethasone	PTOA – bovine cartilage explants	Increased dexamethasone uptake eight-fold, prevented IL-1-induced cartilage degradation	[49]
PAMAM	IGF-1	PTOA – rat ACLT	10-fold increase in joint residence time for 30 days; reduced cartilage degradation and osteophyte burden	[51]
Type II collagen				
WYRGRL	Dexamethasone	PTOA – bovine cartilage explants	Deep zone retention; reduced inflammatory markers, GAG loss	[53▪]
WRYGRL	Hydroxychloroquine	OA – mice papain injection	14-day retention; suppression of synovial inflammation	[54▪]
WRYGRL	Metformin	OA – mice papain injection	3–4-week retention; reduced inflammation	[55]
WYRGRL	–	PTOA – rat MMT	Specific binding to bovine articular cartilage, increased intra-joint half-life and retention *in vivo* for 26 days	[30▪▪]
mAbCII	MMP13 siRNA	PTOA – mice repetitive joint loading	Enhanced reduction in MMP13 expression; improved OARSI scores	[56▪]
Avimer M26	IL-1Ra	PTOA – rat IL-1β injection	1-month retention; enhanced suppression of IL-6	[57▪▪]
Chondrocyte				
DWRVIIPPRPSA	Hesperetin	PTOA – mice ACLT	Alleviation of gradual degeneration of cartilage via TLR-2 inhibition	[61▪]
p5RHH	NF-κB p65 siRNA	PTOA – bovine cartilage explants	3-week suppression of p65; attenuation of cell death	[63▪]
Gene delivery				
AAV9	Follistatin	PTOA – high-fat diet mice DMM	Reduction in cartilage degeneration, synovitis, pro-inflammatory cytokine expression and mechanical algesia at 12 weeks; enhanced muscle growth	[70▪]
AV	RHEB	PTOA – mouse DMM	Inhibition of OA progression at 8 weeks, regulation of ADAMTS5 and MMP13, reduction in apoptosis	[71▪]
HDAV w/Ef1 or NF-κB promoter	PRG4, IL-1Ra	PTOA – mouse DMM or CLT	Enhanced preservation of articular cartilage volume, surface area, increased expression of cartilage matrix genes with combination therapy at 10 weeks	[72▪]
HDAV w/NF-κB promoter	IL-1Ra	PTOA – mouse CLT	Lowered OA scores, increased cartilage volume and surface area	[73]

Targeted carrier	Target gene	Model	Major outcomes	Referencess
Gene editing				
AAV w/ CRISPR-Cas9	MMP13, IL-1β, NGF	PTOA – mouse partial meniscectomy	Alleviation of pain but worsening of joint damage with NGF ablation, attenuation of structural damage with deletion of MMP13 and IL-1β; combination therapy mitigates adverse events of NGF ablation at 3 months	[74▪▪]
Ribonucleoprotein complexes w/ CRISP-Cas9	MMP13	Healthy and OA – human chondrocytes	Significant reduction in MMP13 secretion and activity levels, enhanced type II collagen accumulation for 7 days	[75]
PLGA NP w/ siRNA	p66shc	OA – rat MIA injection	96.4% release in 48 h *in vitro*; attenuation of ROS production, amelioration of pain behavior, cartilage damage and IL-1β, TNFα, COX2 production levels for 21 days	[76▪]
PLGA NP w/ siRNA	P47phox	OA – Rat MIA injection	53.2% burst release at 24 h; attenuation of oxidative stress, proteoglycan loss, articular cartilage calcification and apoptosis for 14 days	[77]

AAV, adeno-associated virus; ACLT, anterior cruciate ligament transection; AV, adenovirus; CDP, cysteine dense peptide; CLT, cruciate ligaments transection; CPC, cationic peptide carrier; DMAB, didodecyldimethylammonium bromide; DMM, destabilization of the medial meniscus; Ef1, elongation factor 1; GAG, glycosaminoglycan; HDAV, helper-dependent adenovirus; IGF-1, insulin-like growth factor-1; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; IV, intravenous; LBP, low back pain; MIA, monosodium Iodoacetate; MMP, matrix metalloproteinase; MnO_2, manganese dioxide; NF-κB, nuclear factor κB; NGF, nerve growth factor; NO, nitric oxide; OA, osteoarthritis; PAMAM, polyamidoamine; PBAE, poly-beta-amino-ester; PLGA, poly-lactic co-glycolic acid; PRG4, proteoglycan 4; PTOA, posttraumatic OA; RA, rheumatoid arthritis; RHEB, Ras homolog enriched in brain; S-GFP, supercharged green fluorescent protein; siRNA, small interfering RNA; TCA, triamcinolone acetonide; TNFα, tumor Necrosis Factor α.

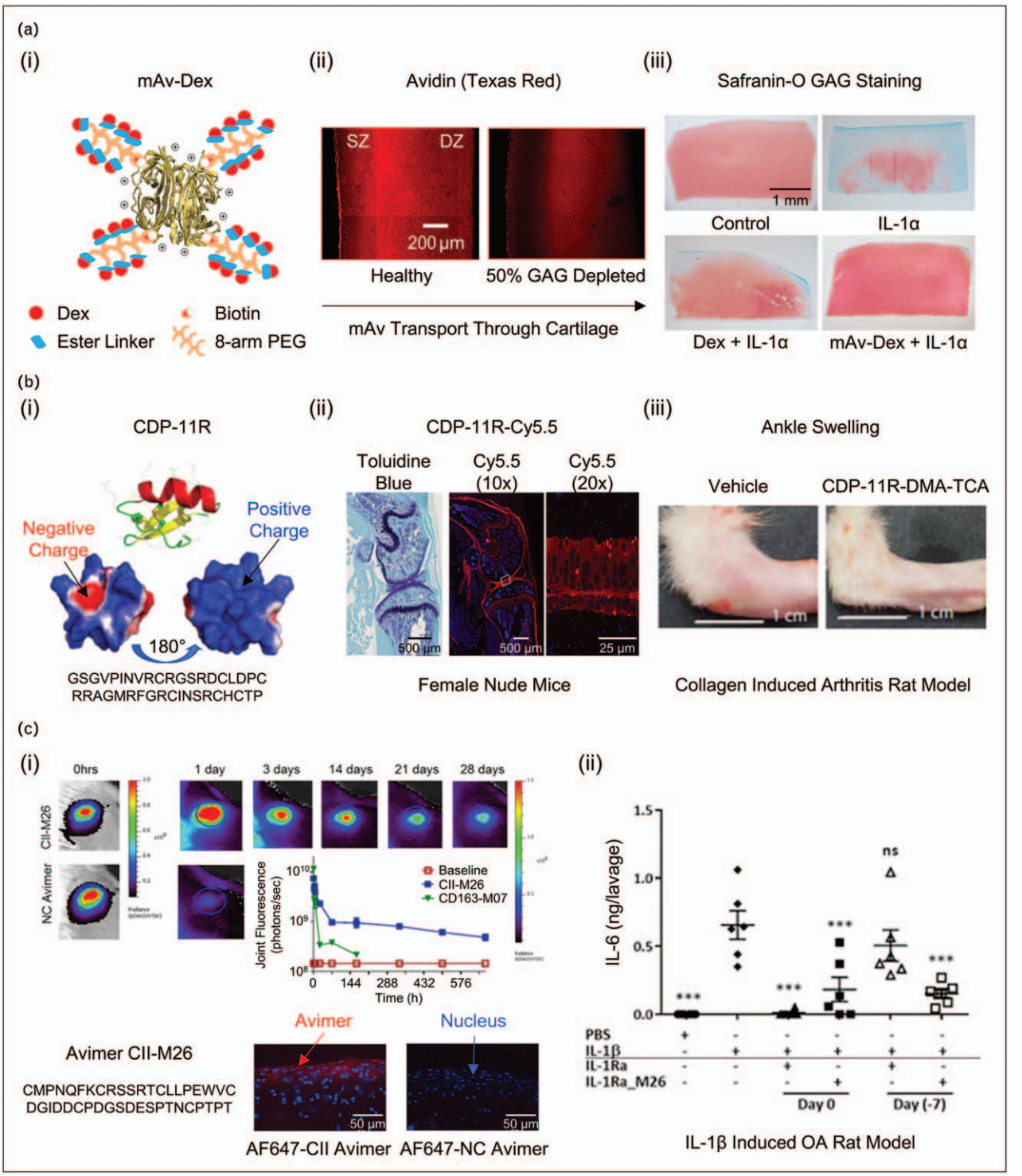

FIGURE 2. (a, I) Multiarm Avidin conjugated to Dex (mAv-Dex) via controlled release ester linkers. (ii) Confocal imaging showing full thickness penetration from superficial zone (SZ) to deep zone (DZ) of mAv-Dex in healthy and GAG-depleted cartilage explants within 24 h. (iii) A single low dose of mAv-Dex suppressed IL-1-induced GAG loss significantly greater than free Dex at 16 days as shown by Safranin-O/fast green staining of cartilage explants. Adapted with permission from reference [40▪▪]. (b, I) X-ray crystallography showing structure ribbon (top) and molecular surface representations (bottom) of CDP-11R (α-helices: red; β-strands: yellow; random coil: green; disulfide bonds: gold). Positive (blue) and negative (red) electrostatic potentials are also shown. (ii) Mouse knee joint stained with Toluidine Blue (left). Cy5.5 and DAPI channel fluorescent images of CDP-11R-Cy5.5 (red) localized to articular cartilage following intravenous injection. (iii) Suppression of CIA rat ankle joint swelling following intravenous injection of CDP-11R–DMA–TCA on treatment day 4. Adapted with

dose suppressed IL-1-induced GAG loss [Fig. 2a(iii)] and chondrocyte death in cartilage explants significantly more effectively than free Dex [40▪▪]. Avidin-based targeting strategies have also been used for targeting other negatively charged and aggrecan-rich tissues, such as the nucleus pulposus of intervertebral disks. Wagner *et al.* [42▪] designed Avidin-grafted dextran nanostructures with multiple drug conjugation sites to enable electrostatic binding with aggrecans, providing a month-long intra-discal retention. Using short-length arginine and lysine-rich cationic peptide carriers (CPCs, ~3 kDa) of varying net charge, it was recently shown that there exists an optimal net cationic charge that a drug of given size should possess to target a tissue of known negative FCD to rapidly penetrate through the full thickness of tissue in high concentrations [43▪▪]. Intra-cartilage uptake did not monotonically increase with net charge of CPCs; CPC + 14 had the highest uptake (400× higher than an uncharged solute), greater than both CPC + 8 and CPC + 20, all of which had similar sizes [43▪▪]. CPC + 8 and CPC + 14 penetrated through the full cartilage thickness whereas CPC + 20 did not, owing to stronger binding interactions with negatively charged aggrecans that hindered its penetrability and uptake [43▪▪]. This work highlighted that the optimal net charge on a carrier should be chosen to take advantage of Donnan partitioning-induced enhanced transport, such that charge interactions are weak enough for the carriers to rapidly get past the tissue superficial zones but strong enough to bind within tissue-deep zones for long-term retention [10,11▪,43▪▪]. Another key finding was that short-range binding interactions like hydrophobic and H-bonds can synergistically stabilize long-range charge interactions, and thus can enhance drug retention within arthritic tissues, which have lost a majority of GAGs, and thus have lower negative FCD [43▪▪]; a feature that can be incorporated in carrier design.

Sangar *et al.* [44▪▪] recently identified cationic cysteine-dense peptide, CDP-11R, as a carrier that accumulates within cartilage because of its distribution of positive charge and disulfide-bonded tertiary structure, even when administered systemically via intravenous injection in healthy mice [Fig. 2b(i and ii)]. Upon conjugation of CDP-11R with triamcinolone acetonide (TCA), ankle joint inflammation [Fig. 2b(iii)] and off-target toxicities in RA rats were suppressed for 4 days following treatment [44▪▪]. Another study explored the use of variously charged green fluorescent proteins for enhancing cartilage penetration and retention as well [45▪]. Sharma and co-workers modified PLGA nanoparticles (260–290 nm) with didodecyldimethylammonium bromide (DMAB) containing a quaternary ammonium cation and showed six-fold greater retention in healthy bovine cartilage explants because of binding with the tissue superficial zone compared with anionic polyvinyl alcohol (PVA)-modified PLGA nanoparticles. However, retention of cationic nanoparticles was reduced two-fold in presence of negatively charged synovial fluid and 2.9-fold in arthritic tissue, indicating the charge-dependency of nanoparticle retention [46]. They also demonstrated cationic PLGA nanoparticles for delivering KGN, a chondrogenic drug, coupled with a cartilage-binding bioadhesive to improve retention [47]. A more recent study focused on the scavenging of reactive oxygen species (ROS) utilized cationic manganese dioxide (MnO_2) nanoparticles for full depth cartilage penetration and chondrocyte targeting, leading to suppression of IL-1β-induced GAG loss and NO release from cartilage explants [48▪]. In-vivo rat studies revealed intra-joint residence for over 1 week with the nanoparticle accumulating on chondral surfaces [48▪].

PBAEs have also been considered as they are inexpensive, biocompatible, cationic and can be end-capped with therapeutics [49]. Perni *et al.* recently chemically modified and optimized PBAE components (amine, acrylate and end-capping) for enhancing their ability to target and bind with cartilage. The optimized PBAE chain conjugated to Dex showed an eight-fold increase in cartilage uptake compared with free Dex [49,50], likely owing to adsorption within the cartilage superficial zones. This DDS resulted in significantly reduced IL-1-induced cartilage degradation compared with free drug *in vitro* [49]. Positively charged sixth generation polyamidoamine (PAMAM) dendrimers (6.7 nm in diameter) have also been utilized for enhancing intra-cartilage penetration and retention of insulin-like growth factor-1 (IGF-1) in rat knee joints. PAMAM-IGF-1 significantly suppressed cartilage degeneration and osteophyte formation compared

permission from reference [44▪▪]. (c, I) IVIS imaging showing collagen II-binding Avimer (CII-M26) being retained within rat knee joint for 28 days after intra-articular injection compared with negative control (NC), which was cleared within 1 day. Region of interest of each image is plotted as fluorescence vs. time. DAPI (nucleus: blue) and AF657 staining (Avimer: red) of articular cartilage confirms intra-tissue presence of CII-M26 for 28 days through confocal imaging. (ii) IL-1Ra-M26 [given either at the same time as (day 0) or 7 days prior to IL-1β intra-articular injection) suppresses IL-6 expression in synovial fluid of rat knees at 4 h following treatment. ***P 0.001 or less. Adapted with permission from from reference [57▪▪].

with untreated control and unmodified IGF-1 in a rat ACLT model at 4 weeks postsurgery [51].

Collagen II targeting

Using phage display, a collagen II-binding peptide sequence, WYRGRL was discovered and it has been widely used for cartilage targeting [52]. This peptide was shown to be retained within the deep zones of healthy and GAG-depleted osteoarthritis cartilage for 48 h, whereas cationic chitosan suffered from a significant drop in retention in GAG-depleted cartilage compared with that in normal tissue [53▪]. Following conjugation to Dex using ester linkers, the collagen-targeting prodrug demonstrated a drug-release half-life of 35.8 ± 9.0 h in presence of PBS that remained unchanged in low concentration esterase solution [53▪]. The prodrug was able to significantly reduce IL-1β induced GAG loss in an in-vitro bovine cartilage explant model [53▪]. In another study, WRYGRL was genetically displayed onto an MMP13 and pH-responsive ferritin nanocage for delivery of anti-inflammatory drug hydroxychloroquine (HCQ) to cartilage [54▪]. This HCQ nanostructure was retained for 14 days in osteoarthritis mice cartilage, resulting in suppression of synovial inflammation [54▪]. Ferritin nanocages functionalized with this peptide have also been used for metformin delivery [55]. A recent study utilized this peptide for delivering microgels containing PLGA nanoparticles tagged with rhodamine B to healthy and osteoarthritis rats, leading to significant binding with articular cartilage as well as increased residence time (up to 26 days) compared with free dye [30▪▪]. Bedingfield *et al.* [56▪] recently utilized a monoclonal antibody that specifically targets type II collagen (mAbCII) for delivering MMP13 siRNA. In a mouse PTOA model, significantly higher MMP13 silencing was achieved compared with noncollagen-targeting siRNA, contributing towards improved OARSI scores [56▪]. A newly devised strategy for targeting type II collagen is the use of Avimers, which are small derivations of cell surface protein A-domains involved in protein–protein interactions [57▪▪]. Avimer M26 displayed high collagen II specificity, allowing for 1 month intra-joint and intra-cartilage retention following intra-articular injection into rat knees [Fig. 2c(i)] [57▪▪]. Furthermore, intra-articular delivery of IL-1Ra fused M26 suppressed IL-1 induced IL-6 expression more significantly than free IL-1Ra [Fig. 2c(ii)] [57▪▪]. It should be noted that unlike charge interactions, strong binding of nanocarriers with collagen II can hinder their transport and penetration through the full thickness of normal or early-stage osteoarthritis cartilage [11▪]. Most of the above discussed work has utilized mouse or rat models that have very thin cartilage, and thus the transport data from these studies should be interpreted cautiously as these models are not appropriate for studying intra-articular transport kinetics and drug delivery [37,38]. Solutes penetrate much faster through thin cartilage than thick, as the diffusion time scales as the square of tissue thickness [38]. It is imperative to validate these results using larger animal models with thicker cartilage more like that of humans as the performance of the DDS not only depends on its size and surface properties but also on the biophysical properties of the animal joint [10,38]. Therefore, targeting collagen II for drug binding should be considered especially in later stages of the disease where a majority of aggrecans in cartilage have been degraded and results should be validated in large animal models [11▪].

Chondrocyte targeting

As the drug ultimately has to be delivered to cell receptors, drug carriers are functionalized with chondrocyte- targeting motifs in combination with aggrecan and collagen-targeting strategies to facilitate multistage drug delivery [58,59,60▪]. A chondrocyte affinity peptide, DWRVIIPPRPSA, discovered by Pi *et al.* [59] was recently functionalized on hesperetin-loaded $GD_2(CO_3)_3^-$ nanoparticles [61▪]. In ACLT mice, the construct exhibited strong cartilage specificity, alleviating cartilage degradation and IL-1-induced apoptosis and inflammation [61▪]. Melittin-derived positively charged peptide VLTTGLPALISWIRRRHRRHC (p5RHH) was previously shown to have strong chondrocyte and cartilage-penetrating ability (up to 700 μm depth) [62]; it was recently modified for delivery of NF-κB p65 siRNA to IL-1 treated cartilage explants, resulting in suppression of p65 for 3 weeks and attenuating cell death [63▪].

Gene delivery

Gene therapy has emerged as a promising strategy for manipulation of expression levels of disease-associated genes via a controlled and targeted mechanism [64]. Genetic materials are introduced into cells via viral or nonviral vectors to induce long-term overexpression or silence a selected gene, thereby creating a prolonged therapeutic response. This technique is especially advantageous for slow-progressing diseases like osteoarthritis, where ablation of catabolic (ADAMTS5, MMP13) and pro-inflammatory (IL-1, TNFα) genes or introduction of anabolic [IGF-1, transforming growth factor β (TGFβ)] and anti-inflammatory [IL-1 receptor

antagonist (*IL-1Ra*), *IL-4*] genes have the potential to prevent further cartilage damage while promoting tissue regeneration [64,65].

Early gene therapy strategies utilized recombinant adenoviruses to encode human IGF-1 into rabbit knees, resulting in increased matrix synthesis from the joint cartilage [66]. Frisbie *et al.* [67] used an adenoviral vector to overexpress intra-articular IL-1Ra in an equine osteoarthritis model and showed elevated expression for 28 days leading to significant improvements in pain, cartilage preservation and synovial membrane histological parameters. However, the most successful strategy to date has been the use of adeno-associated (AAV) or helper-dependent adeno-viruses (HDAV) as carriers for genes for in-vivo transduction. These are small (20–25 nm diameter), nonenveloped, single-stranded DNA viruses that are dependent upon a helper virus – either adenovirus or herpesvirus, for replication [68]. Therefore, despite their limited transgene capacity (<4.8 kb), the absence of all viral-coding sequences makes AAVs less immunogenic and a more attractive option for a well tolerated gene delivery system [69]. Recently, Tang *et al.* [70▪] showed that AAV-mediated delivery of follistatin – a protein involved in enhancing muscle formation by neutralizing members of the TGF-β superfamily, to mice prior to medial meniscus destabilization prevented posttraumatic osteoarthritis like changes within the joint. In a similar mouse model, Ashraf *et al.* delivered Ras homolog enriched in brain (*RHEB*) gene via intra-articular injection, resulting in suppression of ADAMTS5 and MMP13 and overexpression of COL2A1 immunohistochemical staining, while reducing apoptosis [71▪]. It was recently suggested that a combination of genes, such as IL-1Ra with proteoglycan 4 (PRG4) on individual HDAV carriers can provide enhanced therapeutic benefit over monotherapy when delivered to mice suffering from PTOA [72▪]. Recent work has incorporated promoters within the delivery systems to ensure that only diseased cells express and secrete the desired gene [72▪,73].

Gene editing

A new development in osteoarthritis therapy is the use of CRISPR/Cas9 technology to ablate disease-causing genes. An efficient gene-editing technique, this strategy employs a complex of Cas9 proteins and an engineered single guide RNA, which recognize and introduce a double-stranded break in the target DNA [74▪▪]. The DNA undergoes a repair process, which causes insertions or deletions resulting in disruption, thereby eliminating gene expression [74▪▪]. Zhao *et al.* [74▪▪] delivered an AAV expressing CRISPR/Cas9 to mice via intra-articular injection to target genes encoding MMP13, IL-1β and nerve growth factor (NGF). NGF ablation was able to mitigate pain induced by partial meniscectomy while disruption of MMP13 and IL-1β reduced the expression levels of cartilage-degrading enzymes [74▪▪]. Similarly, Seidl *et al.* [75] demonstrated reduced MMP13 levels and enhanced type II collagen accumulation in healthy and osteoarthritis human articular chondrocytes when administered ribonucleoprotein complexes containing CRISPR/Cas9 technology targeting the *MMP13* gene. RNA interference (RNAi) is another strategy for inhibiting gene expression or translation via targeting of mRNA molecules. Small interfering RNA (siRNA) – double-stranded noncoding RNA molecules of 20–25 bp length, targeting the silencing of the *p66shc* gene were encapsulated within PLGA nanoparticles and delivered via intra-articular injection to osteoarthritic mice [76▪]. Silencing of the *p66shc* gene, which is implicated in the generation of mitochondrial reactive oxygen species (mtROS), resulted in alleviation of cartilage damage and pain behavior as well was suppression of IL-1β, TNFα and Cyclooxygenase 2 (COX2) expression levels [76▪]. Using the same PLGA-based nanoparticle system, Shin *et al.* [77] also silenced *p47phox* to reduce ROS-induced chondrocyte damage in an osteoarthritis rat model.

On the basis of the success shown in preclinical models and its current development in clinical trials, AAV and HDAV seem to be the most well tolerated and promising carriers for genes for osteoarthritis therapy. Overexpression of anabolic and anti-inflammatory genes have the potential to prevent further cartilage damage and related catabolic activity within the joint, while gene ablation and silencing through CRISPR/Cas9 and RNAi approaches remain interesting areas of future research.

OSTEOARTHRITIS DRUG DELIVERY SYSTEMS IN CLINICAL TRIALS

Particle-based delivery systems

Taiwan Liposome Company (TLC) developed TLC599, a Dex-sodium phosphate incorporated liposome (~130 nm), for intra-articular delivery for knee osteoarthritis patients (Table 3). The hydrophobic surface of liposome particles enhances their binding within the hydrophobic synovial fluid and their large size prevents them from exiting via the lymphatics. In a phase II clinical trial (NCT03005873), intra-articular injection of 12 mg TLC599 resulted in greater suppression of pain from week 1 through week 24 compared with placebo

Table 3. Ongoing clinical trials evaluating drug delivery systems for treatment of knee osteoarthritis

Trial (start year)	Phase	Sponsor	Product	Drug	Delivery system	Clinical outcomes
Particle-based delivery systems						
NCT03754049 (2019)	II	Taiwan Liposome Company	TLC599	Dexamethasone	Liposome	**PK parameters**, AE
NCT04123561 (2019)	III					**WOMAC**, PGIC
NCT03529942 (2018)	III	Flexion Therapeutics	Zilretta (FX006)	Triamcinolone Acetonide	PLGA	**Synovial volume**
NCT03895840 (2018)	IV					**Chair standing test, Fast paced walking test, Stair climb**, KOOS, NRS for pain
NCT04120402 (2020)	II	Eupraxia Pharmaceuticals	EP-104IAR	Fluticasone propionate	PVA	**WOMAC**, OMERACT-OARSI
Hydrogel-based delivery systems						
NCT04231318 (2020)	III	Anika Therapeutics	Cingal	Triamcinolone Hexacetonide	Crosslinked HA hydrogel	**WOMAC**
NCT03209362 (2017)	II	Seikagaku Corporation	SI-613	Diclofenac	HA hydrogel	**WOMAC**
Gene delivery						
NCT02790723 (2019)	I	Mayo Clinic	sc-rAAV2.5 IL-1Ra	Interleukin-1 Receptor Antagonist (IL-1Ra)	Self-complementary recombinant adeno-associated virus	**AE**
NCT04119687 (2019)	I	Flexion Therapeutics	FX201	IL-1Ra	Helper-dependent adenoviral vector	**AE**, systemic biodistribution
NCT03769662 (2019)	I	Xalud Therapeutics	XT-150	Interleukin-10 (IL-10)	Plasmid DNA	**AE**, KOOS, Verbal Numeric Rating Score, Clinical Global Improvement
NCT03203330 (2018)	III	Kolon TissueGene	TissueGene-C	Transforming Growth Factor β-1 (TGF-β1)	Transduced and nontransduced chondrocytes	**WOMAC, VAS**, MRI, Physical Component Score, Health Assessment

Primary clinical outcomes bolded. AE, adverse events; HA, hyaluronic acid; KOOS, Knee Injury and Osteoarthritis Outcome Score; NRS, Numeric Rating Scale; OMERACT-OARSI, Outcome Measures in Rheumatology-Osteoarthritis Research Society International; PGIC, Patient Global Impression of Change; PK, pharmacokinetics; PLGA, poly-lactic-co-glycolic acid; PVA, polyvinyl alcohol; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

[78▪]. Currently, there are two ongoing clinical trials targeting knee osteoarthritis with TLC599. NCT03754049 is a 90 participant phase II study focused on pharmacokinetic evaluation [79] whereas NCT04123561 is a 500 participant phase III study focused on efficacy [80].

Recently, intra-joint sustained release formulation of TCA encapsulated within micron-sized PLGA particles (Flexion Therapeutics product FX006, Zilretta) received clinical approval for osteoarthritis pain relief as it showed prolonged synovial fluid joint residency for 12 weeks, owing to its large micron size (20–100 μm), following a single intra-articular injection in patients with knee osteoarthritis [81]. The efficacy of FX006 in patients with unilateral knee osteoarthritis was evaluated in a phase III study with results showing significant improvements in WOMAC and ADP (average-daily-pain) scores compared with saline and free drug over 24 weeks [82▪▪]. Following approval, this product has been under review in clinical trials for 24-week synovial inflammation (NCT03529942) [83▪] and performance measures in bilateral knee osteoarthritis patients (NCT03895840) [84]. Another microparticle product, EP-104IAR (60–150 μm) developed by Eupraxia Pharmaceuticals, formulated fluticasone propionate and PVA for intra-articular treatment of osteoarthritis [85]. A 238 patient phase II study evaluating safety, efficacy and pharmacokinetics is currently in the prerecruitment stage (NCT04120402) [86].

Hydrogel-based delivery systems

Cingal, a chemically cross-linked hyaluronic acid gel loaded with triamcinolone hexacetonide is

currently under review in a phase III trial for pain relief evaluation at 26 weeks (NCT04231318) [87▪]. A clinical trial for knee osteoarthritis pain, NCT03209362, evaluated intra-articular injections of SI-613, an injectable hyaluronic acid-based formulation incorporating diclofenac, however, results are still pending [88]. A similar DDS, encapsulating polynucleotides, was administered to knee osteoarthritis patients, however, no significant differences in WOMAC score were reported at 6 months time point (NCT02417610) [89].

Gene delivery systems

Clinically, IL-1Ra (interleukin-1 receptor antagonist) is most commonly employed osteoarthritis therapeutic for gene delivery; currently there are two ongoing trials for intra-articular delivery using an AAV (NCT02790723) [90▪] and an HDAV (NCT04119687) [91▪]. A phase I study delivering XT-150 – a plasmid DNA with a variant of IL-10, was recently completed, however, results have not yet been published [92]. Use of most other types of viral and nonviral vectors for osteoarthritis therapy have faced difficulty in transducing chondrocytes in their in-vivo environment [93]. Thus, their use in ex-vivo approaches, where patient cells can be extracted and then transduced with a gene prior to depositing them back into the joint space, is of interest [93]. This strategy has been commonly employed with synoviocytes to deliver IL-1Ra and IL-10 genes in experimental models of osteoarthritis [94,95]. Invossa, a product combining TGF-β1 transduced and nontransduced chondrocytes for intra-articular delivery has been approved for treating osteoarthritis in South Korea and is currently under review in a phase III clinical trial (NCT03203330) [96▪]. A complete list of ongoing clinical trials evaluating drug delivery systems for treatment of knee osteoarthritis can be found in Table 3.

CONCLUSION

Synovial joint and cartilage-targeting strategies can enable clinical translation of a variety of osteoarthritis drugs that despite strong preclinical evidence have not translated to practice yet. Recent years have witnessed significant increase in both basic science and clinical studies evaluating drug delivery systems for osteoarthritis treatment. Steroid-encapsulating polymeric micron particles for providing longer lasting pain relief were recently approved for clinical use. Electrically charged biomaterials for intra-cartilage targeting and delivery of DMOADs have shown promising results in preclinical models warranting studies with larger animal models. With ongoing clinical trials, gene delivery has the potential to become an effective therapy especially if disease biomarkers at various stages of osteoarthritis can be detected and targeted at early timepoint to prevent further disease progression.

Acknowledgements

We would like to acknowledge our funding sources, Yang Wenhui for assistance in artwork and the rest of the Bajpayee Lab for helpful discussions.

Financial support and sponsorship

Funding was received from National Institutes of Health (NIH) Trailblazer R21 (EB028385-01), NIH R03 (EB025903-1) and NIH R01 (1R01AR075121-01A1). Funding sources had no involvement in the preparation of this manuscript.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Blyth FM, Briggs AM, Schneider CH, *et al.* The global burden of musculoskeletal pain—where to from here? Am J Public Health 2019; 109:35–40.
2. Sebbag E, Felten R, Sagez F, *et al.* The world-wide burden of musculoskeletal diseases: a systematic analysis of the World Health Organization Burden of Diseases Database. Ann Rheum Dis 2019; 78:844–848.
3. Al Maini M, Adelowo F, Al Saleh J, *et al.* The global challenges and opportunities in the practice of rheumatology: white paper by the World Forum on Rheumatic and Musculoskeletal Diseases. Clin Rheumatol 2015; 34:819–829.
4. Lieberthal J, Sambamurthy N, Scanzello CR. Inflammation in joint injury and posttraumatic osteoarthritis. Osteoarthritis Cartilage 2015; 23:1825–1834.
5. Mehta S, Akhtar S, Porter RM, *et al.* Interleukin-1 receptor antagonist (IL-1Ra) is more effective in suppressing cytokine-induced catabolism in cartilage-synovium co-culture than in cartilage monoculture. Arthritis Res Ther 2019; 21:238.
6. Maudens P, Jordan O, Allémann E. Recent advances in intra-articular drug delivery systems for osteoarthritis therapy. Drug Discov Today 2018; 23:1761–1775.
7. Glyn-Jones S, Palmer A, Agricola R, *et al.* Osteoarthritis. Lancet 2015; 386:376–387.
8. He T, Li B, Colombani T, et al. Hyaluronic acid-based shape-memory cryogel scaffolds for focal cartilage defect repair. Tissue Engineering. 2020 Oct 27(ja)
9. Hunter DJ. Pharmacologic therapy for osteoarthritis—the era of disease modification. Nat Rev Rheumatol 2011; 7:13.
10. Bajpayee AG, Grodzinsky AJ. Cartilage-targeting drug delivery: can electrostatic interactions help? Nat Rev Rheumatol 2017; 13:183.
11. ▪ Vedadghavami A, Zhang C, Bajpayee AG. Overcoming negatively charged tissue barriers: drug delivery using cationic peptides and proteins. Nano Today 2020; 34:100898.

This review extensively details the drug delivery strategies that have been utilized for targeting negatively charged tissues based on electrostatic interactions for treating various diseases related to musculoskeletal tissues, such as cartilage, meniscus and intervertebral disks.

12. Evans CH, Kraus VB, Setton LA. Progress in intra-articular therapy. Nat Rev Rheumatol 2014; 10:11.
13. Rai MF, Pham CT. Intra-articular drug delivery systems for joint diseases. Curr Opin Pharmacol 2018; 40:67–73.
14. Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. Nat Rev Rheumatol 2012; 8:390.

15. Tsubosaka M, Kihara S, Hayashi S, *et al.* Gelatin hydrogels with eicosapentaenoic acid can prevent osteoarthritis progression in vivo in a mouse model. J Orthop Res 2020; 38:2157–2169.
This study emphasizes the advantages of using drug-encapsulated hydrogel-based systems for providing a controlled-release mechanism.
16. Sarkar A, Carvalho E, D'souza AA, Banerjee R. Liposome-encapsulated fish oil protein-tagged gold nanoparticles for intra-articular therapy in osteoarthritis. Nanomedicine 2019; 14:871–887.
This is the first study to report DPPC liposomal encapsulation of antioxidant gold nanoparticles tagged with fish oil protein for increased retention time and anti-osteoarthritis therapeutic efficacy.
17. Yang J, Zhu Y, Wang F, *et al.* Microfluidic liposomes-anchored microgels as extended delivery platform for treatment of osteoarthritis. Chem Eng J 2020; 400:126004.
Liposomal-anchored microgels have the potential to provide sustained delivery of several drugs, thereby minimizing numbers of injections required and associated side effects.
18. Jin T, Wu D, Liu X-M, *et al.* Intra-articular delivery of celastrol by hollow mesoporous silica nanoparticles for pH-sensitive anti-inflammatory therapy against knee osteoarthritis. Res Square 2020; 18:592.
HMS nanoparticles are strong candidates for intra-articular delivery of hydrophobic and natural drugs because of their pH-sensitivity and ability to improve drug solubility.
19. Zerrillo L, Que I, Vepris O, *et al.* pH-responsive poly (lactide-co-glycolide) nanoparticles containing near-infrared dye for visualization and hyaluronic acid for treatment of osteoarthritis. J Control Release 2019; 309:265–276.
This study designed PLGA nanoparticles encapsulated with pH-sensitive ammonium bicarbonate for sustained release of hyaluronic acid. Highlighted in this work, is the therapeutic potential of delivering a combination of pH-responsive and non-pH-responsive nanoparticles to provide an initial burst release of drug followed by slow drug release.
20. Xiong F, Qin Z, Lan Q, *et al.* pH-responsive and hyaluronic acid-functionalized metal-organic frameworks for therapy of osteoarthritis. J NanoBiotech 2020; 139:367.
MOFs as drug and diagnostic carriers are of interest for osteoarthritis therapy because of their excellent biocompatibility, high drug-loading capacity and pH-sensitive property.
21. Kang C, Jung E, Hyeon H, *et al.* Acid-activatable polymeric curcumin nanoparticles as therapeutic agents for osteoarthritis. Nanomedicine 2020; 23:102104.
This is the first study to evaluate an acid-activatable therapeutic polymeric prodrug of curcumin for osteoarthritis therapy.
22. Zhao Y, Wei C, Chen X, *et al.* Drug delivery system based on near-infrared light-responsive molybdenum disulfide nanosheets controls the high-efficiency release of dexamethasone to inhibit inflammation and treat osteoarthritis. ACS Appl Mater Interfaces 2019; 11:11587–11601.
The ability of NIR radiation to trigger photothermal conversion of MoS_2 is shown to increase drug release efficiency and prolong localized release of drug to minimize off-target side effects.
23. Chen X, Liu Y, Wen Y, *et al.* A photothermal-triggered nitric oxide nanogenerator combined with siRNA for precise therapy of osteoarthritis by suppressing macrophage inflammation. Nanoscale 2019; 11:6693–6709.
This novel nanogenerator combining photothermal hemoglobin and anti-osteoarthritis therapeutics NO and Notch1-siRNA provided controlled drug release. The ability to prevent cartilage erosion and inflammatory activity upon NIR light application has profound therapeutic potential for treating inflammatory diseases.
24. Deloney M, Smart K, Christiansen BA, Panitch A. Thermoresponsive, hollow, degradable core-shell nanoparticles for intra-articular delivery of anti-inflammatory peptide. J Control Release 2020; 323:47–58.
This study shows the opportunity to thermally control the design of nanoparticles, as they swell under colder temperatures to create more space for drug loading. Upon in-vivo delivery and at higher temperatures, the particles deswell to mitigate any immune response and provide week-long retention. Thus, thermoresponsive, hollow core nanoparticles encapsulating anti-osteoarthritis drugs are promising for therapy.
25. Yang G, Fan M, Zhu J, *et al.* A multifunctional anti-inflammatory drug that can specifically target activated macrophages, massively deplete intracellular H_2O_2, and produce large amounts CO for a highly efficient treatment of osteoarthritis. Biomaterials 2020; 255:120155.
This study shows that FA modification of nanoparticles can be used for specifically targeting and entering macrophages for large amounts of drug release.
26. Zhou F, Mei J, Yang S, *et al.* Modified ZIF-8 nanoparticles attenuate osteoarthritis by reprogramming the metabolic pathway of synovial macrophages. ACS Appl Mater Interfaces 2019; 12:2009–2022.
Another MOF, ZIF-8 possessing pH-sensitivity is modified with anti-CD16/32 antibody for targeting M1 macrophages for drug release to promote a shift to M2 macrophages. This study provides a new strategy by combining pH-responsiveness and M1 macrophagial targeting, thus further utilizing the diseased environment for enhanced osteoarthritis therapy.
27. She P, Bian S, Cheng Y, *et al.* Dextran sulfate-triamcinolone acetonide conjugate nanoparticles for targeted treatment of osteoarthritis. Int J Bio Macromolecules 2020; 158:1082–1089.
The strategy to utilize receptors on activated macrophages, such as SR-A for targeted osteoarthritis therapy is shown here for the first time.
28. Liu X, Corciulo C, Arabagian S, *et al.* Adenosine-functionalized biodegradable PLA-b-PEG nanoparticles ameliorate osteoarthritis in rats. Sci Rep 2019; 9:7430.
29. Vanniasinghe A, Manolios N, Schibeci S, *et al.* Targeting fibroblast-like synovial cells at sites of inflammation with peptide targeted liposomes results in inhibition of experimental arthritis. Clin Immunol 2014; 151:43–54.
30. Mancipe Castro LM, Sequeira A, Garcia AJ, Guldberg RE. Articular cartilage- and synoviocyte-binding poly (ethylene glycol) nano-composite microgels as intra-articular drug delivery vehicles for the treatment of osteoarthritis. ACS Biomat Sci Eng 2020.
Encapsulation of synovial or cartilage-targeting peptides within microgels represents a promising strategy for providing long-term intra-tissue retention following intra-articular injection. This study provides intrigue for new studies to investigate the effects of drugs delivered in this DDS and possibilities for multitissue targeting to enhance retention.
31. Colombo F, Durigutto P, De Maso L, *et al.* Targeting CD34+ cells of the inflamed synovial endothelium by guided nanoparticles for the treatment of rheumatoid arthritis. J Autoimmun 2019; 103:102288.
Targeting and blocking of neoangiogenesis in RA is presented here as drug-loaded biodegradable nanoparticles are locally delivered to the ÿignalli synovial tissue endothelium
32. Meka RR, Venkatesha SH, Acharya B, Moudgil KD. Peptide-targeted liposomal delivery of dexamethasone for arthritis therapy. Nanomedicine 2019; 14:1455–1469.
33. Bhosale AM, Richardson JB. Articular cartilage: structure, injuries and review of management. Br Med Bull 2008; 87:77–95.
34. Vedadghavami A, Mehta S, Bajpayee AG. Characterization of intra-cartilage transport properties of cationic peptide carriers. J Vis Exp 2020; 162:e61340.
This video shows the methodology researchers can use for evaluating intra-tissue transport of drugs and their carriers, a key component for achieving long-term drug retention and prolonging therapeutic efficacy.
35. Bajpayee AG, Wong CR, Bawendi MG, *et al.* Avidin as a model for charge driven transport into cartilage and drug delivery for treating early stage posttraumatic osteoarthritis. Biomaterials 2014; 35:538–549.
36. Bajpayee AG, Quadir MA, Hammond PT, Grodzinsky AJ. Charge based intra-cartilage delivery of single dose dexamethasone using Avidin nano-carriers suppresses cytokine-induced catabolism long term. Osteoarthritis Cartilage 2016; 24:71–81.
37. Bajpayee AG, Scheu M, Grodzinsky AJ, Porter RM. Electrostatic interactions enable rapid penetration, enhanced uptake and retention of intra-articular injected avidin in rat knee joints. J Orthop Res 2014; 32:1044–1051.
38. Bajpayee AG, Scheu M, Grodzinsky AJ, Porter RM. A rabbit model demonstrates the influence of cartilage thickness on intra-articular drug delivery and retention within cartilage. J Orthop Res 2015; 33:660–667.
39. Bajpayee AG, Rodolfo E, Scheu M, *et al.* Sustained intra-cartilage delivery of low dose dexamethasone using a cationic carrier for treatment of post traumatic osteoarthritis. Eur Cell Mater 2017; 34:341.
40. He T, Zhang C, Vedadghavami A, *et al.* Multiarm Avidin nano-construct for intra-cartilage delivery of small molecule drugs. J Control Rel 2020; 318:109–123.
A DDS utilizing electrostatic interactions to delivery a small molecule drug to the deep zones of cartilage has been developed here. This design increases the drug-loading content compared with previous designs and provides a combination of burst and slow drug release via ester linker modification for preventing PTOA.
41. Zhang C, He T, Vedadghavami A, Bajpayee AG. Avidin-biotin technology to synthesize multiarm nano-construct for drug delivery. MethodsX 2020; 7:100882.
42. Wagner EK, Vedadghavami A, Jacobsen TD, *et al.* Avidin grafted dextran nanostructure enables a month-long intra-discal retention. Sci Rep 2020; 10:12017.
A novel study showing the use of avidin and dextran for electrostatically enabling intra-discal retention based on particle charge and size.
43. Vedadghavami A, Wagner EK, Mehta S, *et al.* Cartilage penetrating cationic peptide carriers for applications in drug delivery to avascular negatively charged tissues. Acta Biomater 2019; 93:258–269.
A novel study detailing the design of custom cationic peptide carriers of similar size but varying charge for intra-cartilage uptake, retention and penetration. Shown here for the first time is the additional dependency upon short-range hydrophobic and H-bond interactions for achieving high uptake of carriers in synergy with charge.
44. Sangar MLC, Girard EJ, Hopping G, *et al.* A potent peptide-steroid conjugate accumulates in cartilage and reverses arthritis without evidence of systemic corticosteroid exposure. Sci Transl Med 2020; 12:533.
An RA therapy study emphasizing the cationic and disulfide-bonded tertiary structure of a peptide for enabling cartilage accumulation and retention despite systemic delivery. Conjugation with steroid TCA, alleviated joint inflammation while mitigating off-target toxicities, thereby showing promise as an RA DDS.
45. Krishnan Y, Rees HA, Rossitto CP, *et al.* Green fluorescent proteins engineered for cartilage-targeted drug delivery: insights for transport into highly charged avascular tissues. Biomaterials 2018; 183:218–233.
A mathematical model for predicting the transport of S-GFPs in human knee cartilage is described here.

46. Brown S, Pistiner J, Adjei IM, Sharma B. Nanoparticle properties for delivery to cartilage: the implications of disease state, synovial fluid, and off-target uptake. Mol Pharm 2017; 16:469–479.
47. Sharma B, Brown SB. Chondroprotective nanoparticles for the treatment of osteoarthritis. US Patent. 2019. US20190224132A1.
48. ▪ Kumar S, Adjei IM, Brown SB, *et al.* Manganese dioxide nanoparticles protect cartilage from inflammation-induced oxidative stress. Biomaterials 2019; 224:119467.
This study shows the potential of using dual-functional compounds, such as MnO_2, which possess cationic and ROS scavenging abilities for nanoparticle-based osteoarthritis treatment.
49. Perni S, Prokopovich P. Optimisation and feature selection of poly-beta-amino-ester as a drug delivery system for cartilage. J Materials Chem B 2020; 8:5096–5108.
50. Perni S, Prokopovich P. Poly-beta-amino-esters nano-vehicles based drug delivery system for cartilage. Nanomedicine 2017; 13:539–548.
51. Geiger BC, Wang S, Padera RF, *et al.* Cartilage-penetrating nanocarriers improve delivery and efficacy of growth factor treatment of osteoarthritis. Sci Transl Med 2018; 10:eaat8800.
52. Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA. Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. Nat Mater 2008; 7:248–254.
53. ▪ Formica FA, Barreto G, Zenobi-Wong M. Cartilage-targeting dexamethasone prodrugs increase the efficacy of dexamethasone. J Control Release 2019; 295:118–129.
The ability of collagen-binding peptides to be retained within healthy and aggrecan-depleted cartilage is promising for treatment of latter stages of osteoarthritis where collagen II is still present within the tissue matrix.
54. ▪ Chen H, Qin Z, Zhao J, *et al.* Cartilage-targeting and dual MMP-13/pH responsive theranostic nanoprobes for osteoarthritis imaging and precision therapy. Biomaterials 2019; 225:119520.
The opportunity to combine collagen targeting approaches and osteoarthritis environment sensitivity for delivery of osteoarthritis diagnostics and drugs is described here.
55. He Y, Ren E, Lu Z, *et al.* Rational engineering of ferritin Nanocages for targeted therapy of osteoarthritis. Nanomedicine 2020; 28:102210.
56. ▪ Bedingfield SK, Yu F, Liu DD, *et al.* Matrix-targeted nanoparticles for MMP13 RNA interference blocks post-traumatic osteoarthritis. bioRxiv 2020; https://doi.org/10.1101/2020.01.30.925321.
This study shows the use of an antibody for enhancing collagen II binding and delivering siRNA for preventing PTOA-induced changes.
57. ▪▪ Hulme JT, D'Souza WN, McBride HJ, *et al.* Novel protein therapeutic joint retention strategy based on collagen-binding Avimers. J Orthop Res 2018; 36:1238–1247.
A novel strategy for targeting collagen II with the use of Avimers is detailed here. The high collagen II specificity and month-long cartilage retention shown by Avimer M26 is encouraging for carrying DMOADs and eliciting a long-term therapeutic response.
58. Cheung CS, Lui JC, Baron J. Identification of chondrocyte-binding peptides by phage display. J Orthop Res 2013; 31:1053–1058.
59. Pi Y, Zhang X, Shi J, *et al.* Targeted delivery of nonviral vectors to cartilage in vivo using a chondrocyte-homing peptide identified by phage display. Biomaterials 2011; 32:6324–6332.
60. ▪ Young CC, Vedadghavami A, Bajpayee AG. Bioelectricity for drug delivery: the promise of cationic therapeutics. Bioelectricity 2020; 2: 68–81.
This review presents a perspective on the safety and rational design of cationic carriers for targeted drug delivery to negatively charged tissues.
61. ▪ Ouyang Z, Tan T, Liu C, *et al.* Targeted delivery of hesperetin to cartilage attenuates osteoarthritis by bimodal imaging with Gd2 (CO3) 3@ PDA nanoparticles via TLR-2/NF-(B/Akt ÿignalling. Biomaterials 2019; 205: 50–63.
A chondrocyte-targeted peptide carrier is presented here, which is able to deliver hesperetin to alleviate PTOA-induced cartilage degradation in mice.
62. Hou KK, Pan H, Lanza GM, Wickline SA. Melittin derived peptides for nanoparticle based siRNA transfection. Biomaterials 2013; 34:3110–3119.
63. ▪ Yan H, Duan X, Pan H, *et al.* Development of a peptide-siRNA nanocomplex targeting NF-(B for efficient cartilage delivery. Sci Rep 2019; 9:1–7.
The chondrocyte-penetrating peptide used in this study also possesses the ability to penetrate deep through the cartilage matrix, therefore, it is able to target more chondrocytes and elicit a more effective biological response.
64. Madry H, Cucchiarini M. Advances and challenges in gene-based approaches for osteoarthritis. J Gene Med 2013; 15:343–355.
65. Evans CH, Gouze J, Gouze E, *et al.* Osteoarthritis gene therapy. Gene Ther 2004; 11:379–389.
66. Mi Z, Ghivizzani SC, Lechman ER, *et al.* Adenovirus-mediated gene transfer of insulin-like growth factor 1 stimulates proteoglycan synthesis in rabbit joints. Arthritis Rheum 2000; 43:2563–2570.
67. Frisbie D, Ghivizzani S, Robbins PD, *et al.* Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. Gene Ther 2002; 9:12–20.
68. Lai CM, Lai YK, Rakoczy PE. Adenovirus and adeno-associated virus vectors. DNA Cell Biol 2002; 21:895–913.
69. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. Adv Biomed Res 2012; 1:27.
70. ▪ Tang R, Harasymowicz NS, Wu C-L, *et al.* Gene therapy for follistatin mitigates systemic metabolic inflammation and posttraumatic arthritis in high-fat diet–induced obesity. Sci Adv 2020; 6:eaaz7492.
This study shows the ability of AAV-follistatin delivery to not only mitigate osteoarthritis but also to alleviate obesity-induced changes in mice.
71. ▪ Ashraf S, Kim B, Park S, *et al.* RHEB gene therapy maintains the chondrogenic characteristics and protects cartilage tissue from degenerative damage during experimental murine osteoarthritis. Osteoarthritis Cartilage 2019; 27:1508–1517.
This is the first study to show the anti-osteoarthritis activity of *RHEB* gene *in vivo*.
72. ▪ Stone A, Grol MW, Ruan M, *et al.* Combinatorial Prg4 and IL-1ra gene therapy protects against hyperalgesia and cartilage degeneration in posttraumatic osteoarthritis. Human Gene Ther 2019; 30:225–235.
Delivery of a combination of genes on HDAVs is shown to have improved benefit over single gene administration.
73. Lee B, Guse K, Ruan Z. Adenoviral-based biological delivery and expression system for use in the treatment of osteoarthritis. US Patent. 2019. US10301647B2.
74. ▪▪ Zhao L, Huang J, Fan Y, *et al.* Exploration of CRISPR/Cas9-based gene editing as therapy for osteoarthritis. Ann Rheum Dis 2019; 78:676–682.
This is the first study to show the in-vivo efficacy of CRISPR/Cas9 technology in treating preventing PTOA-induced changes. Further, a combination of multiple gene ablation provides increased therapeutic benefit as suppression of both pain and degradative changes can be achieved.
75. Seidl C, Fulga T, Murphy C. CRISPR-Cas9 targeting of MMP13 in human chondrocytes leads to significantly reduced levels of the metalloproteinase and enhanced type II collagen accumulation. Osteoarthritis Cartilage 2019; 27:140–147.
76. ▪ Shin HJ, Park H, Shin N, *et al.* p66shc siRNA nanoparticles ameliorate chondrocytic mitochondrial dysfunction in osteoarthritis. Int J Nanomed 2020; 15:2379.
Application of PLGA nanoparticles encapsulating siRNA-silencing oxidative stress-related genes for osteoarthritis therapy is described here.
77. Shin HJ, Park H, Shin N, *et al.* p47phox siRNA-Loaded PLGA nanoparticles suppress ROS/oxidative stress-induced chondrocyte damage in osteoarthritis. Polymers 2020; 12:443.
78. ▪ Brown C, Wu C-F, Chuang W, Shih S-F. Single intra-articular injection of TLC599 in patients with osteoarthritis knee pain: subgroup analyses of a placebo-controlled 24-week phase 2 trial. Osteoarthritis Cartilage 2020; 28:S481–S482.
This is the first completed clinical trial to show significant long-term pain suppression following intra-articular delivery of liposomal encapsulated Dex.
79. ClinicalTrials.gov. A phase 2, open label, PK study of TLC599 in subject with osteoarthritis of the knee. NCT03754049. 2019.
80. ClinicalTrials.gov. Extended and controlled release liposomal formulated dexamethasone for chronic knee OA pain. NCT04123561. 2019.
81. Byers-Kraus V, Aazami H, Mehra P, *et al.* Synovial and systemic pharmacokinetics of triamcinolone acetonide following intra-articular injection of an extended release formulation (FX006) or standard crystalline suspension in patients with knee osteoarthritis. Osteoarthritis Cartilage 2017; 25: S431.
82. ▪▪ Langworthy MJ, Conaghan PG, Ruane JJ, *et al.* Efficacy of triamcinolone acetonide extended-release in participants with unilateral knee osteoarthritis: a post hoc analysis. Adv Ther 2019; 36:1398–1411.
This is the only clinically approved corticosteroid delivery system for treating osteoarthritis-related knee pain. FX006 is a microsphere PLGA particle system that delivers TCA via intra-articular injection for prolonged joint residency, resulting in significant pain improvement over 24 weeks.
83. ▪ ClinicalTrials.gov. Study to evaluate the effect of FX006 on synovial inflammation in patients with OA of the knee. NCT03529942. 2018.
FX006 is under review in this trial for synovial inflammation, which is a key component to diagnosing early stage osteoarthritis for early intervention.
84. ClinicalTrials.gov. The effect of intra-articular bilateral knee injections of zilretta on performance measures in adults with knee OA. NCT03895840. 2018.
85. Helliwell JA, Malone AM, Smith TJ, Baum MM. Injectable sustained release composition and method of using the same for treating inflammation in joints and pain associated therewith. US Patent. 2018. US20180071222A1.
86. ClinicalTrials.gov. Study to evaluate the efficacy and safety of EP-104IAR in patients with osteoarthritis of the knee. NCT04120402. 2020.
87. ▪ ClinicalTrials.gov. Study of Cingal® and triamcinolone hexacetonide for the relief of knee osteoarthritis pain. NCT04231318. 2020.
This clinical trial represents two common osteoarthritis treatments combining intra-articular corticosteroid delivery via hydrogel encapsulation.
88. ClinicalTrials.gov. SI-613 study for knee osteoarthritis. NCT03209362. 2017.
89. ClinicalTrials.gov. Comparative assessment of viscosupplementation with polynucleotides and hyaluronic acid. NCT02417610. 2014.

90. ClinicalTrials.gov. Safety of intra-articular Sc-rAAV2.5IL-1Ra in subjects with ■ moderate knee OA. NCT02790723. 2019.
This clinical trial delivers IL-1Ra cDNA on a self-complementary adeno-associated virus for knee osteoarthritis therapy.
91. ClinicalTrials.gov. Study to evaluate the safety and tolerability of FX201 in ■ patients with osteoarthritis of the knee. NCT04119687. 2019.
Flexion Therapeutics, in addition to FX006, is also investigating the safety of *IL-1Ra* gene delivery for treating knee OA.
92. ClinicalTrials.gov. Follow on extension of XT-150-1-0201. NCT03769662. 2019. https://clinicaltrials.gov/ct2/show/NCT03769662
93. Madry H, Cucchiarini M. Gene therapy for human osteoarthritis: principles and clinical translation. Expert Opin Biol Ther 2016; 16:331–346.
94. Bandara G, Mueller G, Galea-Lauri J, *et al.* Intraarticular expression of biologically active interleukin 1-receptor-antagonist protein by ex vivo gene transfer. Proc Natl Acad Sci U S A 1993; 90:10764–10768.
95. Zhang X, Mao Z, Yu C. Suppression of early experimental osteoarthritis by gene transfer of interleukin-1 receptor antagonist and interleukin-10. J Orthop Res 2004; 22:742–750.
96. ClinicalTrials.gov. A study to determine the safety and efficacy of TG-C in ■ subjects with Kellgren and Lawrence grade 2 or 3 osteoarthritis of the knee. NCT03203330. 2018.
This is a clinically approved (in South Korea) ex-vivo gene therapy strategy combining transduced and nontransduced chondrocytes for knee osteoarthritis therapy.