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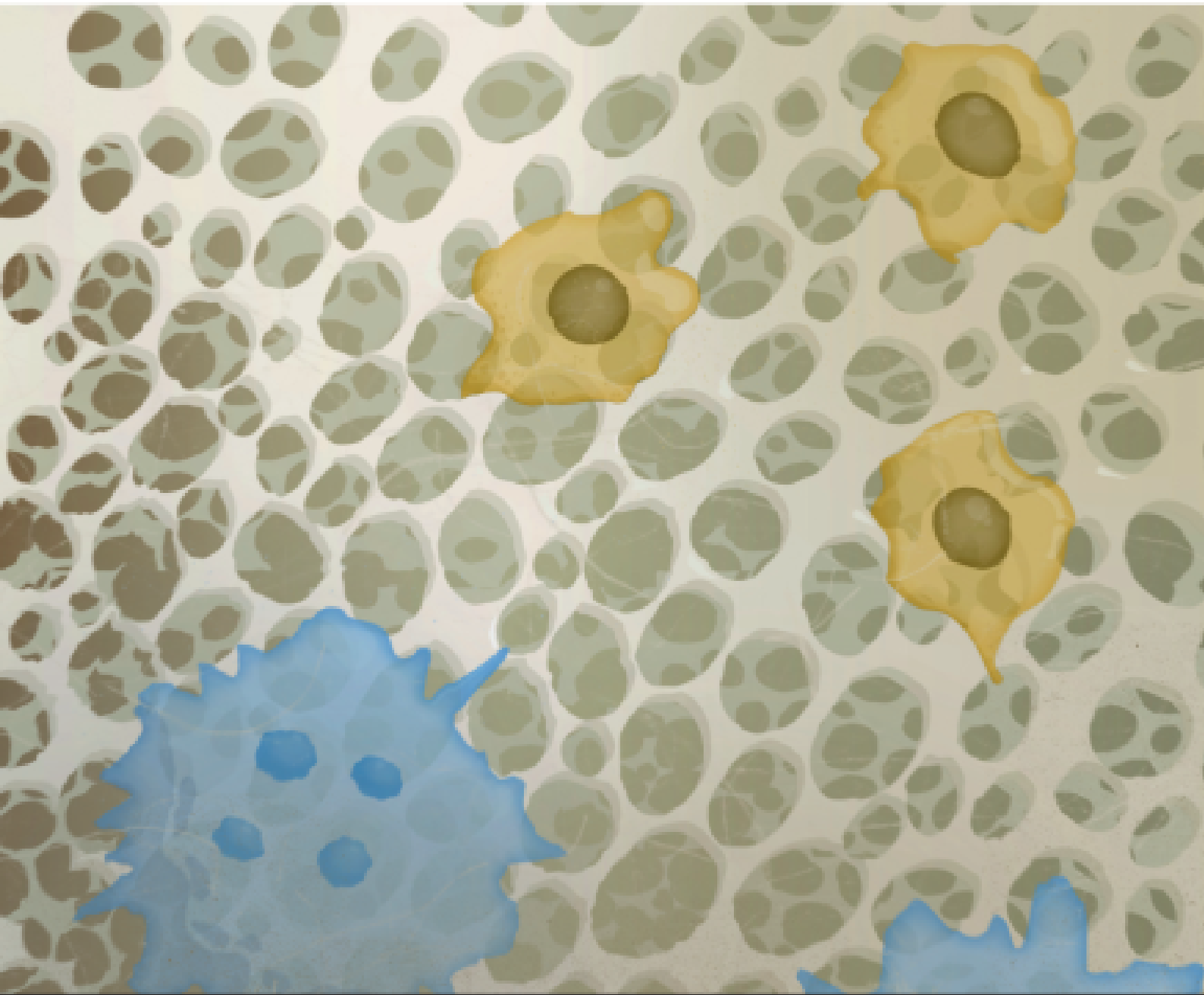


OSTEOARTHRITIS

Examining the role of synovial inflammation in OA progression

Effects of targeted therapies on bone

Local and generalized influences

OSTEOARTHRITIS

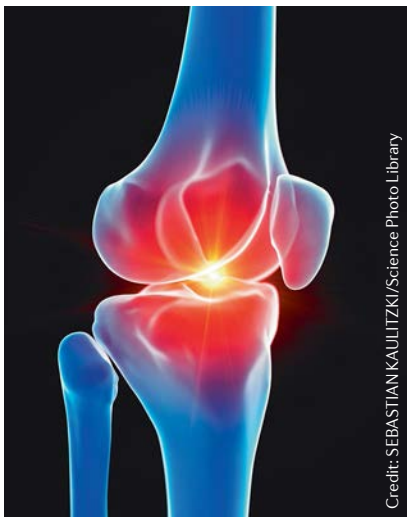
PGE_2 receptor antagonist has potential to treat osteoarthritis

In newly published results from two related studies, prostaglandin E_2 (PGE_2) and its receptor EP4 were associated with both osteoclast activity in osteoarthritis (OA) progression and tissue degeneration in cartilage injury. In both studies, the EP4 antagonist HL-43 inhibited these pathological activities and reduced associated pain in murine models, suggesting HL-43 has therapeutic potential in OA.

OA is characterized by pain, articular cartilage degeneration, osteophyte growth and subchondral sclerosis. PGE_2 is associated with OA pathology, but although NSAIDS (which affect production of PGE_2 and other prostanoids) offer symptomatic relief in OA, they can also have severe adverse effects.

In the new studies, EP4 expression was higher in injured articular cartilage and in subchondral bone in samples from patients with OA undergoing total knee replacement than in uninjured OA cartilage and subchondral bone from patients without OA. In both studies, tissue-specific deletion of *Ptger4*, which encodes EP4, improved OA-associated pathology in murine models. Knockout of *Ptger4* in osteoclasts reduced disease progression and osteophyte formation, as well as pain, in a mouse anterior cruciate ligament transection model of OA. Similarly, in mice with cartilage defects induced by microfracture surgery or destabilization of the medial meniscus, knockout of *Ptger4* promoted regeneration of stable, mature articular cartilage and reduced joint pain compared with control mice without *Ptger4* deletion.

> knockout of *Ptger4* promoted regeneration of stable, mature articular cartilage



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The EP4 antagonist HL-43 was identified by screens for the most effective 1H-1,2,3-triazole-based small-molecule inhibitor of PGE_2-induced osteoclast differentiation and IL-1β-mediated changes in chondrocyte gene expression. In the murine models, HL-43 treatment had similar effects to *Ptger4* knockout. "HL-43 had the best efficacy of the screened compounds on articular cartilage regeneration and lower toxicity than FDA-approved NSAIDs celecoxib and grapiprant," explains Jian Luo, corresponding author on both studies. "The work of setting up clinical trials testing HL-43 in OA treatment is ongoing."

Robert Phillips

ORIGINAL ARTICLES Jiang, W. et al. PGE2 activates EP4 in subchondral bone osteoclasts to regulate osteoarthritis. *Bone Res.* **10**, 27 (2022) | Jin, Y. et al. A novel prostaglandin E receptor 4 (EP4) small molecule antagonist induces articular cartilage regeneration. *Cell Discov.* **8**, 24 (2022)

AUTOINFLAMMATORY DISEASES

NEMO splice variant causes distinct autoinflammatory syndrome

Loss-of-function mutations in *IKBKG*, encoding NF-κB essential modulator (NEMO), typically cause an immunodeficiency syndrome. However, new research reveals that mutations that mediate alternative mRNA splicing of *IKBKG* lead to an inflammatory disease phenotype that does not seem to be associated with increased susceptibility to viral or bacterial infection. As the NEMO isoform overexpressed by patients with this phenotype lacks the domain encoded by exon 5, the researchers propose to name the newly discovered syndrome NEMO deleted exon 5 autoinflammatory syndrome (NDAS).

The discovery arose from studies of three unrelated male patients with a similar severe, early onset inflammatory disease, the features of which suggested an underlying defect in NEMO. Genetic studies using several different testing modalities determined that the patients had de novo mutations that led to production of the same NEMO isoform, termed NEMO-Δex5. Expression of the NEMO-Δex5 isoform in cells from patients with NDAS was associated with activation of NF-κB response genes.

In vitro, expression of NEMO-Δex5 in dermal fibroblasts was associated with dampened antiviral responses, whereas in immune cells such as T cells and macrophages it led to increased NF-κB activation and type I interferon production, as well as resistance to viral infection.

> the researchers propose to name the … syndrome NEMO deleted exon 5 autoinflammatory syndrome (NDAS)

Credit: Alex Whitworth/Springer Nature Limited

Further investigations revealed that NEMO-Δex5 enhances NF-κB signalling in immune cells by forming a complex with and stabilizing inducible IκB kinase (IKK)-related kinase IKKi. In dermal fibroblasts from patients with NDAS, stimulation with TNF was able to induce IKKi protein expression and rescue type I interferon production. The findings suggest a mechanism by which TNF blockade, which has some efficacy in patients with NDAS, might reduce NF-κB activity and inflammatory disease.

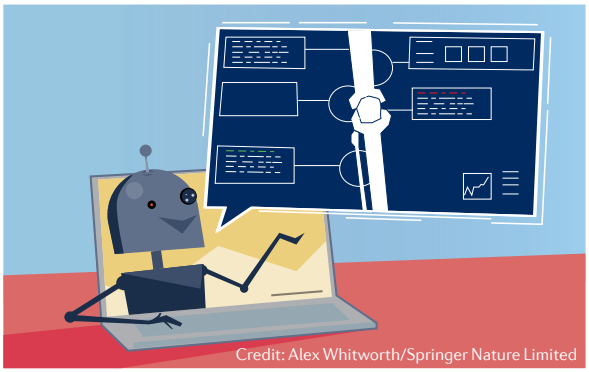
"Characterization of the NEMO isoform expressed in [patients with NDAS] has taught us more about how type I interferon and antiviral immunity and the pro-inflammatory NF-κB pathway may be fine-tuned or regulated," reports corresponding author Eric Hanson.

Sarah Onuora

ORIGINAL ARTICLE Lee, Y. et al. Genetically programmed alternative splicing of NEMO mediates an autoinflammatory disease phenotype. *J. Clin. Invest.* **132**, e128808 (2022)

OSTEOARTHRITIS

Identifying OA subgroups based on biochemical data


Credit: Alex Whitworth/Springer Nature Limited

Despite much research, progress in developing disease-modifying drugs for osteoarthritis (OA) has been slow, owing in part to the heterogeneity of the disease. In a new study, Angelini et al. used a machine learning approach to stratify patients with OA into distinct subsets that correspond to their underlying pathophysiology, with potential implications for individualized treatments that target specific disease mechanisms.

The authors studied 297 patients with knee OA. At baseline, serum and urine samples were collected for the analysis of 16 biochemical markers, each reflecting different aspects of joint tissue turnover and OA pathology. A machine learning approach was used to identify subgroups of patients based on these biochemical data. Moreover, to identify clinically meaningful differences between subgroups, a variety of clinical assessments, physical examinations and questionnaires were collected.

Three distinct OA subtypes (clusters) were identified, reflecting differences in their underlying biology: a low tissue turnover subtype, a structural damage subtype and a systemic inflammation subtype. Replication of the clustering analysis on a distinct OA cohort, based on a common set of 11 biomarkers, confirmed the presence of these three subtypes. As well as biochemical data, clinical scores differed between each cluster when analysing 2-year follow-up data. The low tissue turnover cluster had the highest proportion of patients with non-progressive symptomology, whereas the structural damage cluster had the highest proportion of structural progressors, and the inflammatory cluster had the highest proportion of patients with sustained or progressive pain. Thus, stratification of patients with OA based on baseline biochemical clustering could be used to predict disease progression.

> “Three distinct OA subtypes (clusters) were identified, reflecting differences in their underlying biology”

These findings support the existence of distinct OA subtypes and emphasize the need to move away from the one-size-fits-all treatment approach that is often used in clinical trials. “With precisely defined disease phenotypes, future clinical trials may be able to define more refined inclusion and exclusion criteria, leading to a more effective evaluation of potential OA treatments”, explains Jaume Bacardit, corresponding author of the study.

Michael Attwaters

ORIGINAL ARTICLE Angelini, F. et al. Osteoarthritis endotype discovery via clustering of biochemical marker data. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-221763 (2022)

AUTOIMMUNITY

C4A copy number is associated with autoimmune disease

New research demonstrates that the presence or absence of anti-SSA/Ro and/or anti-SSB/La autoantibodies greatly influences the relationship between complement-component gene *C4A* copy number and the likelihood of having a systemic autoimmune inflammatory disease (SAID).

Deficiencies in components of the classic complement pathway, such as C4, are known to be associated with the occurrence of systemic lupus erythematosus (SLE). C4 is encoded by two genes, *C4A* and *C4B*, which demonstrate considerable copy-number variation. *C4A* and *C4B* are located between the *HLA* class I and class II regions on chromosome 6, and linkage disequilibrium has made it difficult to determine which of these genetic elements is associated with the presence of SAIDs.

In the new study, low (≤1) *C4A* copy number was more common among patients with SLE, primary Sjögren syndrome or myositis ($n = 2,290$) than among healthy individuals ($n = 1,251$). The pattern of *C4A* copy number was similar in each of the three SAIDs. *C4A* copy number in patients with SAIDs was inversely associated with the presence of anti-SSA/Ro and anti-SSB/La autoantibodies. Furthermore, for each decrease in *C4A* copy number, the additional risk of having an SAID was highest in individuals with both autoantibodies (OR = 5.9; 95% CI 4.8–7.2), intermediate in individuals with only one type of autoantibody (OR = 2.4; 95% CI 2.0–2.8) and lowest in individuals with neither autoantibody (OR = 1.5; 95% CI 1.4–1.7).

> “A lack of C4b binding could prevent removal of immune complexes”


Credit: ValentynVolkov/iStock/Getty Images Plus

A pathogenic mechanism linking *C4A* deficiency with impaired removal of immune complexes in SAID was suggested by the results of in vitro experiments in which deposition of C4b on aggregated IgG was greater with serum from *C4A*-only carriers than from *C4B*-only carriers. A lack of C4b binding could prevent removal of immune complexes, leading to autoantibody generation and SAID development.

“The presence of anti-SSA/Ro or anti-SSB/La autoantibodies may be a common mediator of disease in a subgroup of patients,” notes the first author of the study, Christian Lundtoft. Lars Rönnblom, the corresponding author, adds “genetic profiling of patients may be important when classifying and stratifying them for a more individualized treatment.”

Robert Phillips

ORIGINAL ARTICLE Lundtoft, C. et al. Complement *C4* copy number variation is linked to SSA/Ro and SSB/La autoantibodies in systemic inflammatory autoimmune diseases. *Arthritis Rheumatol.* https://doi.org/10.1002/art.42122 (2022)

CARDIOVASCULAR DISEASE

Cardio-rheumatology: it's time to collaborate

Lihi Eder and Paula Harvey

New EULAR recommendations offer useful guidance for improving cardiovascular health in patients with rheumatic and musculoskeletal disease. However, an interdisciplinary model of care is crucial to the optimal management of cardiovascular risk in these patients.

Refers to Drosos G. C. et al. EULAR recommendations for cardiovascular risk management in rheumatic and musculoskeletal diseases, including systemic lupus erythematosus and antiphospholipid syndrome. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-221733 (2022).

It is now well accepted that most patients with rheumatic and musculoskeletal diseases (RMDs) have an increased cardiovascular risk. This risk is related to both the burden of traditional cardiovascular risk factors and additional RMD-related factors, particularly chronic inflammation. New EULAR recommendations for the management of cardiovascular risk in RMDs aim to guide clinicians regarding the management of these important co-morbidities while also highlighting the many gaps in evidence and need for further research[1]. Although they provide much-needed practical recommendations, we believe the implementation of these recommendations in the 'real world' merits further discussion and highlights the need to enhance interdisciplinary cardio-rheumatology care to optimize the management of cardiovascular risk in RMDs.

The new guidelines are an important addition to previous EULAR recommendations, which focused on cardiovascular risk management in patients with inflammatory joint disorders[2]. The current article focuses, for the first time, on a large number of RMDs that have not been addressed in previous publications, such as gout, vasculitis and systemic autoimmune rheumatic diseases including systemic lupus erythematosus (SLE) and anti-phospholipid syndrome (APS). In addition to several overarching principles, the guidelines discuss RMD-specific approaches to cardiovascular risk stratification, management of traditional modifiable cardiovascular risk factors, and considerations regarding the use of immune-modulating therapies from a cardiovascular standpoint.

One of the main questions that arises when discussing such recommendations is who should be responsible for the regular surveillance of cardiovascular risk and interventions for optimization of traditional cardiovascular risk factors in patients with RMD? An overarching principle of the recommendations states that "rheumatologists are responsible for [cardiovascular risk] assessment and management in collaboration with primary care providers, internists or cardiologists…"[1]. This statement clearly puts the rheumatologist at the center of cardiovascular preventive care, which has some limitations when translated to the real-world clinical environment and calls for some re-thinking and flexibility of this approach. Substantial gaps in the management of cardiovascular risk remain a major problem despite an increased awareness of this problem among rheumatologists; a persistently high proportion of patients remain underdiagnosed and undertreated for hypertension and dyslipidemia, two important modifiable risk factors[3,4]. Studies that attempted to identify barriers to cardiovascular risk management among rheumatologists and primary care physicians indicated lack of time, lack of knowledge of current guidelines for cardiovascular risk factors, and lack of care coordination as some of the main reasons[5,6]. Inter-disciplinary collaborations and shared models of care have been developed to better manage patients with complex medical conditions and could provide insight into effective models for coordinated cardiovascular risk management in patients with RMD. A successful example of such shared care includes the co-management of psoriatic arthritis by rheumatologists and dermatologists and the establishment of dermatology–rheumatology care models[7]. An approach to shared care that involves rheumatologists, primary care physicians and preventive cardiologists could be an important step for optimizing cardiovascular risk management in RMD[8] (FIG. 1).

EULAR recommends that on the basis of current (and lack of) evidence, the cornerstone of cardiovascular risk reduction in RMD should be similar to that for the general population[1]. This approach relies on the identification of patients who are at high cardiovascular risk by use of established risk scores developed and validated in the general population. The guidelines recognize the suboptimal performance of such risk scores when applied to patients with some RMDs, leading to underestimation of cardiovascular risk in these populations. Hence, EULAR recommends considering the use of disease-specific risk prediction models or consideration of disease-specific risk factors for risk stratification in patients with SLE, ANCA-associated vasculitis or APS. However, the low level of evidence regarding cardiovascular risk stratification, in particular the lack of external validation of these specific prediction approaches, precludes the endorsement of any particular disease-specific risk-prediction equations. Furthermore, owing to lack of data, the guidelines suggest using the same cardiovascular risk scores as for the general population in patients with the other RMDs covered by the recommendations. This guidance is in contrast to the prior EULAR recommendation to apply a 1.5 multiplication factor to improve cardiovascular risk stratification in rheumatoid arthritis[2].

Regardless of the selected approach to risk stratification, regular screening for cardiovascular risk factors and tailoring treatment on the basis of estimated cardiovascular risk remain critical to all approaches to primary prevention of cardiovascular events in RMDs. Patients should undergo regular measurement of blood pressure, lipid and glucose profiles, and evaluation of lifestyle factors for input into a risk-prediction calculator to estimate cardiovascular risk. Additionally, knowledge of local guidelines for management of hypertension, dyslipidemia and diabetes mellitus is needed to

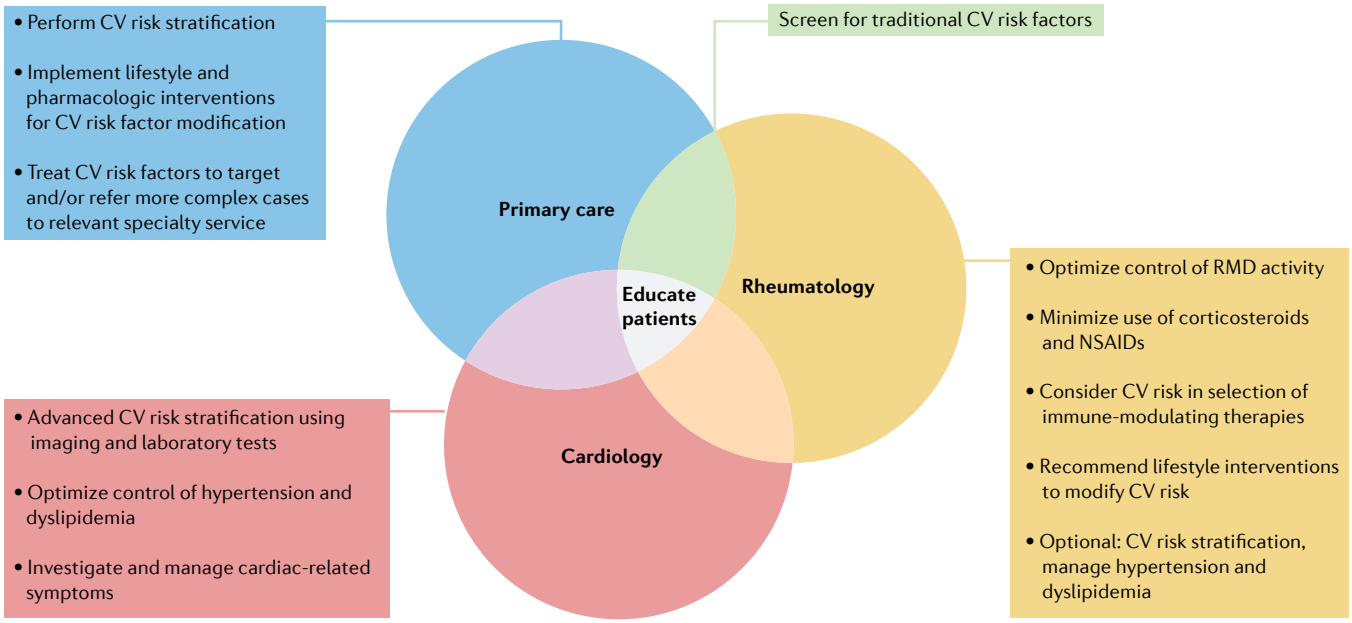


Fig. 1 | **Cardio-rheumatology: a new model of shared care.** Optimal management of cardiovascular (CV) risk in patients with rheumatic and musculoskeletal diseases requires collaboration and coordination of care between rheumatologists, primary care physicians and cardiologists.

recognize thresholds and targets for tailoring treatment according to the calculated cardiovascular risk. These tasks might be beyond the scope of practice of the typical rheumatologist.

Conversely, the EULAR recommendations[1] highlight several issues regarding cardiovascular risk prevention that clearly fall within the scope of practice of all rheumatologists. Achieving optimal control of rheumatic disease activity is an important treatment goal from a cardiovascular standpoint, as studies have found an association between various measures of disease activity and cardiovascular risk[9]. Titration of urate-lowering therapy to reach specific targets in gout, prioritizing the use of hydroxycholoquine in SLE, and minimizing the use of corticosteroids in various RMDs are some specific recommendations that rheumatologists could adopt to reduce cardiovascular risk in their patients. The EULAR recommendations also recognize the general lack of knowledge to guide selection of specific immune-modulating therapies when considering cardiovascular risk reduction[1], although research is being undertaken in this field[10]. These considerations are beyond the scope of practice of primary care physicians and cardiologists and highlight the important role of rheumatologists in tailoring treatment of RMD to improve the overall health of patients, including cardiovascular disease.

In summary, the new EULAR recommendations are an important step towards improving awareness and optimizing screening, evaluation and management of cardiovascular risk in patients with RMD. Important gaps in knowledge still exist regarding the approach to risk stratification, treatment targets for modifiable risk factors and the selection of immune-modulating therapies. In addition, these gaps signal the need for enhanced collaboration and coordination of care between rheumatologists, primary care physicians and cardiologists. Establishment of cardio-rheumatology models of care (see FIG. 1) whereby responsibilities are shared and coordinated between different specialties could improve adherence to current treatment recommendations. Such programmes could also increase awareness and knowledge across specialties and trigger collaborative research efforts to inform future guidelines.

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1. Drosos, G. C. et al. EULAR recommendations for cardiovascular risk management in rheumatic and musculoskeletal diseases, including systemic lupus erythematosus and antiphospholipid syndrome. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-221733 (2022).
2. Agca, R. et al. EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. *Ann. Rheum. Dis.* **76**, 17–28 (2017).
3. Eder, L. et al. Gaps in diagnosis and treatment of cardiovascular risk factors in patients with psoriatic disease: an international multicenter study. *J. Rheumatol.* **45**, 378–384 (2018).
4. Bartels, C. M. et al. Low frequency of primary lipid screening among medicare patients with rheumatoid arthritis. *Arthritis Rheum.* **63**, 1221–1130 (2011).
5. Navarro-Millan, I. et al. Barriers and facilitators for screening and treatment of hyperlipidemia among patients with inflammatory arthritis. *BMC Rheumatol.* **4**, 26 (2020).
6. Esmaeilbeigi, F. & Pope, J. E. Appropriate cardiovascular disease risk assessment in systemic lupus erythematosus may be lacking in rheumatology practice. *Clin. Exp. Rheumatol.* **36**, 526–532 (2018).
7. Okhovat, J. P. et al. Psoriasis and Psoriatic Arthritis Clinics Multicenter Advancement Network Consortium (PPACMAN) survey: benefits and challenges of combined rheumatology-dermatology clinics. *J. Rheumatol.* **44**, 693–694 (2017).
8. Rollefstad, S. et al. Treatment to lipid targets in patients with inflammatory joint diseases in a preventive cardio-rheuma clinic. *Ann. Rheum. Dis.* **72**, 1968–1974 (2013).
9. Nikpour, M., Gladman, D. D. & Urowitz, M. B. Premature coronary heart disease in systemic lupus erythematosus: what risk factors do we understand? *Lupus* **22**, 1243–1250 (2013).
10. Ytterberg, S. R. et al. Cardiovascular and cancer risk with tofacitinib in rheumatoid Arthritis. *N. Engl. J. Med.* **386**, 316–326 (2022).

Competing interests

L.E. declares that she has received research and educational grants from Abbvie, Eli Lilly, Janssen, Novartis, Pfizer and UCB. P.H. declares no competing interests.

Effects of targeted therapies on bone in rheumatic and musculoskeletal diseases

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Abstract | Generalized bone loss (osteoporosis) and fragility fractures can occur in rheumatic and musculoskeletal diseases including rheumatoid arthritis and spondyloarthritis (SpA; including ankylosing spondylitis and psoriatic arthritis). In addition, rheumatoid arthritis can involve localized, periarticular bone erosion and, in SpA, local (pathological) bone formation can occur. The RANK–RANKL–osteoprotegerin axis and the Wnt–β-catenin signalling pathway (along with its inhibitors sclerostin and Dickkopf 1) have been implicated in inflammatory bone loss and formation, respectively. Targeted therapies including biologic DMARDs and Janus kinase (JAK) inhibitors can stabilize bone turnover and inhibit radiographic joint damage, and potentially also prevent generalized bone loss. Targeted therapies interfere at various points in the mechanisms of local and generalized bone changes in systemic rheumatic diseases, and they effect biomarkers of bone resorption and formation, bone mass and risk of fragility fractures. Studies on the effects of targeted therapies on rates of fragility fracture are scarce. The efficacy of biologic DMARDs for arresting bone formation in axial SpA is debated. Improved understanding of the most relevant therapeutic targets and identification of important targeted therapies could lead to the preservation of bone in inflammatory rheumatic and musculoskeletal diseases.

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Inflammatory rheumatic and musculoskeletal diseases (RMDs), such as rheumatoid arthritis (RA) and spondyloarthritis (SpA; including ankylosing spondylitis (AS) and psoriatic arthritis (PsA)), as well as other arthritides, have been associated with both generalized osteoporosis and fragility fractures, as well as with localized inflammatory bone resorption (erosions) and/or pathological bone formation[1–7]. Targeted therapies, including biologic DMARDs (bDMARDs) and targeted synthetic DMARDs (such as Janus kinase (JAK) inhibitors), not only inhibit localized bone resorption and formation but might also slow down generalized bone loss and inhibit the development of fragility fractures in patients with these diseases[1,3–5,8–11]. Systemic inflammation involving inflammatory cells and mediators is a main cause of arthritis-associated inflammatory bone resorption and formation[1–4]. Therefore, in addition to their possible direct effects on bone, targeted therapies for RMDs might indirectly halt bone loss by dampening inflammation in RA[3,12]. However, the effects of targeted therapies on bone formation in axial SpA (axSpA) are rather more controversial[8,9,13,14].

In this Review, we briefly introduce the cellular and molecular factors involved in inflammatory bone resorption and formation, and then discuss the effects of targeted therapies on laboratory biomarkers of bone metabolism as well as on bone density. Finally, we present data on the clinical effects of targeted therapies on osteoporosis and fragility fractures in arthritides. The main goal of this Review is to present the most important mechanisms involved in pathological bone metabolism underlying RA and SpA. In addition, we discuss which targeted therapies might be suitable for normalizing pathological bone turnover in these RMDs.

Bone metabolism in inflammatory RMDs

Receptor activator of nuclear factor-κB (RANK), its ligand (RANKL) and osteoprotegerin are important mediators of bone turnover in the context of inflammation[12,15]. The pro-inflammatory cytokines TNF, IL-1β, IL-6, IL-17 and IL-23 are involved in RANKL-dependent osteoclastogenesis, osteoclast differentiation and activation[2,3,12,16] (FIG. 1). Among these cytokines, TNF has an exceptional role as it directly induces osteoclast function, indirectly enhances osteoclastogenesis via RANKL and stimulates RANKL expression by osteoblasts, T cells and B cells[12,17]. Inhibition of TNF results in increased osteoprotegerin and decreased

Key points

- Several molecules, especially inflammatory mediators, contribute to localized bone resorption and formation and to generalized osteoporosis associated with inflammatory rheumatic and musculoskeletal diseases.
- Targeted therapies could balance the pathological bone turnover in rheumatoid arthritis; however, their effects on bone formation in spondyloarthritis are not equivocal and might depend on the disease stage.
- Most targeted therapies, particularly TNF inhibitors, might attenuate generalized bone loss in inflammatory rheumatic and musculoskeletal diseases, but more information is needed on the effects of other biologic DMARDs and Janus kinase (JAK) inhibitors.
- Few studies have assessed the effects of targeted therapies on the risk of fragility fractures; more trials need to be conducted.

RANKL expression in RMDs[18,19]. The IL-17–IL-23 axis has also been implicated in osteoclastogenesis and inflammatory bone erosion[16,20–22]. Anti-IL-6 receptor treatment also exerts inhibitory effects on osteoclasts, underscoring the role of IL-6 in bone resorption[23]. In addition, multiple JAK–signal transducer and activator of transcription (STAT) pathways have been implicated in RANKL-dependent inflammatory bone destruction[24,25]. Moreover, JAK inhibition might ameliorate pathological bone loss and increase bone mass by stimulating osteoblast function[26] (FIG. 1). Preclinical studies revealed that tofacitinib and baricitinib attenuated RANKL-dependent osteoclast activation[24,25] and exerted bone-preserving effects in animal models[26–28]. Under conditions of hypoxia, tofacitinib promoted bone formation from mesenchymal stem cells[27].

In addition to the role of immune cells and inflammatory mediators in bone destruction, the possibility of autoantibody-mediated, inflammation-independent bone loss has also been postulated in RA[29,30]. Bone destruction mediated directly by anti-citrullinated protein antibodies (ACPAs) or rheumatoid factor could precede the development of inflammation and might contribute to the early development of bony erosions[29] (FIG. 1). ACPAs can directly activate osteoclasts by various mechanisms including the stimulation of TNF production, synovial fibroblast migration and subsequent osteoclast differentiation[31,32]. ACPA seropositivity has been associated with impaired bone strength and increased risk of fragility fractures in RA[33], suggesting that autoimmunity is involved in generalized, as well as localized, bone loss in arthritis.

Wnt proteins, as well as other proteins associated with the canonical Wnt–β catenin signalling pathway, are important regulators of bone formation in the context of inflammation, especially in SpA[34]. Dickkopf 1 (DKK1) and sclerostin are important inhibitors of the Wnt axis and thus promote bone loss[34–36]. Sclerostin (an osteocyte-specific protein) also stimulates bone resorption through an autologous effect on osteocyte RANKL production[37]. Production of DKK1 is increased in RA[38,39]; however, a meta-analysis did not find increased sclerostin production in this disease[40]. One explanation of the latter phenomenon might be that although osteocytes produce high levels of sclerostin, the increased death rate of these cells could result in the absence of high sclerostin concentrations in active RA[41]. The interaction between DKK1 and sclerostin could have an additive effect, as DKK1 blockade resulted in inhibition of sclerostin production[42].

Among the pro-inflammatory cytokines implicated in pathological bone metabolism, TNF stimulates DKK1 production and consequently inhibits the canonical Wnt signalling pathway[12,35]. The effects of IL-6 on Wnt-dependent bone formation are somewhat controversial[43–45]. On the one hand, in contrast to TNF, IL-6 inhibits DKK1 expression by synovial fibroblasts[43]. On the other hand, IL-6 blockade has been associated with decreased DKK1 production in RA[46], suggesting that IL-6 might induce DKK1 expression[12]. The regulation of DKK1 and other members of the canonical Wnt signalling axis by IL-6 needs further clarification.

The Wnt–DKK1–SOST system and its regulation by TNF and other pro-inflammatory cytokines have a peculiar role in localized bone formation and the development of syndesmophytes in SpA, with therapeutic relevance[9,13] (FIG. 2). TNF-induced production of DKK1 and consequently of sclerostin (which can suppress Wnt-mediated bone formation) is limited in SpA[47,48]. However, low concentrations or blockade of sclerostin and DKK1 have been associated with persistent inflammation and syndesmophyte formation in AS[47,49]. Similar to DKK1, sclerostin is also found at low concentrations in the blood of patients with SpA[49,50]. HLA-B27 has been associated with low concentrations of DKK1 and sclerostin levels in AS[51]. Low concentrations of DKK1 have been found in patients with PsA as well[39]. As discussed further below, these molecular mechanisms could be, in part, responsible for the inability of TNF inhibitors to halt syndesmophyte formation in SpA[9,13]. With respect to IL-17, one study showed that concentrations of the Wnt inhibitors DKK1 and sclerostin increased after 6 months of treatment with the IL-17A inhibitor secukinumab in patients with PsA[52], supporting a role for IL-17 in the stimulation of Wnt-mediated osteogenesis. Moreover, bimekizumab, a dual inhibitor of IL-17A and IL-17F, was able to inhibit inflammation-driven osteogenic differentiation of human periosteal cells[22]. These results might have important relevance for the use of IL-17 inhibitors to halt new bone formation in SpA (FIG. 2).

In conclusion, the RANK–RANKL and the canonical Wnt–β catenin pathways are critically involved in inflammatory bone resorption and formation, respectively. Inflammatory mediators, primarily TNF, IL-6, the IL-23–IL-17 axis and JAK–STAT-mediated mechanisms, have a crucial role in the regulation of these processes. Targeted therapies directed against these mediators might effectively restore bone balance in inflammatory RMDs.

Targeted therapies and bone biomarkers

Targeted therapies used in RMDs such as RA or SpA can influence bone turnover and the release of circulating biomarkers (FIG. 2). These biomarkers include markers of bone formation, namely osteocalcin and procollagen type I N-propeptide (P1NP), as well as of bone resorption, such as C-terminal telopeptide (CTX) of type I collagen, cathepsin K, matrix metalloproteinases (MMPs) and RANKL[3,19,53–56]. Inhibitors of bone resorption,

such as osteoprotegerin, and those of bone formation, including DKK1 and sclerostin, have also been studied as markers of bone metabolism[19,49,55] (TABLE 1). Most anti-cytokine therapeutics favourably affect the pathological bone turnover underlying RA and SpA. However, for some of the effects of targeted therapies, especially with regard to bone formation, controversy remains that needs to be clarified by future therapeutic trials. As RA and SpA differ from each other in many ways, in this section we discuss the effects of targeted therapies on these two types of arthritis separately.

Effects of TNF inhibitors. A great amount of data has been published on the favourable effects of anti-TNF therapy on bone biomarkers in RA, beginning in the early 2000s[55,57]. At that time, it was clear that TNF inhibitors induced a reduction in bone resorption[55,57], but their Wnt-mediated effect, resulting in an increase in bone formation, has become understood only recently[9,13]. The initial data on bone resorption effects were confirmed in studies with etanercept, adalimumab and also some with golimumab[3]. Anti-TNF agents have been shown to increase serum concentrations of osteocalcin and P1NP and to suppress CTX and RANKL levels in RA[3,53,55–58]. These findings were not always consistent, as in some studies anti-TNF agents showed no effects on osteocalcin, P1NP or CTX levels in some studies[3,59,60], whereas in other studies TNF inhibitors increased osteoprotegerin:RANKL, osteocalcin:CTX and P1NP:CTX ratios[55,58,59]. Moreover, TNF inhibitors also suppressed DKK1 production, leading to increased bone formation in patients with RA[60,61]. In some studies, anti-TNF treatment increased sclerostin production in RA[19,62]. In most studies in RA, changes in bone biomarkers induced by anti-TNF therapy were associated with or accompanied by improvements in disease activity and inflammatory markers (for example, C-reactive protein (CRP) concentration)[3] (TABLE 1).

TNF inhibition also increased serum concentrations of osteocalcin, P1NP and sclerostin and decreased

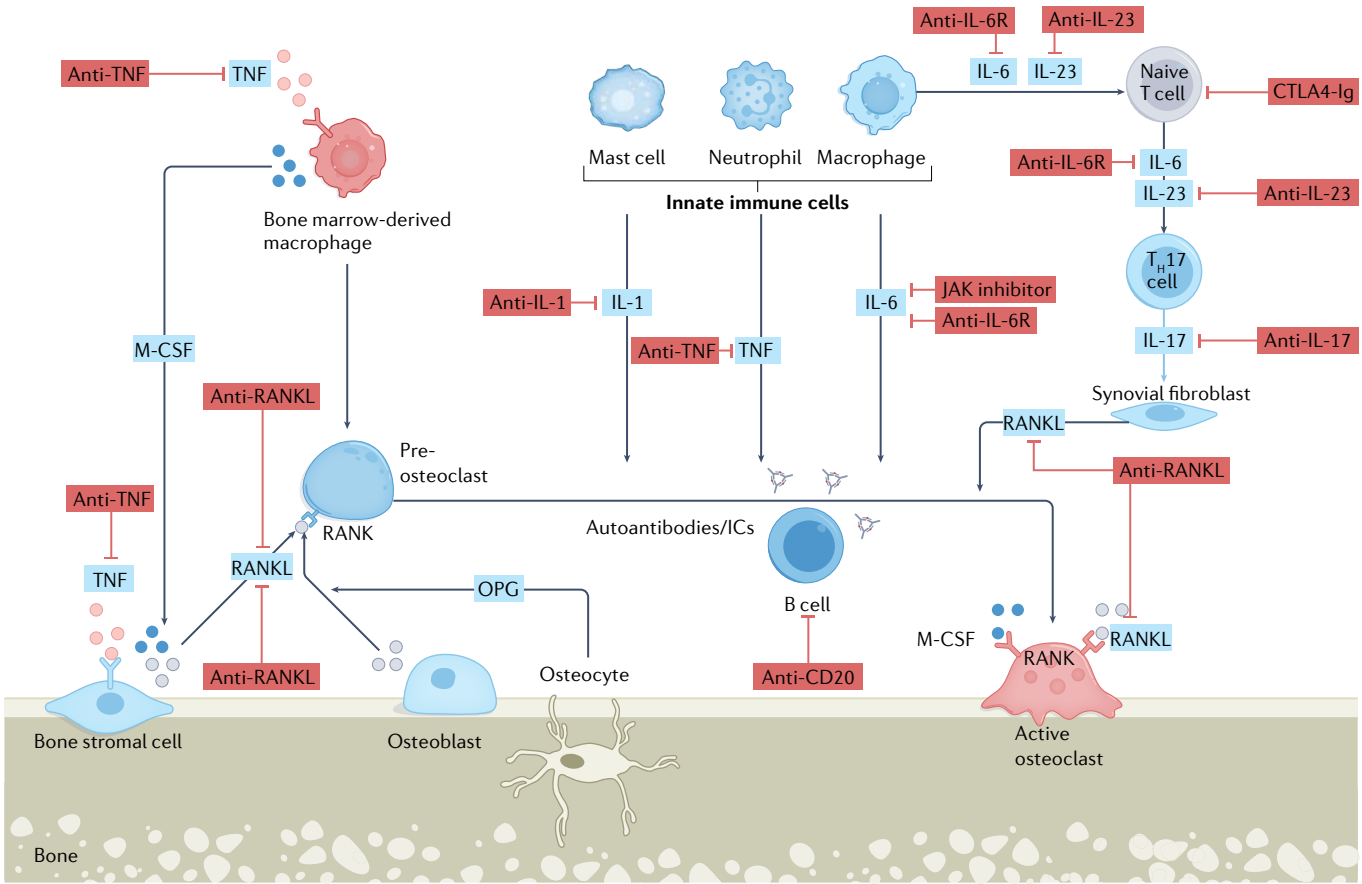

Fig. 1 | **Effects of targeted therapies on cellular and molecular pathways involved in inflammatory bone resorption.** Osteoclasts are derived from TNF-stimulated bone marrow-derived macrophages and then osteoclast precursor cells. Osteoblasts and bone stromal cells release receptor activator of nuclear factor-κB ligand (RANKL), which binds to RANK on osteoclast precursor cells and stimulates osteoclast differentiation and activation. RANKL-dependent osteoclast differentiation is also mediated by pro-inflammatory cytokines (TNF, IL-1, IL-6 and IL-17) produced by innate immune cells, including macrophages. IL-6 and IL-23 promote the differentiation of naive T cells into T helper 17 (T_H17) cells. T_H17-produced IL-17 stimulates synovial fibroblasts to release RANKL and thus promote osteoclast activation. Inflammation-independent, autoantibody-driven bone loss might also exist. B cells produce autoantibodies that can form immune complexes and stimulate osteoclast growth. Osteocytes are major producers of osteoprotegerin (OPG), which binds to and neutralizes RANKL, thus inhibiting the transition of osteoclast precursor cells to active osteoclasts. Targeted therapies, including inhibitors of TNF, IL-1, IL-6, IL-17, IL-23, T cells (CTLA4-Ig), B cells (anti-CD20), Janus kinases (JAKs) and RANKL, can interfere with osteoclastogenesis and inflammatory bone resorption at different points in the pathways involved. IC, immune cell; M-CSF, macrophage colony-stimulating factor 1.

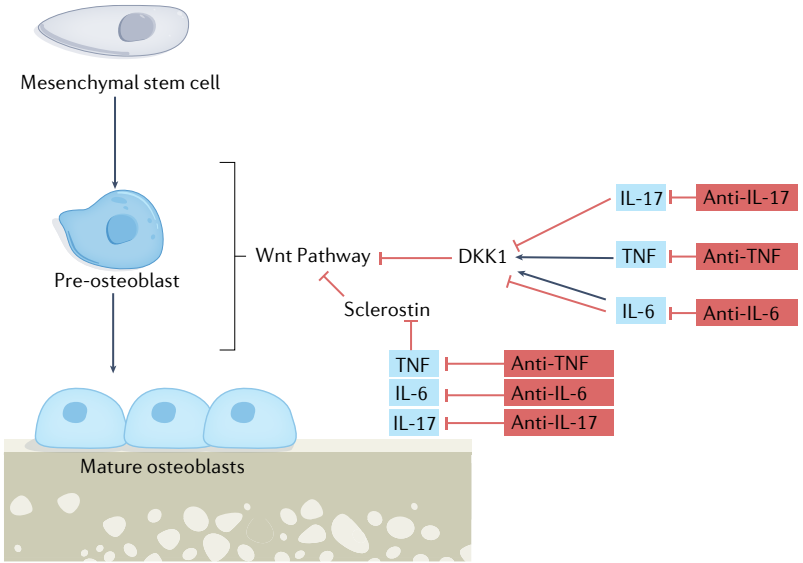

Fig. 2 | **Effects of targeted therapies on inflammatory bone formation.** Mature osteoblasts derive from mesenchymal stem cells and pre-osteoblasts. The Wnt signalling pathway has a crucial role in osteoblast differentiation. Dickkopf 1 (DKK1) and sclerostin block Wnt-dependent osteoblast maturation. TNF directly stimulates DKK1 and thus inhibits bone formation. IL-17 blocks DKK1 and thus enables Wnt-mediated bone formation. The role of IL-6 is controversial as different studies have shown that it can either stimulate or inhibit DKK1. DKK1 also stimulates sclerostin production; thus, TNF, IL-17 and IL-6 might act indirectly on sclerostin as well. Targeted therapies against these cytokines can interfere with the molecular events underlying Wnt-dependent inflammatory osteoblastogenesis and bone formation. Inhibitors of TNF, IL-6 and IL-17 may increase sclerostin levels.

concentrations of CTX and RANKL in SpA[19,53,56,58] (TABLE 1).

In a study comparing the effects of anti-TNF treatment on bone markers in RA and PsA, serum concentrations of DKK1 were lower in patients with PsA than in those with RA[63]. As described above, low DKK1 concentrations have also been found and have been associated with increased syndesmophyte formation in studies of AS, suggesting that similar mechanisms could be involved in the bone formation underlying PsA[48,50]. In a separate study of treatment with etanercept or certolizumab pegol for 1 year in a mixed cohort of patients with RA or AS, anti-TNF therapy favourably influenced bone metabolism in the overall (mixed) cohort as indicated by several biomarkers[19].

Effects of other targeted therapies. Among bDMARDs other than TNF inhibitors, the anti-IL-6 receptor antibody tocilizumab lowered concentrations of CTX[64] and DKK1 (REF.[46]) in RA, whereas it also increased concentrations of biomarkers of bone formation including P1NP, osteoprotegerin, osteocalcin and sclerostin[46,64,65]. The anti-CD20 antibody rituximab (a B cell-depleting agent) decreased CTX concentrations, but increased P1NP concentrations and the osteoprotegerin:RANKL ratio in patients with RA[66,67]. JAK inhibitors decreased concentrations of some biomarkers of bone destruction[68] and increased those of osteocalcin and proteins in the Wnt signalling pathway in RA[26,69] (TABLE 1).

With respect to SpA, the IL-17A inhibitor secukinumab decreased MMP3 levels in peripheral SpA[70], which suggests that this therapy might be involved in the prevention of bone destruction by MMPs in this disease. As mentioned above, treatment with secukinumab also increased the production of DKK1 and sclerostin in patients with PsA[52] (TABLE 1).

Interactions between DKK1 and sclerostin. The relationship between DKK1 and sclerostin has become an interesting issue. The traditional concept was that both DKK1 and sclerostin inhibit Wnt-mediated osteoblast activation[12,34,35]. As discussed above, in general, TNF and IL-6 stimulate production of both DKK1 and sclerostin[12,34,35]. The neutralization of DKK1 also reduced sclerostin production[12,35,42]. These findings indicate that DKK1 and SOST might act in parallel during bone formation. Conversely, a number of studies[19,49,52,61,62] found that inhibition of TNF, IL-6 or IL-17 stimulated sclerostin production in most arthritides. Moreover, DKK1 production was decreased by TNF inhibition[19], but increased by IL-17 inhibition[19,52]. As the inhibition of DKK1 by TNF inhibitors might result in increased Wnt-mediated bone formation, whereas stimulation of DKK1 production by IL-17 blockade might attenuate this process, the different effects of anti-TNF and anti-IL-17 agents on production of DKK1 could, at least in part, explain the disparate effects of these two types of cytokine inhibitors on radiographic progression in SpA.

These observations also suggest that pro-inflammatory cytokines can differentially regulate DKK1 and sclerostin under inflammatory and non-inflammatory conditions. In general, sclerostin levels are lower in patients with SpA than in those with RA despite TNF blockade[19,49]. Moreover, low levels of sclerostin and DKK1 could reflect persistent inflammation and syndesmophyte formation in SpA, including AS[48,49]. Low serum concentrations of sclerostin despite TNF inhibition might explain, at least in part, why anti-TNF treatment is unable to control syndesmophyte formation in SpA[49].

All these data suggest that in addition to interactions of DKK1 and sclerostin with each other and with pro-inflammatory cytokines, other mechanisms might also exist that lead to disparate changes in DKK1 and sclerostin release upon administration of targeted therapies (FIG. 2; TABLE 1).

Prediction of therapeutic response. Some of the biomarkers of bone turnover described above could also be used to predict response to targeted therapies. For example, in RA, low baseline concentration of RANKL and higher osteoprotegerin:RANKL ratio were associated with remission in patients receiving anti-TNF therapy[71]. In SpA, as discussed above, low baseline concentrations of sclerostin predict sustained inflammation in patients treated with bDMARDs[49]. Finally, baseline MMP3 concentrations have been associated with radiographic progression in AS[72].

Targeted therapies and bone loss

Localized bone destruction and formation. The assessment of erosions and localized bone formation is a usual end point in clinical trials of targeted therapies. Therefore, here we only very briefly mention the focal effects of bDMARDs and JAK inhibitors on bone. In general, most

targeted therapies, including bDMARDs and JAK inhibitors, have been shown to attenuate the development of bony erosions and slow down radiographic progression in both RA and peripheral SpA (including PsA)[3,73–75]. Considering the dichotomy of inflammation-dependent versus inflammation-independent, autoantibody-mediated development of erosions[12,29,32] (as discussed above), the former mechanism might account for the efficacy of cytokine inhibitors in delaying radiographic progression[3,12,73], whereas the latter could be involved in the effects of abatacept or rituximab on joint destruction in RA[29,32].

Data on the effects of targeted therapies on bone formation suggest the presence of an optimal bone turnover rate that limits the extent of inhibition of radiographic progression. Early studies have suggested that erosion healing occurs in some patients with RA or SpA treated with targeted therapies[12,76,77]. Erosion repair might occur mostly in non-inflamed joints[76,77] and is observed more frequently in patients with RA treated with anti-TNF therapy than those treated with methotrexate[77]. The lack of repair in most patients treated with anti-TNF agents could suggest that cytokines other than TNF are important in the development of erosions[12]. Indeed, in a 2019 study in RA, tocilizumab monotherapy resulted in better erosion repair than the combination of adalimumab and methotrexate[78].

The molecular mechanisms possibly underlying the inefficacy of bDMARDs in halting new bone formation in AS and PsA are discussed above. In these diseases, localized bone formation occurs in areas of enthesitis[9,13,22,79]. There is abundant production of pro-inflammatory cytokines, including TNF and IL-17, at entheseal sites in SpA[13,79]. These cytokines are important drivers of inflammation-dependent new bone formation[13,22]. The low levels of DKK1 and sclerostin, as well as insufficient blockade of Wnt signalling by DKK1 and sclerostin, are responsible for uncontrolled bone formation and the limited effects of anti-TNF agents on radiographic progression in advanced SpA[9,48,49,80]. By contrast, in the early, inflammatory stages of the disease with no definitive radiographic damage of the sacroiliac joints, new bone formation might be more effectively controlled by bDMARDs including TNF and IL-17 inhibitors[11,13,81–88] or tofacitinib[89] as determined by MRI. The positive effect of bDMARDs on early radiographic progression has been primarily observed in conjunction with improvement of disease activity, suggesting that new bone formation is indeed mostly driven by local inflammation[83,85]. In a study published in 2020, IL-17 blockade with bimekizumab inhibited inflammation-driven osteogenesis[22]. In addition, new bone formation, as determined by MRI, correlated with low concentrations of DKK1 in a study of patients with SpA[80]. Bone formation in patients with AS treated with bDMARDs has been evaluated by use of ^{18}F-fluorodeoxyglucose (FDG) PET–CT (BOX 1). Among patients with AS, 12 weeks of anti-TNF treatment decreased the number of PET-positive lesions as well as FDG uptake in the costovertebral and sacroiliac joints of clinical responders[90]. In a 2020 study, localized bone erosions, focal bone formation and generalized bone loss were evaluated in a spontaneous transgenic animal model of SpA (TgA86). Early anti-TNF treatment significantly reduced the inflammatory phase, as well as bone loss, in these mice. This model may be suitable for preclinical evaluation of compounds being developed for the treatment of SpA[91].

The results described above indicate that whereas radiographic progression in advanced AS is refractory to most targeted therapies, early inflammatory lesions that

Table 1 | **Effects of targeted therapies on biomarkers of bone resorption and formation in RA and SpA**

Biomarker	Effect indicated	Targeted therapy					Refs
		TNF inhibitor	IL-6 inhibitor	IL-17 inhibitor	Anti-CD20 antibody	JAK inhibitor	
Bone resorption							
Osteocalcin	Bone formation	↑ or ↔	↑		↓	↑	26,55–58,64,65,67
P1NP	Bone formation	↑ or ↔	↑	↔	↑		19,46,52,57,59,60,66
CTX	Bone resorption	↓ or ↔	↓	↔			46,52,55–60,64
RANKL	Bone resorption	↓ or ↔					58
Osteoprotegerin	Inhibition of bone resorption	↔	↑				46,58,64
MMP3	Bone resorption	↓	↓	↓			58,65
Osteoprotegerin:RANKL	Bone turnover balance (high ratio favours formation)	↑			↑	↑	19,26,53,67
Osteocalcin:CTX	Bone turnover balance (high ratio favours formation)	↑					19,53
P1NP:CTX	Bone turnover balance (high ratio favours formation)	↑					19,59
Bone formation							
DKK1	Inhibition of bone formation	↓	↓	↑			19,46,52,60,61
Sclerostin	Inhibition of bone formation	↑	↑	↑			19,46,52,62
Wnt signalling proteins	Bone formation					↑	26

↑, increase; ↓, decrease; ↔, no change; CTX, C-terminal telopeptide; DKK1, Dickkopf 1; JAK, Janus kinase; MMP3, matrix metalloproteinase 3; P1NP, type 1 procollagen N-terminal propeptide; RA, rheumatoid arthritis; RANKL, receptor activator nuclear factor-κB ligand; SpA, spondyloarthritis.

would later undergo ossification could be responsive to such treatments[8,9,83,84,90].

In conclusion, although most targeted therapies suppress localized bone loss and the development of erosions, there is still controversy with respect to the effects of these agents on syndesmophyte formation. The nature of the targeted therapy used and the stage of the disease might be important determinants of its effect on localized bone destruction and formation.

Generalized osteoporosis. Early publications in the 2000s reported favourable effects of anti-TNF therapies and other bDMARDs on osteoporosis associated with RA or SpA (reviewed elsewhere[3,91]) (TABLE 2). In most studies, anti-TNF treatment, in contrast to methotrexate monotherapy, slowed or halted generalized bone loss in association with anti-inflammatory and clinical effects in both RA and SpA[3,10,11,92]. The prevention of further loss of bone mineral density (BMD) was also observed in those who did not have a clinical response to bDMARD therapy, as well as those who did, as measured by dual-energy X-ray absorptiometry (DXA)[93]. Very limited data are available on the possible favourable effects of targeted therapies other than TNF inhibitors, such as tocilizumab[46,93], rituximab[94,95], abatacept[94], the IL-1 inhibitor anakinra[94], and the JAK inhibitors tofacitinib[24,26,27] and baricitinib[25,26], on arthritis-related systemic bone loss. In one study, micro-finite element analysis together with peripheral quantitative CT (QCT) (BOX 1) found that bDMARDs were more effective than methotrexate in preserving bone structure and bone strength in PsA[96]. To date, no studies on the effects of IL-17 and IL-23 inhibitors on osteoporosis associated with RMDs have been published[11].

Studies have also evaluated the effects of targeted therapies on bone density as determined by DXA (BOX 1). In one study, RA was associated with decreased hand BMD, which did not change over time with TNF inhibition[63]. In another study evaluating hand BMD by use of digital X-ray radiogrammetry in bDMARD-treated patients with RA or PsA, those with RA had sustained bone loss despite treatment, whereas in those with PsA periarticular BMD was unchanged following anti-TNF therapy[97]. Thus, different processes could be involved in hand bone remodelling in RA versus PsA[98]. Moreover, in the mixed cohort of patients with RA and AS described above[19], TNF inhibition prevented bone loss at the lumbar spine and femoral neck in the overall cohort as assessed by DXA and this favourable effect was accompanied by the attenuation of disease activity and systemic inflammation[19] (TABLE 2).

QCT determines volumetric BMD and is suitable for the assessment of cortical and trabecular bone loss in RMDs[98,99] (BOX 1). QCT has also been used to assess peripheral bone density and the effects of treatment on osteoporosis in some arthritis studies[98–100]. However, to our knowledge, there have been no studies on targeted therapies in relation to RA. Therefore, we performed a QCT study on the aforementioned mixed cohort of patients with RA and AS. Anti-TNF treatment was able to arrest further bone loss in the forearm of these patients, and the effects of treatment on radial QCT were associated with vitamin D3 status, as well as cathepsin K levels at baseline[101].

Very few studies have explored the possible effects of JAK inhibitors on bone (TABLE 2). Theoretically, JAK inhibitors could inhibit bone loss and promote bone formation[24–28]. In our 2021 study in which patients with RA were treated with tofacitinib (either 5 mg or 10 mg twice daily) for 1 year, good clinical efficacy was associated with arrest of further bone loss as determined by DXA and QCT. As mentioned above, treatment with tofacitinib increased concentrations of osteocalcin, osteoprotegerin and vitamin D3, and decreased concentrations of CTX. CRP concentration, 28-joint disease activity score and serum concentration of RANKL correlated inversely with volumetric BMD; moreover, patient age and concentrations of CRP, ACPA and DKK1 influenced the effects of tofacitinib therapy on BMD changes[69]. We have not found any other reports on the effects of JAK inhibitors on bone loss in arthritides.

In conclusion, targeted therapies could dampen the development of secondary osteoporosis in RA and SpA. Most of those available concern TNF inhibitors. More clinical data are needed to determine the effects of JAK inhibitors and bDMARDs other than TNF inhibitors.

Fragility fractures. An increased risk of fragility fractures has been similarly observed in patients with RA, SpA and other inflammatory RMDs[1,4–7]. Therefore, regular vertebral fracture assessment by use of DXA or conventional radiography, as well as calculation of 10-year fragility fractures risk using the FRAX tool, is crucial[4,5]. Every patient with RMD who has osteoporosis and a high risk of fragility fractures should receive calcium and vitamin D supplementation in addition to the necessary anti-osteoporotic pharmacotherapy[4,5]. As well as reaching remission or low disease activity of arthritis, corticosteroid therapy should be used at the lowest effective dose[3–5].

Very few studies have assessed the efficacy of targeted therapies for the prevention of fragility fractures[10]. In a 2020 cohort study that used data from an insurance database, the occurrence of non-vertebral fractures and

Box 1 | **Imaging tools for the clinical assessment of bone**

- Localized bone destruction (erosions): radiography, ultrasonography, MRI
  - Early inflammatory and destructive lesions can be detected only by ultrasonography and MRI
  - Radiography is suitable only to evaluate bony erosions
  - High-resolution peripheral quantitative CT (QCT) can detect localized bone destruction and is highly effective in determining spatial resolution
- Localized bone formation (erosion healing and syndesmophytes): radiography, MRI
  - Radiography can demonstrate advanced bone formation
  - Early bone formation can be visualized with MRI
  - In a research setting, early bone formation can be assessed using ^{18}F-fluorodeoxyglucose (FDG) PET–CT
- Generalized osteoporosis: dual-energy X-ray absorptiometry (DXA), digital X-ray radiogrammetry, ultrasonography, peripheral QCT
  - DXA is used in everyday clinical practice
  - Peripheral QCT is suitable for assessing bone structure and for separately studying trabecular and cortical bone
  - Ultrasonography is an easy-to-use technique that can assess generalized osteoporosis, especially if DXA is not available
  - Digital X-ray radiogrammetry is suitable for assessing generalized osteoporosis in the hands

Table 2 | **Effects of targeted therapies on localized bone loss or bone formation and generalized bone loss**

Disease	Targeted therapy					Refs
	TNF inhibitor	IL-6 inhibitor	IL-17 inhibitor	Anti-CD20 antibody	JAK inhibitor	
Localized bone loss						
RA	Progression ↓, erosion repair ↑ or ↔	Erosion ↓, erosion repair ↑↑		Progression ↓	Progression ↓	12,24,67, 73–75,77,78
SpA	Early SpA: progression ↓ Advanced SpA: ↔		Early SpA: progression ↓		Early SpA: progression ↓	11,22,48,49, 81–85,87–90
Generalized bone loss						
RA	Bone loss ↓	Bone loss ↓		Bone loss ↓	Bone loss ↓	10,19,24,26,27,46, 54,62,66,69,92–94
SpA	Bone loss ↓	Bone loss ↓		Bone loss ↓	Bone loss ↓	11,19,24, 26,27,54,94

↑, increase; ↓, decrease; ↔, no change; JAK, Janus kinase; RA, rheumatoid arthritis; SpA, spondyloarthritis.

vertebral fractures requiring hospitalization was similar among patients with RA treated with TNF inhibitors, tocilizumab or abatacept[102].

Little information is available on JAK inhibitors and risk of fragility fractures. The effects of these drugs on osteoporosis has been a matter of debate. On the basis of preliminary in vitro experiments and animal studies, JAK inhibitors might attenuate bone loss and promote bone formation[24,25,27]; therefore, stopping these medications in patients with RA who have osteoporotic fractures might be unnecessary[27].

With respect to SpA, in a prospective longitudinal study carried out in patients with AS treatment with etanercept increased BMD; however, the number of patients with vertebral fractures and the severity of fractures increased, as did radiographic progression in the spine, underscoring that spondylitis and vertebral fractures are related[103]. A 2019 systematic literature review also confirmed that anti-TNF treatment improved BMD but did not affect rates of vertebral fractures in axSpA[11].

In conclusion, although targeted therapies might slow or halt generalized bone loss, very few studies have included fragility fractures as an end point. More trials using fragility fractures need to be conducted in inflammatory RMDs.

Conclusions

Inflammatory RMDs have been associated with localized and generalized bone loss, as well as with localized new bone formation. Systemic inflammation is an important contributor to inflammatory bone resorption. In RA and peripheral SpA, the RANK–RANKL–osteoprotegerin axis is involved in bone destruction, whereas in SpA the Wnt-β-catenin pathway, DKK1 and sclerostin are important for syndesmophyte formation. Targeted therapies, including various bDMARDs and JAK inhibitors, inhibit osteoclast-mediated bone resorption. These treatments can stabilize bone metabolism, as indicated by their effects on bone resorption and formation. Targeted therapies can also improve BMD and halt radiographic progression in RA and peripheral SpA (including PsA). In axSpA, however, anti-TNF agents might not be able to prevent syndesmophyte formation. In early axSpA, inflammation-driven bone formation may be attenuated by anti-TNF and anti-IL-17 agents. More clinical studies are needed in order to assess the effects of other targeted therapies on localized bone formation. The stage of SpA might also be important in this respect as bDMARDs might be more effective in early axSpA than in established AS. Although multiple studies confirmed the favourable effects of bDMARDs, primarily TNF inhibitors, on secondary osteoporosis associated with inflammatory RMDs, rather few studies have assessed the possible effects of targeted therapies on fragility fractures. The future research agenda should include the determination of the effects of inhibitors of IL-17, IL-23 and JAKs and other targeted therapies on bone, including fragility fractures; the effects of combinations of targeted therapies and antiresorptive and/or osteoanabolic drugs; as well as the effects of targeted therapies on the prevention of syndesmophyte formation in the early stages of SpA and repair of small, early erosions in RA.

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1. Lems, W. F. & Dijkmans, B. A. Should we look for osteoporosis in patients with rheumatoid arthritis? *Ann. Rheum. Dis.* **57**, 325–327 (1998).
2. Walsh, N. C., Crotti, T. N., Goldring, S. R. & Gravallese, E. M. Rheumatic diseases: the effects of inflammation on bone. *Immunol. Rev.* **208**, 228–251 (2005).
3. Szentpetery, A. et al. Effects of targeted therapies on the bone in arthritides. *Autoimmun. Rev.* **16**, 313–320 (2017).
4. Raterman, H. G., Bultink, I. E. & Lems, W. F. Osteoporosis in patients with rheumatoid arthritis: an update in epidemiology, pathogenesis, and fracture prevention. *Expert Opin. Pharmacother.* **21**, 1725–1737 (2020).
5. Raterman, H. G. & Lems, W. F. Pharmacological management of osteoporosis in rheumatoid arthritis patients: a review of the literature and practical guide. *Drugs Aging* **36**, 1061–1072 (2019).
6. Magrey, M. & Khan, M. A. Osteoporosis in ankylosing spondylitis. *Curr. Rheumatol. Rep.* **12**, 332–336 (2010).
7. Petho, Z. et al. Characterization of bone metabolism in Hungarian psoriatic arthritis patients: a case-control study. *BMC Musculoskelet. Disord.* **22**, 70 (2021).
8. Baraliakos, X. & Braun, J. Biologic therapies for spondyloarthritis: what is new? *Curr. Rheumatol. Rep.* **14**, 422–427 (2012).
9. Lories, R. J., de Vlam, K. & Luyten, F. P. Are current available therapies disease-modifying in spondyloarthritis? *Best Pract. Res. Clin. Rheumatol.* **24**, 625–635 (2010).
10. Zerbini, C. A. F. et al. Biologic therapies and bone loss in rheumatoid arthritis. *Osteoporos. Int.* **28**, 429–446 (2017).
11. Ashany, D., Stein, E. M., Goto, R. & Goodman, S. M. The effect of TNF inhibition on bone density and fracture risk and of IL17 inhibition on radiographic progression and bone density in patients with axial spondyloarthritis: a systematic literature review. *Curr. Rheumatol. Rep.* **21**, 20 (2019).
12. Schett, G. & Gravallese, E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nat. Rev. Rheumatol.* **8**, 656–664 (2012).

13. Lories, R. J. & Haroon, N. Bone formation in axial spondyloarthritis. *Best Pract. Res. Clin. Rheumatol.* **28**, 765–777 (2014).
14. Boers, N., Michielsens, C. A. J., van der Heijde, D., den Broeder, A. A. & Welsing, P. M. J. The effect of tumour necrosis factor inhibitors on radiographic progression in axial spondyloarthritis: a systematic literature review. *Rheumatology* **58**, 1907–1922 (2019).
15. Geusens, P. P. et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum.* **54**, 1772–1777 (2006).
16. van den Berg, W. B. & Miossec, P. IL-17 as a future therapeutic target for rheumatoid arthritis. *Nat. Rev. Rheumatol.* **5**, 549–553 (2009).
17. Lam, J. et al. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J. Clin. Invest.* **106**, 1481–1488 (2000).
18. Sennels, H. et al. Circulating levels of osteopontin, osteoprotegerin, total soluble receptor activator of nuclear factor-kappa B ligand, and high-sensitivity C-reactive protein in patients with active rheumatoid arthritis randomized to etanercept alone or in combination with methotrexate. *Scand. J. Rheumatol.* **37**, 241–247 (2008).
19. Gulyas, K. et al. Effects of 1-year anti-TNF-alpha therapies on bone mineral density and bone biomarkers in rheumatoid arthritis and ankylosing spondylitis. *Clin. Rheumatol.* **39**, 167–175 (2020).
20. Yago, T. et al. IL-23 induces human osteoclastogenesis via IL-17 in vitro, and anti-IL-23 antibody attenuates collagen-induced arthritis in rats. *Arthritis Res. Ther.* **9**, R96 (2007).
21. Le Goff, B. et al. Implication of IL-17 in bone loss and structural damage in inflammatory rheumatic diseases. *Mediators Inflamm.* **2019**, 8659302 (2019).
22. Shah, M. et al. Dual neutralisation of IL-17F and IL-17A with bimekizumab blocks inflammation-driven osteogenic differentiation of human periosteal cells. *RMD Open* **6**, e001306 (2020).
23. Axmann, R. et al. Inhibition of interleukin-6 receptor directly blocks osteoclast formation in vitro and in vivo. *Arthritis Rheum.* **60**, 2747–2756 (2009).
24. LaBranche, T. P. et al. JAK inhibition with tofacitinib suppresses arthritic joint structural damage through decreased RANKL production. *Arthritis Rheum.* **64**, 3531–3542 (2012).
25. Murakami, K. et al. A Jak1/2 inhibitor, baricitinib, inhibits osteoclastogenesis by suppressing RANKL expression in osteoblasts in vitro. *PLoS ONE* **12**, e0181126 (2017).
26. Adam, S. et al. JAK inhibition increases bone mass in steady-state conditions and ameliorates pathological bone loss by stimulating osteoblast function. *Sci. Transl Med.* **12**, eaay4447 (2020).
27. Gaber, T. et al. Impact of Janus kinase inhibition with tofacitinib on fundamental processes of bone healing. *Int. J. Mol. Sci.* **21**, 865 (2020).
28. Vidal, B. et al. Effects of tofacitinib in early arthritis-induced bone loss in an adjuvant-induced arthritis rat model. *Rheumatology* **57**, 1461–1471 (2018).
29. Kleyer, A. et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. *Ann. Rheum. Dis.* **73**, 854–860 (2014).
30. Harre, U. et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J. Clin. Invest.* **122**, 1791–1802 (2012).
31. Sun, M. et al. Anticitrullinated protein antibodies facilitate migration of synovial tissue-derived fibroblasts. *Ann. Rheum. Dis.* **78**, 1621–1631 (2019).
32. Kleyer, A. & Schett, G. Arthritis and bone loss: a hen and egg story. *Curr. Opin. Rheumatol.* **26**, 80–84 (2014).
33. Stemmler, F. et al. Biomechanical properties of bone are impaired in patients with ACPA-positive rheumatoid arthritis and associated with the occurrence of fractures. *Ann. Rheum. Dis.* **77**, 973–980 (2018).
34. Schett, G., Zwerina, J. & David, J. P. The role of Wnt proteins in arthritis. *Nat. Clin. Pract. Rheumatol.* **4**, 473–480 (2008).
35. Diarra, D. et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat. Med.* **13**, 156–163 (2007).
36. Chen, X. X. et al. Sclerostin inhibition reverses systemic, periarticular and local bone loss in arthritis. *Ann. Rheum. Dis.* **72**, 1732–1736 (2013).
37. Cosman, F. & Dempster, D. W. Anabolic agents for postmenopausal osteoporosis: how do you choose? *Curr. Osteoporos. Rep.* **19**, 189–205 (2021).
38. Ma, Y. et al. The serum level of Dickkopf-1 in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Int. Immunopharmacol.* **59**, 227–232 (2018).
39. Fassio, A. et al. In psoriatic arthritis Dkk-1 and PTH are lower than in rheumatoid arthritis and healthy controls. *Clin. Rheumatol.* **36**, 2377–2381 (2017).
40. Shi, J., Ying, H., Du, J. & Shen, B. Serum sclerostin levels in patients with ankylosing spondylitis and rheumatoid arthritis: a systematic review and meta-analysis. *Biomed. Res. Int.* **2017**, 9295313 (2017).
41. Appel, H. et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. *Arthritis Rheum.* **60**, 3257–3262 (2009).
42. Heiland, G. R. et al. Neutralisation of Dkk-1 protects from systemic bone loss during inflammation and reduces sclerostin expression. *Ann. Rheum. Dis.* **69**, 2152–2159 (2010).
43. Yeremenko, N. et al. TNF-alpha and IL-6 differentially regulate Dkk-1 in the inflamed arthritic joint. *Arthritis Rheumatol.* **67**, 865 (2015).
44. Fassio, A. et al. Acute effects of glucocorticoid treatment, TNFα or IL-6R blockade on bone turnover markers and Wnt inhibitors in early rheumatoid arthritis: a pilot study. *Calcif. Tissue Int.* **106**, 371–377 (2020).
45. Terpos, E. et al. Early effects of IL-6 receptor inhibition on bone homeostasis: a pilot study in women with rheumatoid arthritis. *Clin. Exp. Rheumatol.* **29**, 921–925 (2011).
46. Briot, K. et al. The effect of tocilizumab on bone mineral density, serum levels of Dickkopf-1 and bone remodeling markers in patients with rheumatoid arthritis. *Joint Bone Spine* **82**, 109–115 (2015).
47. Kwon, S. R. et al. Dickkopf-1 level is lower in patients with ankylosing spondylitis than in healthy people and is not influenced by anti-tumor necrosis factor therapy. *Rheumatol. Int.* **32**, 2523–2527 (2012).
48. Heiland, G. R. et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. *Ann. Rheum. Dis.* **71**, 572–574 (2012).
49. Saad, C. G. et al. Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? *Arthritis Res. Ther.* **14**, R216 (2012).
50. Wu, M. et al. Dickkopf-1 in ankylosing spondylitis: review and meta-analysis. *Clin. Chim. Acta* **481**, 177–183 (2018).
51. Aschermann, S. et al. Presence of HLA-B27 is associated with changes of serum levels of mediators of the Wnt and hedgehog pathway. *Joint Bone Spine* **83**, 43–46 (2016).
52. Fassio, A. et al. Secukinumab produces a quick increase in WNT signalling antagonists in patients with psoriatic arthritis. *Clin. Exp. Rheumatol.* **37**, 133–136 (2019).
53. Barnabe, C. & Hanley, D. A. Effect of tumor necrosis factor alpha inhibition on bone density and turnover markers in patients with rheumatoid arthritis and spondyloarthropathy. *Semin. Arthritis Rheum.* **39**, 116–122 (2009).
54. Szentpétery, Á., Bhattoa, H. P., Antal-Szalmas, P., Szekanecz, Z. & FitzGerald, O. Circulating mediators of bone remodelling in patients with psoriatic and rheumatoid arthritis treated with anti-TNF-alpha therapy. *Arthritis Rheum.* **63** (Suppl. 10), S200 (2011).
55. Vis, M. et al. Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFκB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **65**, 1495–1499 (2006).
56. Visvanathan, S. et al. Effects of infliximab on markers of inflammation and bone turnover and associations with bone mineral density in patients with ankylosing spondylitis. *Ann. Rheum. Dis.* **68**, 175–182 (2009).
57. Vis, M. et al. Early changes in bone metabolism in rheumatoid arthritis patients treated with infliximab. *Arthritis Rheum.* **48**, 2996–2997 (2003).
58. Woo, J. H., Lee, H. J., Sung, I. H. & Kim, T. H. Changes of clinical response and bone biochemical markers in patients with ankylosing spondylitis taking etanercept. *J. Rheumatol.* **34**, 1753–1759 (2007).
59. Chopin, F. et al. Long-term effects of infliximab on bone and cartilage turnover markers in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **67**, 353–357 (2008).
60. Fassio, A. et al. Inhibition of tumor necrosis factor-alpha (TNF-alpha) in patients with early rheumatoid arthritis results in acute changes of bone modulators. *Int. Immunopharmacol.* **67**, 487–489 (2019).
61. Garnero, P., Tabassi, N. C. & Voorzanger-Rousselot, N. Circulating dickkopf-1 and radiological progression in patients with early rheumatoid arthritis treated with etanercept. *J. Rheumatol.* **35**, 2313–2315 (2008).
62. Lim, M. J. et al. Early effects of tumor necrosis factor inhibition on bone homeostasis after soluble tumor necrosis factor receptor use. *Korean J. Intern. Med.* **29**, 807–813 (2014).
63. Szentpetery, A. et al. Periarticular bone gain at proximal interphalangeal joints and changes in bone turnover markers in response to tumor necrosis factor inhibitors in rheumatoid and psoriatic arthritis. *J. Rheumatol.* **40**, 653–662 (2013).
64. Garnero, P., Thompson, E., Woodworth, T. & Smolen, J. S. Rapid and sustained improvement in bone and cartilage turnover markers with the anti-interleukin-6 receptor inhibitor tocilizumab plus methotrexate in rheumatoid arthritis patients with an inadequate response to methotrexate: results from a substudy of the multicenter double-blind, placebo-controlled trial of tocilizumab in inadequate responders to methotrexate alone. *Arthritis Rheum.* **62**, 33–43 (2010).
65. Bay-Jensen, A. C. et al. Effect of tocilizumab combined with methotrexate on circulating biomarkers of synovium, cartilage, and bone in the LITHE study. *Semin. Arthritis Rheum.* **43**, 470–478 (2014).
66. Wheater, G. et al. Changes in bone density and bone turnover in patients with rheumatoid arthritis treated with rituximab, results from an exploratory, prospective study. *PLoS ONE* **13**, e0201527 (2018).
67. Boumans, M. J. et al. Rituximab abrogates joint destruction in rheumatoid arthritis by inhibiting osteoclastogenesis. *Ann. Rheum. Dis.* **71**, 108–113 (2012).
68. Thudium, C. S. et al. The Janus kinase 1/2 inhibitor baricitinib reduces biomarkers of joint destruction in moderate to severe rheumatoid arthritis. *Arthritis Res. Ther.* **22**, 235 (2020).
69. Hamar, A. et al. Effects of one-year tofacitinib therapy on bone metabolism in rheumatoid arthritis. *Osteoporos. Int.* **32**, 1621–1629 (2021).
70. Kaaij, M. H. et al. Anti-IL-17A treatment reduces serum inflammatory, angiogenic and tissue remodeling biomarkers accompanied by less synovial high endothelial venules in peripheral spondyloarthritis. *Sci. Rep.* **10**, 21094 (2020).
71. Gonzalez-Alvaro, I. et al. Baseline serum RANKL levels may serve to predict remission in rheumatoid arthritis patients treated with TNF antagonists. *Ann. Rheum. Dis.* **66**, 1675–1678 (2007).
72. Maksymowych, W. P. et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum.* **56**, 1846–1853 (2007).
73. Schett, G., Coates, L. C., Ash, Z. R., Finzel, S. & Conaghan, P. G. Structural damage in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: traditional views, novel insights gained from TNF blockade, and concepts for the future. *Arthritis Res. Ther.* **13** (Suppl. 1), S4 (2011).
74. Wu, D. et al. Effect of biologics on radiographic progression of peripheral joint in patients with psoriatic arthritis: meta-analysis. *Rheumatology* **59**, 3172–3180 (2020).
75. Emery, P. et al. Baricitinib inhibits structural joint damage progression in patients with rheumatoid arthritis — a comprehensive review. *Arthritis Res. Ther.* **23**, 3 (2021).
76. van der Linden, M. P. et al. Repair of joint erosions in rheumatoid arthritis: prevalence and patient characteristics in a large inception cohort. *Ann. Rheum. Dis.* **69**, 727–729 (2009).
77. Lukas, C., van der Heijde, D., Fatenajad, S. & Landewe, R. Repair of erosions occurs almost exclusively in damaged joints without swelling. *Ann. Rheum. Dis.* **69**, 851–855 (2010).
78. Finzel, S. et al. Comparison of the effects of tocilizumab monotherapy and adalimumab in combination with methotrexate on bone erosion repair in rheumatoid arthritis. *Ann. Rheum. Dis.* **78**, 1186–1191 (2019).

79. Schett, G. et al. Enthesitis: from pathophysiology to treatment. *Nat. Rev. Rheumatol.* **13**, 731–741 (2017).
80. Zhao, Z. et al. Correlation between magnetic resonance imaging (MRI) findings and the new bone formation factor Dkk-1 in patients with spondyloarthritis. *Clin. Rheumatol.* **38**, 465–475 (2019).
81. Braun, J. et al. Magnetic resonance imaging examinations of the spine in patients with ankylosing spondylitis, before and after successful therapy with infliximab: evaluation of a new scoring system. *Arthritis Rheum.* **48**, 1126–1136 (2003).
82. Baraliakos, X. et al. Long-term effects of secukinumab on MRI findings in relation to clinical efficacy in subjects with active ankylosing spondylitis: an observational study. *Ann. Rheum. Dis.* **75**, 408–412 (2016).
83. Dougados, M. et al. Evaluation of the change in structural radiographic sacroiliac joint damage after 2 years of etanercept therapy (EMBARK trial) in comparison to a contemporary control cohort (DESIR cohort) in recent onset axial spondyloarthritis. *Ann. Rheum. Dis.* **77**, 221–227 (2018).
84. Maksymowych, W. P. et al. Modification of structural lesions on MRI of the sacroiliac joints by etanercept in the EMBARK trial: a 12-week randomised placebo-controlled trial in patients with non-radiographic axial spondyloarthritis. *Ann. Rheum. Dis.* **77**, 78–84 (2018).
85. Molnar, C. et al. TNF blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss Clinical Quality Management cohort. *Ann. Rheum. Dis.* **77**, 63–69 (2018).
86. van der Heijde, D. et al. Limited radiographic progression and sustained reductions in MRI inflammation in patients with axial spondyloarthritis: 4-year imaging outcomes from the RAPID-axSpA phase III randomised trial. *Ann. Rheum. Dis.* **77**, 699–705 (2018).
87. Braun, J. et al. Spinal radiographic progression over 2 years in ankylosing spondylitis patients treated with secukinumab: a historical cohort comparison. *Arthritis Res. Ther.* **21**, 142 (2019).
88. Deodhar, A. et al. Efficacy and safety of ixekizumab in the treatment of radiographic axial spondyloarthritis: sixteen-week results from a phase III randomized, double-blind, placebo-controlled trial in patients with prior inadequate response to or intolerance of tumor necrosis factor inhibitors. *Arthritis Rheumatol.* **71**, 599–611 (2019).
89. Maksymowych, W. P. et al. Tofacitinib is associated with attainment of the minimally important reduction in axial magnetic resonance imaging inflammation in ankylosing spondylitis patients. *Rheumatology* **57**, 1390–1399 (2018).
90. Bruijnen, S. T. G. et al. Bone formation in ankylosing spondylitis during anti-tumour necrosis factor therapy imaged by 18F-fluoride positron emission tomography. *Rheumatology* **57**, 631–638 (2018).
91. Christodoulou-Vafeiadou, E. et al. Ectopic bone formation and systemic bone loss in a transmembrane TNF-driven model of human spondyloarthritis. *Arthritis Res. Ther.* **22**, 232 (2020).
92. Sambrook, P. Tumour necrosis factor blockade and the risk of osteoporosis: back to the future. *Arthritis Res. Ther.* **9**, 107 (2007).
93. Marotte, H. et al. A 1-year case-control study in patients with rheumatoid arthritis indicates prevention of loss of bone mineral density in both responders and nonresponders to infliximab. *Arthritis Res. Ther.* **9**, R61 (2007).
94. Confavreux, C. B. & Chapurlat, R. D. Systemic bone effects of biologic therapies in rheumatoid arthritis and ankylosing spondylitis. *Osteoporos. Int.* **22**, 1023–1036 (2011).
95. Hein, G. et al. Influence of rituximab on markers of bone remodeling in patients with rheumatoid arthritis: a prospective open-label pilot study. *Rheumatol. Int.* **31**, 269–272 (2011).
96. Simon, D. et al. Effect of disease-modifying anti-rheumatic drugs on bone structure and strength in psoriatic arthritis patients. *Arthritis Res. Ther.* **21**, 162 (2019).
97. Szentpetery, A. et al. Striking difference of periarticular bone density change in early psoriatic arthritis and rheumatoid arthritis following anti-rheumatic treatment as measured by digital X-ray radiogrammetry. *Rheumatology* **55**, 891–896 (2016).
98. Juhasz, B. et al. Comparison of peripheral quantitative computed tomography forearm bone density versus DXA in rheumatoid arthritis patients and controls. *Osteoporos. Int.* **28**, 1271–1277 (2017).
99. Neumann, A. et al. Cortical bone loss is an early feature of nonradiographic axial spondyloarthritis. *Arthritis Res. Ther.* **20**, 202 (2018).
100. Yue, J. et al. Repair of bone erosion in rheumatoid arthritis by denosumab: a high-resolution peripheral quantitative computed tomography study. *Arthritis Care Res.* **69**, 1156–1163 (2017).
101. Juhász, B. et al. Peripheral quantitative computed tomography in the assessment of bone mineral density in anti-TNF-treated rheumatoid arthritis and ankylosing spondylitis patients. *BMC Musculoskelet. Disord.* **22**, 817 (2021).
102. Shin, A. et al. Comparative risk of osteoporotic fracture among patients with rheumatoid arthritis receiving TNF inhibitors versus other biologics: a cohort study. *Osteoporos. Int.* **31**, 2131–2139 (2020).
103. van der Weijden, M. A. et al. Etanercept increases bone mineral density in ankylosing spondylitis, but does not prevent vertebral fractures: results of a prospective observational cohort study. *J. Rheumatol.* **43**, 758–764 (2016).

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Synovial inflammation in osteoarthritis progression

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Abstract | Osteoarthritis (OA) is a progressive degenerative disease resulting in joint deterioration. Synovial inflammation is present in the OA joint and has been associated with radiographic and pain progression. Several OA risk factors, including ageing, obesity, trauma and mechanical loading, play a role in OA pathogenesis, likely by modifying synovial biology. In addition, other factors, such as mitochondrial dysfunction, damage-associated molecular patterns, cytokines, metabolites and crystals in the synovium, activate synovial cells and mediate synovial inflammation. An understanding of the activated pathways that are involved in OA-related synovial inflammation could form the basis for the stratification of patients and the development of novel therapeutics. This Review focuses on the biology of the OA synovium, how the cells residing in or recruited to the synovium interact with each other, how they become activated, how they contribute to OA progression and their interplay with other joint structures.

Osteoarthritis (OA) is the most common form of arthritis, affecting more than 500 million people worldwide (~7% of the global population), with particularly high prevalence in those of advanced age (>65 years of age)[1]. Epidemiological studies report an increasing incidence of OA in individuals <65 years of age owing to rising obesity, an increasing number of post-traumatic OA (PTOA) cases and diagnosis at an earlier stage[2]. OA is a complex disease characterized by pathological changes across all the joint tissues, including cartilage, subchondral bone, ligaments, menisci, the joint capsule and the synovial membrane[3]. The widely accepted hypothesis of OA pathogenesis implicates an initial injury, frequently biomechanical, of any of these structures, which results in the release of mediators that lead to activation of different inflammatory pathways that damage cartilage. However, increasing evidence indicates that low-grade synovial inflammation (synovitis) contributes to radiographic and pain progression in OA.

Baseline synovitis detected by magnetic resonance imaging (MRI) or ultrasonography is associated with radiographic progression of OA, as defined by worsening of Kallgren and Lawrence (KL) grade or narrowing of joint space[4–11]. Synovitis progression is also associated with more cartilage damage[12]. Radiographic progression and development of erosions in hand OA[13–16] and accelerated knee osteoarthritis (AKOA; defined as a transition from no radiographic knee OA to advanced stage disease within 4 years)[17,18] are also associated with synovitis. More than 2 years before onset, patients with AKOA present with more pain, synovitis-effusion of larger volumes and signal alterations in the infrapatellar fat pad (IFP) compared with patients who develop typical knee OA[17,18]. MRI and ultrasonography have also been used to evaluate associations between synovitis and pain[5,19–27], finding that synovitis contributes to pain in OA. Of note, a study found that synovitis partially mediates the association between cartilage damage loss and worsening pain: each 0.1-mm loss of cartilage over 24 months translated to an increase in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale score of 0.32 (95% CI 0.21–0.44)[28].

However, the results of preclinical studies in animal models and of clinical trials have been contradictory[29]. Although blocking pro-inflammatory mediators secreted by the synovium and cartilage (including IL-6 and IL-1RA) has an analgesic effect and decreases structural progression in several preclinical models of OA[30–33], not all of these studies confirmed a protective role of cytokine blocking in animal models[34]. In addition, in randomized controlled clinical trials in patients with painful erosive hand OA, whose erosive phenotype was associated with the presence of synovitis[13–16], inhibition of the inflammatory mediators IL-1β, IL-6 and tumour necrosis factor (TNF) did not improve pain, synovitis or OA progression, as assessed by MRI or ultrasonography[35–39]. Finally, individuals ($n = 18$) with knee OA without inflammation (by ultrasonography) experienced a more prolonged benefit from intra-articular corticosteroid treatment than individuals with ultrasonography-identified inflammation ($n = 16$)[40].

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Key points

- Imaging studies suggest that synovial inflammation may be present in both early osteoarthritis (OA) and advanced-stage OA and is involved in the development and progression of OA.
- Synovial cells coordinate the production of molecules that initiate and maintain synovial inflammation and contribute to cartilage damage during OA progression.
- Diverse stimuli, including bioactive lipids, prostaglandins, tricarboxylic acid cycle intermediates, cytokines and damage-associated molecular patterns, as well as clinical factors such as obesity, ageing, trauma and excessive mechanical loading, regulate the production of pro-inflammatory and anti-inflammatory mediators by synovial cells.
- There is a need for functional imaging and cellular and molecular studies, together with a more robust histological interpretation at different stages of OA, to better stratify patients with OA and understand the role of synovitis in OA onset and progression.

Taken together, these data raise the question as to whether synovial inflammation is involved in OA pathogenesis, progression or associated joint pain.

In this Review, we describe the current knowledge of synovitis in OA joints, and discuss the pathology (FIG. 1), risk factors (FIG. 2) and cell types associated with synovial inflammation in OA. We focus on the mediators of synovial inflammation (FIG. 3), the crosstalk between synovial cells (FIG. 4) and their clinical relevance (TABLE 1, TABLE 2).

Synovitis in OA

Synovitis scores based on macroscopic features in the OA joint typically assess the presence and abundance of vascularity, villi, fibrin deposits and hyperplasia assessed by visualization of the synovium during arthroscopy[41,42], although other scores use features such as hypertrophy, vascularity and global synovitis[43]. Synovitis scores based on microscopic histological features have also been developed, such as the histological score developed by Krenn, which includes assessment of synovial hyperplasia, stromal cell activation and inflammatory infiltrate extent; this score was able to discriminate between degenerative and inflammatory diseases[44–47]. Other synovial OA scores are being developed that include characteristic features of the OA synovium and are based on the predominance of each feature; synovial changes in OA have thus been classified as hyperplastic (villous hyperplasia), fibrotic (capsular fibrosis), detritus-rich (fibrinous exudate and cartilage and bone debris) and inflammatory (diffuse inflammation and aggregates of lymphoplasmacellular infiltrates), despite all of these features usually coexisting[48]. If present, synovial inflammation (synovitis) is characterized by proliferation of fibroblast-like synoviocytes (FLS) and macrophage recruitment, resulting in hyperplasia of the synovial lining. The synovial sub-lining can also be enriched in macrophages, T cells and, to a lesser extent, mast cells, B cells, plasma cells and endothelial cells (as components of blood vessels)[49].

The two main imaging techniques that are used for synovium assessment, MRI (including contrast-enhanced MRI (CE-MRI) and conventional MRI) and ultrasonography, show good correlation with macroscopic and microscopic histological features of inflammation (BOX 1). Imaging studies revealed that synovitis in OA has a patchy distribution in different anatomical sites of the synovium, including in suprapatellar, infrapatellar, lateral and medial parapatellar and subpopliteal locations, as well as adjacent to posterior cruciate ligaments, and the extent of synovitis can also be different across these different locations[50]. This distribution may be clinically relevant, as different locations and scores of synovial inflammation determined by CE-MRI correlate differently with pain and radiographic OA severity[51]. Synovitis can be present at any disease stage[49], and a study reports a correlation between the patterns of patient-reported knee pain and the location of synovitis; specifically, suprapatellar pain was highly associated with suprapatellar synovitis on MRI[52]. Joint effusions and synovitis may be detected by MRI in subjects with OA joint pain and normal or very minimal damage by joint radiography, indicating that synovitis is not restricted to late stages of disease[11]. A post-mortem study reported a prevalence of synovitis of 11% in patients with no OA history or pain, compared with a prevalence of 67% in synovium from end-stage OA joint replacement surgeries[53]. Interestingly, inflammatory infiltrates coexist with fibrotic changes and angiogenesis in OA, which can be more prevalent in the late stages than in the early stages of the disease[48,54] (FIG. 1).

Cell types in the OA synovium

The inflammatory cell subsets that exist in synovial tissues have been identified by flow cytometry, single-cell transcriptomics and mass cytometry. Evaluation of the synovium from patients with OA undergoing knee replacement showed highly heterogeneous cell populations. Whereas all synovial fibroblasts expressed IL-6, a cytokine independently associated with OA pain and radiographic progression[55,56], $CD34^{+}CD90^{+}$ fibroblasts located in the synovial sub-lining express substantially more IL-6 than $CD34^{-}CD90^{-}$ fibroblasts in the synovial lining[57]. In addition, study participants categorized into clusters based on a high mesenchymal cell content or IL-6 release in the synovial inflammatory response had a history of prior joint surgery[57]. Single-cell RNA sequencing (scRNA-seq) detected 12 different expression profiles in cells of the synovium, including (from most to least abundant) synovial sub-intimal fibroblasts, synovial intimal fibroblasts, *HLA-DRA*$^{+}$ cells (immune regulatory macrophages and inflammatory macrophages, dendritic cells, activated pro-inflammatory *HLA-DRA*$^{+}$ fibroblasts and B cell clusters), smooth muscle cells, endothelial cells, T cells, mast cells and proliferating immune cells[58]. In addition, OA synovial samples contain more $NUPR1^{+}$ monocytes than in leukocyte-rich rheumatoid arthritis (RA) synovial samples ($P < 0.01$), which contain a greater abundance of $IL\text{-}1\beta^{+}$ ($P < 0.001$) and IFN-activated monocytes ($P < 0.01$) than OA synovium. $NUPR1^{+}$ monocytes express high levels of tissue remodelling factors, such as the receptor tyrosine kinase MERTK and the osteoclast progenitor markers osteoactivin and cathepsin K[59]. Together, these studies indicate a considerable heterogeneity in cell subtypes and interaction networks in the OA synovium, which requires further characterization and understanding.

Synovial macrophages in OA

Macrophages are the most abundant immune cells in the synovium, comprising 12–40% of synovial immune cells, depending on the surface markers employed[58,60,61], and they orchestrate the inflammatory and resolution phases after tissue injury[62]. Macrophages are also the main leukocyte population in synovial fluid in human OA knee joints (median = 36.5% of leukocytes), followed by T cells (25%)[63]. In particular, the $CD14^+CD16^+$ macrophage subset (35% of the total macrophage population in synovial fluid) expresses the mature macrophage marker 25F9 (17.3% of the $CD14^+CD16^+$ macrophages), indicating activation[63]. Interestingly, linear modelling (adjusted for sex, BMI and age) showed that the ratio of $CD14^+$ macrophages to total macrophages is a predictor of Knee Injury and Osteoarthritis Outcome Score (KOOS) and WOMAC score, regardless of CD16 expression by this subset of macrophages[63] (TABLE 1). In OA synovia, scRNA-seq of 10,640 synovial cells from 3 patients revealed that ~12.8 % of these cells were $HLA\text{-}DRA^+$, and this subset includes immunoregulatory and pro-inflammatory macrophages, dendritic cells, pro-inflammatory fibroblasts and B cells[58]. The quantity of activated macrophages in OA knee joints detected by single-photon emission computed tomography (SPECT)–CT with the folate receptor-targeting imaging agent ^{99m}Tc-EC20 (etarfolatide) correlated with radiographic OA severity and symptoms, including pain and stiffness (self-reported on a scale from 0 to 3)[64].

Consequently, disruption of pro-inflammatory macrophage infiltration into the synovium has been proposed as a potential therapeutic approach. In a mouse model of OA, inhibition of CC-chemokine receptor type 2 (CCR2; the receptor for the monocyte chemoattractant CCL2) impedes blood monocyte recruitment to injured joints and decreases synovitis and cartilage destruction[65]. In another study, depletion of synovial macrophages by intra-articular injection of anti-CD14-conjugated magnetic beads or clodronate-loaded liposomes decreased production of IL-1β, TNF and matrix metalloproteinases (MMPs) by synovial fibroblasts and reduced cartilage damage and osteophyte formation[66,67]. By contrast, depletion of joint macrophages in *Csf1r*–GFP^+ macrophage FAS-induced apoptosis transgenic mice resulted in increased synovitis but did not inhibit the development of OA, owing partly to increased infiltration of neutrophils (>eightfold) and $CD3^+$ T cells (>fivefold) into the synovium of injured joints, which cause additional damage[68]. These results suggest that a better understanding of macrophage subsets and their role in both healthy and injured or inflamed joints is needed. Identification of pathological macrophage subsets might provide a good opportunity to curtail synovitis and tissue damage.

The pro-inflammatory or anti-inflammatory capacity of macrophages is defined based on their effector function, transcription and metabolic programme, and surface marker expression (reviewed elsewhere[69,70]). Considerable effort has been focused on advancing the identification of macrophage subsets in healthy and inflamed joints and understanding how these populations are associated with clinical outcome. In particular, a study in 184 patients with radiographic knee OA from two different cohorts found that the concentration of the macrophage markers CD14 and CD163 in synovial fluid and blood are associated with OA phenotypic outcomes[71]. Levels of both macrophage markers in the synovial fluid were significantly associated with activated macrophages in the joint detected by ^{99m}Tc-EC20 SPECT–CT, mainly in the capsule ($P = 0.002$ and $P = 0.005$, respectively) and the synovium ($P = 0.0005$ and $P = 0.002$, respectively), of patients with knee OA[71]. Interestingly, CD14 and CD163 levels in the synovial fluid were associated with osteophyte severity,

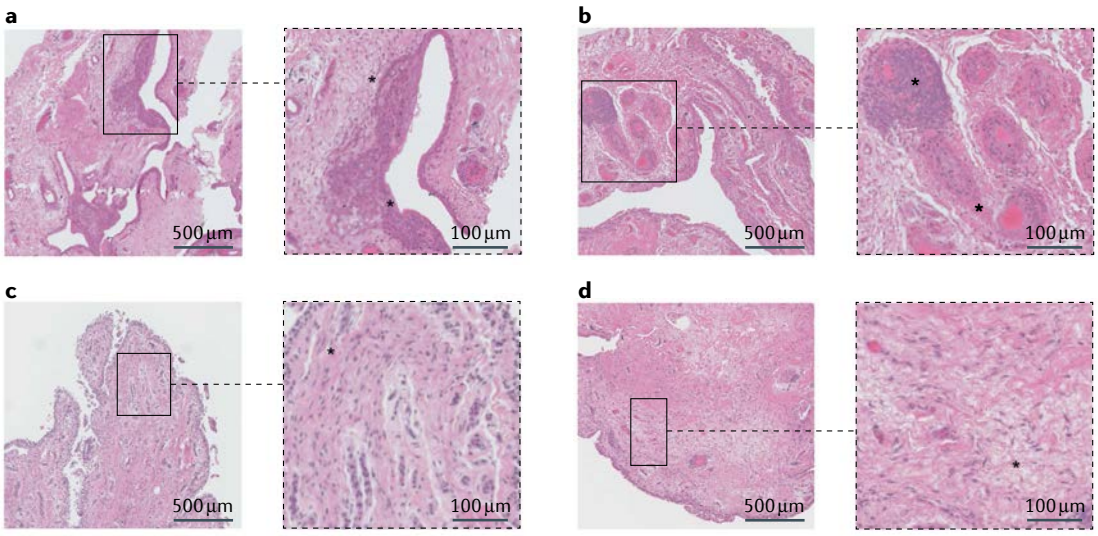

Fig. 1 | **Synovial inflammation and fibrosis in osteoarthritis.** Haematoxylin and eosin staining of synovial tissue from patients who underwent total knee replacement. **a**,**b** | Features of an inflammatory phenotype are highlighted in the magnified insets, including hyperplasia of the synovial lining (asterisk in part **a**), and cellular infiltrates and vascularization in the sublining layer (asterisk in part **b**). **c**,**d** | Features of a fibrotic phenotype are highlighted in the magnified insets, including fibrosis in the sublining layer (asterisks in parts **c** and **d**).

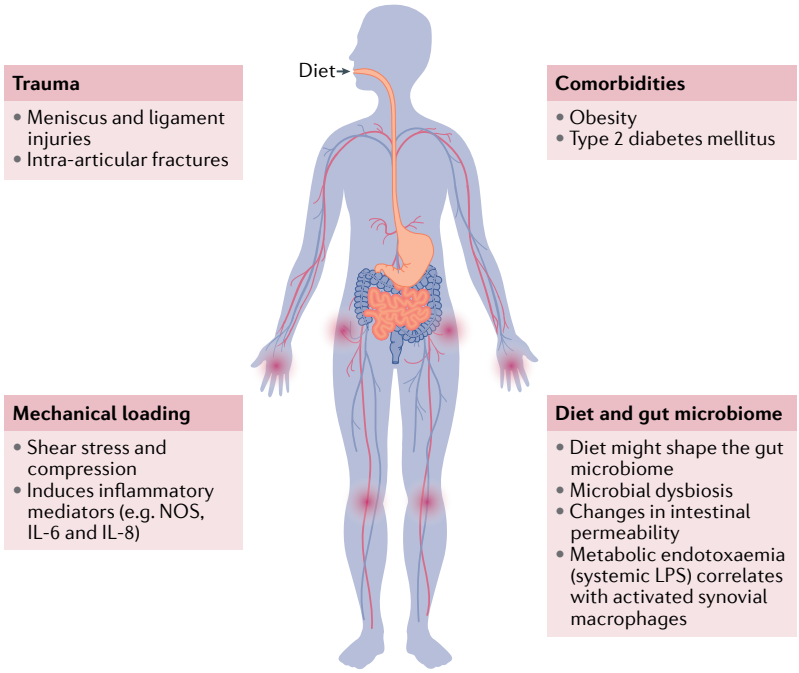

Fig. 2 | **Osteoarthritis risk factors and synovitis.** Among the risk factors associated with osteoarthritis (OA) development and progression, trauma, mechanical loading, comorbidities and diet–microbiome interactions are also related to synovitis. Injury to the meniscus or ligaments and intra-articular fractures lead to the development of synovitis. Aberrant and excessive loading is a known risk factor for developing OA, and subsequent shear stress and compression induce production of inflammatory mediators such as nitric oxide synthase (NOS), IL-6 and IL-8, which contribute to OA pathogenesis. Synovitis has also been related to obesity and type 2 diabetes mellitus. Dietary habit, which has been demonstrated to increase pain prevalence in patients with OA, is one of the factors that influence the composition of the gut microbiome. Microbial dysbiosis (that is, alteration in gut microbiome composition) favours inflammation and metabolic syndrome, as well as changes in intestinal permeability and metabolic endotoxaemia, which correlates with recruitment of activated pro-inflammatory synovial macrophages. LPS, lipopolysaccharide.

whereas synovial fluid CD14 and serum CD163 levels were associated with severity of joint space narrowing. The severity of self-reported knee joint symptoms was associated with CD14 levels in both synovial fluid ($\beta = 0.773$, $P = 0.003$) and serum ($\beta = 0.641$, $P = 0.031$)[71]. The ratio of $CD11c^+$ to $CD206^+$ macrophages or $CD86^+$ to $CD163^+$ macrophages in synovial fluid was associated with KL grading and severity of knee OA in patients[72]. Pro-inflammatory macrophages in OA synovium show upregulated production of matrix metalloproteinases (including MMP1, MMP3, MMP13 and MMP9), aggrecanases (including ADAMTS4 and ADAMTS5) and cyclooxygenase 2, leading to articular degeneration[73]. In addition, secretion of the pro-inflammatory cytokines IL-1β, IL-6, and TNF and oncostatin M stimulates destructive processes in chondrocytes and mesenchymal cells, including downregulating synthesis of type II collagen (an indispensable component of healthy articular cartilage) and aggrecan, limiting chondrogenesis[74]. Interestingly, $CD68^+$ macrophages also contribute to loss of articular type II collagen by engulfing and presenting collagen fragments to $CD4^+$ T cells[75].

Anti-inflammatory macrophages also express the mannose receptors MRC1 and MRC2, which bind to collagen and promote its internalization and lysosomal degradation. The resulting improvement in collagen turnover restores ECM homeostasis in the joint and ameliorates cartilage destruction[76]. Importantly, type II collagen helps to maintain expression of anti-inflammatory genes in macrophages as well as pro-chondrogenic cytokines[77].

Macrophage phenotyping studies have also identified subsets of alternatively activated macrophages (that is, macrophages that are enriched in neither pro-inflammatory nor anti-inflammatory markers), which are more likely to be involved in healing inflammation[78]. Indeed, OA synovial macrophages do not perfectly align with surface marker expression profiles corresponding to classical pro-inflammatory or anti-inflammatory phenotypes, but have been classified as a population that resembles macrophages in RA based on their expression of proliferation genes, and another population is characterized by expression of cartilage-remodelling genes[61]. A scRNA-seq study detected heterogeneous macrophage cell types in the OA synovium, including immunoregulatory (expressing *SEPP1*, *FOLR2*, *STAB1*, *TXNIP* and *CD169*) and pro-inflammatory (expressing *CCL3, CCL4, IL1B* and *TNF*) macrophage subsets[58]. Interestingly, this immunoregulatory population, which does not align with typical pro-inflammatory or anti-inflammatory phenotypes, exhibits a gene expression profile suggestive of enhanced phagocytic activity and immunosuppressive activity.

These results suggest that shifting macrophages towards phenotypes that might contribute to restoration of the damaged articular cartilage could represent a potential treatment for OA. Several therapeutic interventions have the ability to modify macrophage phenotypes in OA synovium. For example, glucocorticoids increase the proportion of $CD163^+FR\beta^+$ synovial macrophages, and slightly reduce the proportion of $CD68^+$ macrophages in the synovial lining, in patients with OA, resulting in decreased osteophyte formation[79,80]. Similarly, a cell-mediated gene therapy that is in phase II trials in patients with OA and allows localized delivery of transforming growth factor β1 (TGFβ1) improved the International Knee Documentation Committee, WOMAC and pain (evaluated on a visual analogue scale from 0 to 10) scores, elevated anti-inflammatory markers in the joints and potentiated IL-10 production[81,82]. In addition, functional imaging techniques (besides 99mTc-EC20 SPECT–CT) that allow the identification of macrophage subsets will help in stratifying patients with OA. Novel probes that target other macrophage markers, including CD206 (REF.[83]), formyl peptide receptor 1 (REF.[84]) or somatostatin subtype receptor 2 (REF.[85]), will improve understanding of macrophage phenotypes in OA.

Synovial FLS in OA

FLS are specialized mesenchymal cells that lubricate the cartilage by producing synovial fluid rich in lubricin and hyaluronic acid (also known as hyaluronan). The concentration of lubricin and hyaluronic acid is decreased in OA synovial fluid, partly owing to changes in synovial membrane permeability, but it is also associated with a change in hyaluronic acid size[86]. Synovial fluid viscosity is decreased in OA and may be related to joint pain, as viscosupplementation therapy with intra-articular

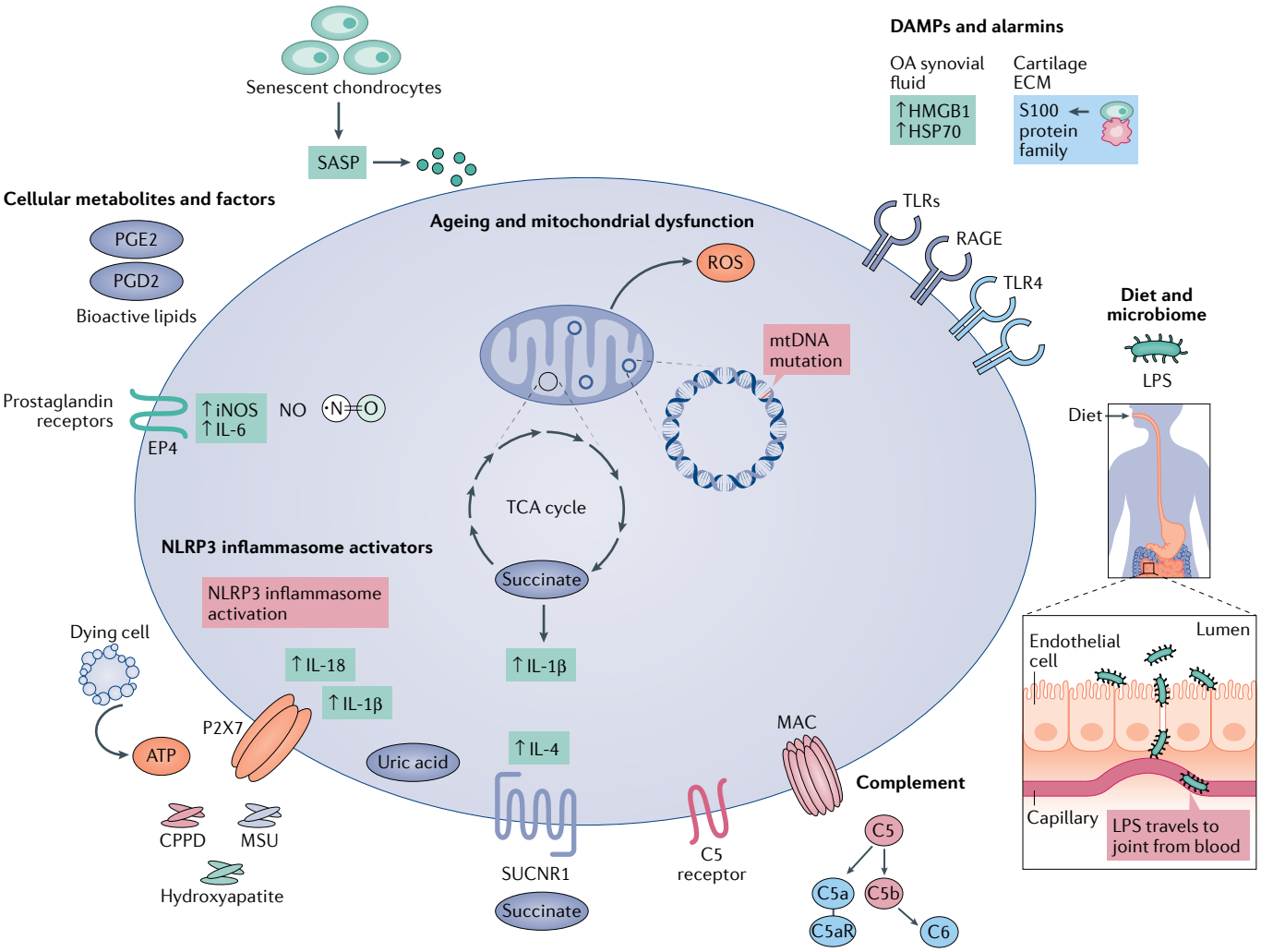

Fig. 3 | **Molecular mediators that contribute to synovial inflammation in osteoarthritis.** Ageing and mitochondrial damage increase reactive oxygen species (ROS) production and mitochondrial DNA mutations and can prolong the production of pro-inflammatory cytokines such as IL-1β and IL-6. Senescent cells are associated with age-related pathological conditions such as osteoarthritis (OA), and several senescence-associated secretory phenotype (SASP) factors are inflammatory mediators. Cellular metabolites, such as nitric oxide (NO), succinate and prostaglandins, as well as other bioactive lipids, contribute to inflammation and cartilage damage. NO levels are elevated in chondrocytes and pro-inflammatory macrophages in patients with OA. Succinate accumulates in inflammatory macrophages and supports their pro-inflammatory phenotype. The succinate receptor SUCNR1 is activated by soluble succinate and boosts IL-4 production. Prostaglandin E2 (PGE2) is considered the major contributor to inflammatory pain in the joint and signals through receptors such as EP4, thereby enhancing production of the pro-inflammatory factors NO (by increasing expression of inducible nitric oxide synthase (iNOS)) and IL-6, which also contributes to synovitis and increases hyperalgesia. PGD2 is also enriched in synovial fluid from patients with OA. Damage-associated molecular patterns (DAMPs) and alarmins, in the context of mechanical stress, interact with Toll-like receptors (TLRs), receptor for advanced glycation end products (RAGE) and other pattern recognition receptors to initiate and propagate inflammation. DAMPs such as high mobility group protein B1 (HMGB1) and heat shock proteins (HSPs) are abundant in OA synovial fluid. S100 family proteins are also upregulated in inflamed synovial tissue. Ectopic deposition of hydroxyapatite crystals, calcium pyrophosphate dihydrate (CPPD) microcrystals and monosodium urate (MSU) crystals, which may signal through P2X7 (depicted) or CD11b, CD16 and CD14 (not shown), as well as ATP released from dying cells, are detected by macrophages and trigger NLRP3 inflammasome activation and IL-1β and IL-18 production. MSU crystals correlate with levels of IL-1β and IL-18 in synovial fluid. Complement factors are highly expressed in OA and play a role in OA pathogenesis. Diet is one of the determining factors of microbiome composition. An altered gut microbial composition is associated with increased intestinal permeability and metabolic endotoxaemia (systemic lipopolysaccharide (LPS)), which is associated with recruitment of pro-inflammatory macrophages in the synovium.

hyaluronic acid decreases pain in patients with OA[87]. The transformation of healthy FLS into activated and pathological cells has been extensively studied in RA and less so in OA. Among the many factors that activate FLS in OA, follistatin-like protein 1 (FSTL1) is overexpressed in the OA synovium, and the levels of this protein correlate with OA severity (assessed by KL and WOMAC scores)[88]. Activated OA FLS secrete pro-inflammatory cytokines, chemokines and proteolytic enzymes (MMPs and aggrecanases), thereby contributing to the propagation of inflammation and destruction of the cartilage matrix[89].

The different FLS phenotypes and their roles in OA pathogenesis have been described in studies in the past few years. A study focusing on RA FLS described

different functional associated phenotypes in FLS isolated from fresh synovial tissue from patients with OA compared with patients with RA who underwent joint replacement[90] (TABLE 1). In a 2021 study, the transcriptomic profiles of synovia and FLS isolated from patients with OA were distinct between patients with early or end-stage OA as well as between patient-reported pain zones and pain-free zones[52]. The transcriptome of synovium from pain zones in patients with early OA was characterized by upregulated expression of pro-fibrotic and pro-inflammatory genes, whereas the transcriptome of both early and end-stage OA showed upregulation of several nociceptive signalling pathways and neuronal growth genes[52]. Interestingly, scRNA-seq analysis of synovial explant FLS revealed that the gene expression profile of an FLS cluster representing the end-stage OA pain zone was associated with eicosanoid signalling, and the most active functions in these cells were "migration of cells" and "cell viability"[52]. Eicosanoid signalling was also associated with a FLS cluster related to early OA pain zone. The end-stage OA FLS had a transcription profile similar to the leukocyte-rich RA FLS described in a previous study[59], whereas the early OA FLS resemble the FLS found to be more predominant in OA in this previous study[59].

Other synovial cells in OA

Neutrophils are another innate immune cell type that is found in the OA knee joint and are highly abundant in synovial fluid compared with synovial tissue[91], although the reason for this distribution is still unknown. The secretion of the key proteolytic enzymes elastase and neutrophil gelatinase-associated lipocalin by activated neutrophils correlates with cartilage damage and radiographic progression[91,92]. Mast cells are also present in the synovium and are associated with inflammation and cartilage destruction in OA[93]. Synovial fluid from individuals with OA is enriched in tryptase (2–25 ng/ml), a mast

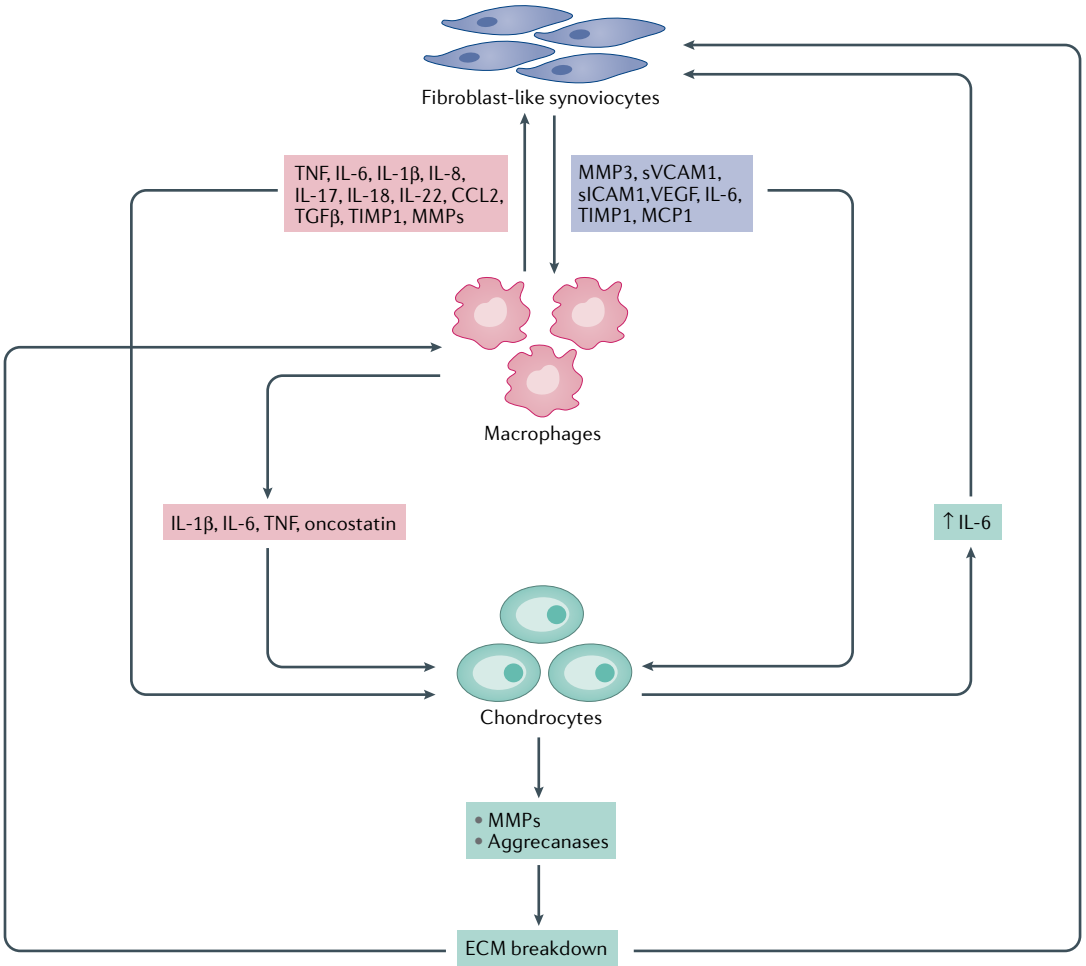

Fig. 4 | **Cellular crosstalk in synovitis and OA progression.** Activated fibroblast-like synoviocytes (FLS) in the osteoarthritis (OA) synovium secrete, among other factors, cytokines, growth factors, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), which contribute to macrophage activation and stimulate catabolic pathways in chondrocytes. Similarly, activated macrophages secrete pro-inflammatory mediators that stimulate FLS and chondrocytes, promoting the degradation of extracellular matrix (ECM) components. ECM degradation products further activate both FLS and macrophages, resulting in a repeating cycle of inflammation and cartilagedegradation. CCL2, CC-chemokine ligand 2; MCP1, monocyte chemoattractant protein 1; sICAM1, soluble intercellular adhesion molecule 1; sVCAM1, soluble vascular cell adhesion molecule 1; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Table 1 | **Clinical relevance of cell types associated with synovitis in OA**

Cell type	Markers	Clinical relevance	Refs
Macrophages	Folate receptor detected by ^{99m}Tc-EC20 SPECT–CT	The quantity of activated macrophages correlated with radiographic OA severity and pain and stiffness	71
	$CD14^+CD16^+$ macrophages in synovial fluid express mature macrophage marker 25F9 (indicating activation)	$CD14^+$ macrophages/total macrophages ratio in synovial fluid is a predictor of KOOS and WOMAC scores, regardless of CD16 expression	63
	CD14 and CD163 in synovial fluid significantly associated with activated macrophages (detected by ^{99m}Tc-EC20 SPECT–CT), in the capsule ($P=0.002$ and $P=0.005$, respectively) and in the synovium ($P=0.0005$ and $P=0.002$, respectively)	CD14 and CD163 presence in the synovial fluid is associated with osteophyte severity Synovial fluid CD14 and serum CD163 associated with severity of joint space narrowing Severity of self-reported knee joint symptoms associated with both synovial fluid ($\beta=0.773$; $P=0.003$) and serum ($\beta=0.641$; $P=0.031$) CD14 levels	71
	$CD11c^+/CD206^+$ or $CD86^+/CD163^+$ ratio in synovial fluid	Associated with KL grading and severity of knee OA in patients	72
	Mannose receptors MRC1 and MRC2	MRC1 and MRC2 recognize collagen, promoting its internalization and lysosomal degradation Resulting improvement in collagen turnover restores ECM homeostasis in the joint and ameliorates cartilage destruction Type II collagen helps to maintain expression of anti-inflammatory macrophage-related genes and pro-chondrogenic cytokines	77
	SEPP1, *FLOR2*, *STAB1*, *TXNIP* and *CD169*	Gene expression profile is indicative of enhanced phagocytic activity and immunosuppressive activity, suggesting an immunoregulatory role	58
	$CCR2^+$ macrophages	Present in human synovium Invasive cells that are associated with cartilage erosion in OA	65
	CCL3, *CCL4*, *IL1B* and *TNF*	Pro-inflammatory macrophages	58
FLS	$CD34^-THY1^+$ FLS	Less abundant in OA synovium than in RA synovium (8% versus 22% of cells, respectively) Perivascular location, proliferative and secrete pro-inflammatory cytokines Proportion of these FLS is correlated with synovitis and synovial hypertrophy assessed by ultrasonography	90
	$CD34^-THY^-$ FLS	Located in synovial lining Express the osteoblastic bone formation promoter BMP6 (involved in osteophyte formation) More abundant in OA than in RA synovium	90
	CADM1, *COL8A2* and *DKK3*	Located in synovial lining DKK3 is a strong inhibitor of cytokine-induced collagen loss	59,259
	PTGDS, *CXCL3*, *RSPO3*, *NRN1*, *NFKBIA*, *CXCL2*, *GEM*, *VCAM1*, *LIF*, *IL6* and *INHBA*	Associated with painful synovial sites in early OA	52
	HSPA1A, *DNAJB1*, *SLC39A8*, *HTRA3*, *ATF3*, *PTGIS* and *BNIP3*	Associated with painful synovial sites in end-stage OA	52

ECM, extracellular matrix; FLS, fibroblast-like synoviocytes; KL, Kellgren and Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; OA, osteoarthritis; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

cell-specific enzyme that is released during degranulation, compared with control individuals[94]. Deficiency of mast cells reduces cartilage loss, osteophyte formation and synovitis in the destabilization of the medial meniscus (DMM) mouse model of OA[94]. In addition, mast cell-dependent production of prostaglandin D2 in response to elevation in nerve growth factor (NGF) levels leads to an increase in nociceptive signalling in OA joints[95].

Endothelial cells are present in joint structures and angiogenesis is implicated in OA pathogenesis[96]. Histological analysis of established OA synovium detected pericytes in all blood vessels, suggesting that these vessels are fully mature and stable, which might explain the persistent inflammation in OA; by contrast, the synovial vasculature in inflammatory arthritis is characterized by a mixture of mature and immature vessels[97]. This study also found that blood vessels were distributed throughout the depth of the synovial membrane in OA, without preferential distribution in synovial lining cells[97]. Endothelial-cell-derived vascular endothelial growth factor (VEGF) seems to play an important part in OA pathogenesis, as the serum and synovial fluid concentration of VEGF correlates positively with WOMAC, radiographic severity (KL score) and the presence of osteophytes and a power Doppler ultrasonography signal of synovitis[98]. Although VEGF is crucial for cartilage formation, its expression seems to be upregulated in the joint of patients with OA and in surgically induced knee OA in mice; increased VEGF expression is associated with catabolic processes in

chondrocytes and synovial cells (related to cartilage destruction)[99]. Furthermore, conditional knockdown of *Vegf* attenuated injury-induced OA in mice and intra-articular anti-VEGF antibodies suppressed OA progression and blocked VEGF signalling, as revealed by reduced levels of phosphorylated VEGFR receptors in articular chondrocytes, synovial cells and dorsal root ganglia[99]. Indeed, oral administration of the VEGFR2 kinase inhibitor vandetanib attenuated OA progression[99].

Studies investigating the composition of the synovial membrane also reported the presence of T cells, including T helper (T_H) 1 cells, T_H2 cells, T_H9 cells, T_H17 cells, T_H22 cells, T regulatory (T_{reg}) cells and cytotoxic T cells[100], even in the earliest stages of disease[101]. Although a change in the profile of T cell subtypes was described to correlate with disease activity and pain[102], the role of T cells in the development and progression of OA has yet to be determined[100].

The presence of the varied cellular players in the synovial tissue might complicate histological evaluation of the OA synovium. As alteration in the equilibrium and interaction between these cell types shape OA progression and symptomatology, understanding of the mediators of this intricate network is therefore crucial.

OA risk factors and synovitis

Trauma

PTOA (FIG. 2) represents ~12% of all cases of symptomatic OA[103]. A study found that patients with a 3–10-year history of sport-related intra-articular knee injury developed OA[104]. Both animal and human studies have demonstrated that joint injuries (to menisci and ligaments, as well as intra-articular fractures) lead to the development of synovitis[105–107]. For example, data from the Osteoarthritis Initiative showed that injury was associated with accelerated OA development, as assessed by KL grade[108,109], whereas other studies found a higher incidence of OA in patients with joint injuries than in those without injuries[110]. As a surrogate of the presence of joint inflammation, pro-inflammatory cytokines, including IL-1β, IL-2, IL-6, IL-8, IL-12, IFNγ and TNF, as well as the cartilage-degrading markers MMP1, MMP3 and MMP9, are substantially elevated immediately after injury in the synovial fluid of patients with joint injuries[111–114], and the elevated cytokine levels persist after bone healing[115]. As pro-inflammatory factors induce the production of cartilage-degrading enzymes, an association between synovial inflammation and PTOA is a prevalent hypothesis. However, in a study of 113 patients with acute anterior cruciate ligament injury, the levels of inflammatory mediators in the synovial fluid or the presence of moderate-to-severe Hoffa synovitis or of effusion synovitis at 2 years after anterior cruciate ligament injury did not predict structural knee OA at the 5-year follow-up[116]. More long-term longitudinal studies are needed to evaluate the contribution of synovial inflammation to the initiation and progression of PTOA.

Mechanical loading

Mechanical loading is essential for healthy joint maintenance. Nonetheless, aberrant excessive loading is a known OA risk factor[117] and is thought to act through

Table 2 | **Risk factors and activators of synovial inflammation in OA**

Risk factor or activator	Clinical relevance	Ref.
Obesity	Patients with obesity have a higher prevalence and severity of synovial inflammation assessed by conventional MRI	25
T2DM	Higher rates of ultrasonography-detected synovitis and effusion in patients with T2DM with end-stage knee OA who underwent arthroplasty compared with patients without T2DM, independent of patient BMI	139
Metabolic endotoxaemia	The presence of LPS in both plasma and synovial fluid from patients with OA correlates with the presence of activated macrophages in the joint capsule and synovium, radiographic severity (by ^{99m}Tc-EC20 SPECT–CT), and total WOMAC score	158
Microbiome — increased intestinal permeability and endotoxaemia	Pro-inflammatory *Streptococcus* species are associated with higher effusion on MRI and WOMAC knee pain, independent of BMI	161
Senescent cells	Positive correlation of the percentage of $p16^{INK4A}$-expressing synoviocytes and IL-6 concentration in the synovial fluid with the degree of synovitis at the site of biopsy	168
Bioactive lipids	11,12-DHET and 14,15-DHET levels are higher in OA knees versus unaffected knees of people with unilateral disease ($P < 0.014$ and $P < 0.003$, respectively) and are associated with radiographic progression over 3.3 years	180
HMGB1	HMGB1 levels in the synovial fluid higher in patients with KL 4 than in those with KL 2 ($P < 0.01$) and KL 3 ($P < 0.05$)	184
	Synovial fluid HMGB1 levels associated with the severity of synovitis and pain	185
HSP70	HSP70 levels higher in both serum and synovial fluid of individuals with knee OA than in healthy controls and both correlate with radiographic severity	189
MSU crystals	MSU crystals in the joint are associated with increased synovial fluid concentrations of IL-1β ($r^2 = 0.34$, $P < 0.0001$) and IL-18 ($r^2 = 0.41$, $P < 0.0001$), OA severity and radiographic progression, and osteophyte formation ($P = 0.001$ and $P < 0.0001$, respectively)	204

KL, Kellgren and Lawrence; LPS, lipopolysaccharide; MSU, monosodium urate; OA, osteoarthritis; SPECT, single-photon emission computed tomography; T2DM, type 2 diabetes mellitus; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

several molecular pathways, including IL-1β, TNF, NF-κB, WNT, microRNA and oxidative stress signalling pathways, which lead to chondrocyte apoptosis and ECM degradation[118]. However, excessive loading might also affect the synovium. For example, in vitro studies on FLS showed that mechanical loading induced the expression of several mediators involved in OA pathogenesis, such as prolyl-4-hydroxylase-α1 (P4HA1), collagen α2 (I) chain (COL1A2), cyclooxygenase 2 (COX2) and IL-6 (REF.[119]). In addition, similar studies in human monocytes revealed that mechanical loading, shear stress and compression induce expression of nitric oxide synthase 2 (NOS2), IL-12B, IL-6 and IL-8 (REF.[120]). Despite all this evidence suggesting that abnormal mechanical loading can facilitate the accumulation of inflammatory mediators in the synovium, the exact mechanism by which aberrant or excessive mechanical loading induces synovitis is still unknown and might involve multiple cellular factors. Of interest, moderate physical activity has been proposed to modulate the immune response by priming circulating monocytes towards an anti-inflammatory macrophage-like differentiation, mediated potentially by peroxisome proliferator-activated receptor-γ (PPARγ) signalling that implicates increased expression of CD36 (1.9 ± 1.5-fold) and liver X receptor-α (LXRα) (5.0 ± 4.7-fold) compared with sedentary individuals[121]. In a randomized controlled trial in women with knee OA, physical activity increased total intra-articular and perisynovial concentration of the anti-inflammatory cytokine IL-10 (REF.[122]), which is mainly produced by anti-inflammatory macrophages[123] and has a chondroprotective role[122].

Box 1 | **Imaging techniques for synovitis assessment**

Magnetic resonance imaging

- Two techniques: conventional MRI and contrast-enhanced MRI (CE-MRI).
- Signs of synovial inflammation in MRI scans include:
  - an increase in synovial membrane thickness and/or volume
  - enhanced signal intensity after intravenous gadolinium injection
  - the presence of effusion
  - alterations in the infrapatellar fat pad[26,258]
- Conventional MRI is still the most frequently used technique in OA, despite being unable to distinguish between synovial hypertrophy and joint effusion
- Synovial hypertrophy and joint effusion were correlated in a study that identified definite synovitis (synovial thickness ≥2 mm by CE-MRI) in 96.3% of knees with an effusion[259]
- A meta-analysis of 8 studies found that both CE-MRI (6 studies) and conventional MRI (2 studies) findings of synovitis correlated with macroscopic (vascularity, hyperplasia and villi) and microscopic (inflammatory infiltrates, synovial lining cells number, oedema and fibrosis) histological features of inflammation[260]

Ultrasonography[261,262]

- Ultrasonography can assess and distinguish between synovial hypertrophy and joint effusion
- Synovitis appears as thickening of the synovial membrane in grey scale (usually scored on a scale of 0–3)
- Power Doppler ultrasonography can detect active synovial inflammation in OA (also scored on a scale of 0–3)
- Power Doppler signal correlates with histologically confirmed inflammatory cell infiltrates, increased synovial lining layer thickness and increased vascularity
- Power Doppler signal also correlates with MRI findings of synovitis (joint effusion: CI = 0.61; $P < 0.001$; synovial thickening: CI = 0.45; $P = 0.01$)[263]

These techniques could possibly be used to stratify patients with synovial inflammation who could benefit from specific anti-inflammatory treatments.

Obesity and T2DM

Obesity is a well-known risk factor for both OA incidence and progression[124], and its role in OA development is different according to sex and also depends on the affected joint[125]. Incidence of knee, hip and hand OA is higher in women[126–128] than in men, and the prevalence of symptomatic OA is higher in women with obesity than in men with obesity[2]. The contribution of obesity to OA occurs not only through so-called mechanoinflammation but also through systemic low-grade inflammation or meta-inflammation, as obesity is linked to OA in weight-bearing joints such as the knees and in non-weight-bearing joints such as the hands[129]. The synovium of individuals with obesity displays marked fibrosis, increased macrophage infiltration and elevated expression of the Toll-like receptor 4 (TLR4) gene, but reduced levels of adiponectin and PPARγ[130]. In addition, abundance of $CD14^+$ and $CD206^+$ macrophages is increased in the synovial tissue of obese individuals[130]. Furthermore, the synovial fluid levels of the pro-inflammatory adipokine leptin are significantly higher in individuals with obesity than in those without obesity and correlate positively with BMI[131]. Finally, levels of mast-cell-produced β-tryptase in synovial fluid are also higher in individuals with obesity than in those without obesity[132]. Studies performed in rat[133] and rabbit[134] models with diet-induced obesity and surgically induced OA also showed an increase in pro-inflammatory macrophages[133] and the pro-inflammatory mediators IL-1β, IL-6 and TNF[134] in the synovium, which promote OA. Some studies also report more pain in patients with obesity with OA[135,136]. FLS isolated from patients with obesity with hip OA who underwent joint replacement surgery secrete higher amounts of IL-6 than FLS from lean patients, which was enhanced by crosstalk with chondrocytes via leptin[137]. Although patients with obesity have a higher prevalence and severity of synovial inflammation, as assessed by conventional MRI[25], improvement in knee pain in patients with obesity with >20% weight loss at 1 year after dietary intervention or bariatric surgery was not mediated by a decrease in synovitis or bone marrow lesions (BMLs), as evaluated by MRI, but was partially explained by improvement in pressure pain threshold (at the patella and wrist) and depression score (CES-D)[138]. Furthermore, there was no noticeable improvement in BMLs (number and volume on MRI) or synovitis score after weight loss, which is in agreement with results of previous studies[139,140]. In fact, weight loss had no effect on synovial inflammation, evaluated by both static conventional MRI and dynamic CE-MRI, or on pain, evaluated by KOOS in a Danish study[141]. The fundamental reasons why obesity seems to facilitate synovitis but weight loss does not reverse this process are still unknown, although it is possible that obesity causes irreversible or long-lasting changes, such as epigenetic modifications or tissue structure alterations, which support OA progression even when individuals lose weight.

Epidemiological studies show a higher prevalence of OA (radiographic and symptomatic) in patients with type 2 diabetes mellitus (T2DM) and a higher rate of arthroplasty[142,143], with a meta-analysis reporting a higher risk of OA development in patients with T2DM than in those without T2DM (OR 1.46; 95% CI 1.08–1.96; $P = 0.01$)[144]. Although some studies neither confirmed these findings after adjustment for BMI[145,146] nor detected an association between T2DM and prevalence or incidence of OA[147,148], some of the studies included in the meta-analysis reported the same increased risk after BMI adjustment, suggesting that T2DM is an independent risk factor for OA development[143]. For example, ultrasonography-detected synovitis and effusion were higher in patients with T2DM and end-stage knee OA who underwent arthroplasty than in those without T2DM, independent of patient BMI[143]. Several reports have described the effect of hyperglycaemia on synovial inflammation. For example, synovial levels of the pro-inflammatory cytokine TNF were higher in obese patients with OA and T2DM than in those without T2DM[149]. FLS in patients with diabetes and obesity with OA are also insulin resistant, implying a diminished ability of insulin to decrease production of pro-inflammatory and catabolic mediators that contribute to OA development[150]. High glucose levels induce VEGF secretion and reactive oxygen species (ROS) production in FLS in OA, increasing angiogenesis, tissue damage and inflammation[151]. Finally, both diabetes and ageing are associated with the accumulation of advanced glycated end-products, which induce an increase in proMMP1 secretion by FLS and in transcription of bone morphogenetic protein (BMP) genes that are involved in osteophyte formation[152].

Diet and the gut microbiome

Although obesity is one of the most important modifiable risk factors to improve outcomes in OA, diet might have a role beyond weight control. A higher dietary inflammatory index score is associated with a higher prevalence of radiographic, symptomatic KOA, independent of patient weight (OR 1.40; 95% CI 1.14–1.72; $P = 0.002$)[153]. Interestingly, a randomized controlled trial of vitamin D supplementation slowed the progression of effusion-synovitis volume increase[154], supporting the premise that micronutrients might have an effect on chronic pain by modulation of intra-articular inflammation. A subsequent randomized controlled trial showed no effect of vitamin D supplementation on BML volume and synovitis[155]. In a randomized, controlled trial, *Curcuma longa* extract, a proposed anti-inflammatory natural product, was superior to placebo in controlling pain but had no effect on knee effusion-synovitis or cartilage damage[156]. The Mediterranean diet is also believed to have positive effects in patients with OA[157], and epidemiological studies from the Osteoarthritis Initiative found that a western diet was associated with progression of OA (higher KL and WOMAC score)[158], although no data on its effect on synovitis were provided.

Diet is one of the modifiable factors that influence the composition of the gut microbiome. As germ-free mice have reduced susceptibility to OA from DMM[159], and microbial DNA signatures have been detected in the cartilage and synovial tissue of patients with OA[160,161], interest in the role of the microbiome in OA development and progression has increased. Western diets lack prebiotic-rich foods, in the form of dietary fibre, other complex carbohydrates and sugar alcohols present in fruits, which might be beneficial in supporting a healthy microbiome. Microbial dysbiosis — adverse alterations of the gut microbiota composition — may favour metabolic syndrome and inflammation. Indeed, obesity is associated with a loss of beneficial *Bifidobacterium* species and an increased abundance of pro-inflammatory bacterial species, which might increase macrophage recruitment from the gut to the synovium and accelerate knee OA[162].

The bacterial endotoxin lipopolysaccharide (LPS) is a known activator of synovial inflammation through TLR4 (REF.[163]). Metabolic endotoxaemia (that is, the presence of bacterial products such as LPS in the blood) has been linked to changes in intestinal permeability induced by diet[164] and has been described in patients with obesity and metabolic syndrome[165]. Interestingly, the presence of LPS in both plasma and synovial fluid from patients with OA correlates with the presence of activated macrophages in the joint capsule and synovium, radiographic severity (by ^{99m}Tc-EC20 SPECT–CT), and total WOMAC score[163]. Another study in individuals with knee OA found an association of the pro-inflammatory *Streptococcus* species with higher effusion (on MRI) and WOMAC knee pain, independent of BMI[166]. Whether changes in the gut microbiota that support inflammation are present in early stages of OA and are a contributing factor in OA radiographic or clinical progression or a consequence, possibly influenced by obesity, needs to be further examined.

Molecular mediators of synovitis in OA

Ageing and mitochondrial damage

Mitochondrial dysfunction (FIG. 3) is characterized by reduced mitochondrial integrity, including decreased mass, number and mitochondrial DNA (mtDNA) content, and impaired mitochondrial respiration, which increases ROS production[167]. Ageing is postulated to play a role in the mitochondrial dysfunction observed in OA. For example, mice that aged prematurely from accumulation of mtDNA mutations exhibited osteopenia, changes in epiphyseal trabecular bone and the subchondral cortical plate, and elevated numbers of hypertrophic chondrocytes in articular calcified cartilage[168]. Evaluation of mtDNA single nucleotide polymorphisms has defined mtDNA haplogroups as potential biomarkers for diagnosis or prognosis of OA[169]. In OA synoviocytes, the frequency of mtDNA mutations is substantially lower than in RA synoviocytes[170], but complete characterization and larger population studies are needed to define the involvement of mtDNA mutations in synoviocytes in OA development. Ageing has also been associated with chronic low-grade inflammation, which could promote OA development, although the exact mechanism of the ageing–inflammation link is still unknown[3]. Mitochondrial dysfunction and deficient ROS scavenging prolong the production of

pro-inflammatory cytokines (such as IL-1β and IL-6) and prevent the repolarization of macrophages from a pro-inflammatory to an anti-inflammatory phenotype in other tissues[171], all of which could influence the development and progression of OA.

Senescent cells are a feature of various age-related pathological conditions, including OA. In particular, senescent chondrocytes in both PTOA and age-related OA accumulate mostly in the articular cartilage and synovium, and their elimination attenuates the development of OA, reduces pain and increases cartilage formation[172]. Several senescent-associated secretory phenotype factors are also inflammatory mediators, supporting the hypothesis that senescent cells in OA synovium could play a role in initiating or maintaining synovial inflammation. Studies in ex vivo human OA knee specimens and in a surgically induced OA mouse model have detected senescent synoviocytes in the OA synovium and demonstrated a positive correlation between the degree of synovitis at the biopsy site and the percentage of $p16^{INK4A}$-expressing synoviocytes and levels of IL-6 in synovial fluid[173]. Together, these results suggest that 'aged synovium', indicated by the presence of senescent cells, is associated with synovitis. Early-stage clinical studies with senolytic agents are now underway, with results forthcoming (NCT04210986, NCT04770064 and NCT04815902).

Metabolites affecting the synovium

The OA synovium is a rich environment containing a wide variety of metabolites and soluble factors that contribute to both inflammation and cartilage damage[174].

Nitric oxide. Synovial fluid from patients with OA contains elevated levels of nitrite and the enzyme responsible for nitric oxide (NO) production, inducible nitric oxide synthase (iNOS; encoded by *NOS2*)[175]. NO, which is mainly produced by chondrocytes in the OA joint[176], mediates inflammatory mediator production, angiogenesis and cartilage destruction[177]. Pro-inflammatory macrophages are also an important source of NO, through the metabolic rewiring of arginine metabolism towards NO and l -citrulline production[70]. Inhibition of iNOS dramatically reduces the production of catabolic and pro-inflammatory factors and prevents OA development in dogs[178]. Although animal studies support the investigation of iNOS inhibitors as a potential disease-modifying intervention for OA, no successful clinical trials of these agents have been reported[179].

Succinate. Pro-inflammatory macrophages exhibit a dysfunctional Krebs cycle that results in accumulation of succinate, which is shuttled from mitochondria to the cytosol to prevent hydroxylation and degradation of hypoxia inducible factor 1α (HIF1α), a key transcription factor involved in IL-1β production[180]. Although intracellular succinate supports the pro-inflammatory phenotype of macrophages, activation of succinate receptor 1 (SUCNR1; also known as GPR91) by soluble succinate boosts IL-4 production[181], a cytokine that induces macrophage polarization towards an anti-inflammatory phenotype. However, succinate signalling through SUCNR1 in FLS links inflammation with fibrosis and angiogenesis and, indeed, exacerbates RA[182]. Despite these animal and in vitro data in RA, there are no studies investigating the relationship between succinate levels in the synovium and radiographic progression or clinical symptoms in OA. Indeed, the role of succinate and other intermediate metabolites in glycolysis and the Krebs cycle in FLS in OA is still unknown, and additional metabolic and functional studies are needed to understand the phenotype of FLS in OA.

Prostaglandins and other bioactive lipids. Both IL-1β and IL-18 substantially increase the production of prostaglandin E2 (PGE2) in the synovium after articular cartilage damage[183]. In synovial fluid from patients with knee OA, the levels of IL-18 and PGE2 correlate greatly[184]. PGE2 is considered the major contributor to inflammatory pain in the OA joint. PGE2 signals through multiple receptors that are expressed differentially in both peripheral sensory neurons and the spinal cord. Through the EP4 receptor, PGE2 has been proposed to participate in enhancing the production of aggrecanases and MMP13 induced by pro-inflammatory cytokines[185]. PGE2 also induces the expression of iNOS and IL-6, which further contributes to maintaining synovitis and increasing hyperalgesia. PGD2 is also enriched in synovial fluid from patients with OA[186] and has been suggested to potentiate nociception[95]. In a study that compared synovial fluid samples from 112 knees of 102 individuals (of whom 58 had knee OA and 44 were healthy controls), including both affected and unaffected knees in those with unilateral OA, increased levels of PGD2, 11,12-dihydroxyeicosatrienoic acid (11,12-DHET) and 14,15-dihydroxyeicosatrienoic acid (14,15-DHET) were associated with the presence of OA[186]. The levels of 11,12-DHET and 14,15-DHET were higher in affected than in unaffected knees of people with unilateral OA ($P < 0.014$ and $P < 0.003$, respectively) and were associated with radiographic progression over 3.3 years of follow-up[186].

DAMPs and alarmins

Excess mechanical stress or injury leads to the release of damage-associated molecular patterns (DAMPs), which interact with pattern recognition receptors (PRRs), including TLRs, receptor for advanced glycation end products (RAGE) and others to initiate the innate immune response and propagate inflammation. DAMPs implicated in OA are very heterogeneous and include cartilage fragments, ECM proteins, secreted intracellular proteins, plasma proteins and crystals (extensively reviewed elsewhere[187,188]).

High mobility group protein B1. HMGB1 is a non-histone nuclear protein that facilitates transcription factor and nucleosome stability. However, HMGB1 acts as an endogenous danger signal and is released from cytokine-activated cells and damaged or dying cells supporting the inflammatory response[189]. In OA joints, HMGB1 is secreted by damaged and necrotic chondrocytes[189]. HMGB1 levels in synovium and synovial fluid are higher in patients with OA than in healthy controls[190,191], and they are also higher in the synovial fluid of patients with KL 4 than in those with

KL 2 ($P < 0.01$) or KL 3 ($P < 0.05$)[190]. HMGB1 levels in synovial fluid have also been associated with the severity of synovitis, pain and daily activity reduction[191]. The therapeutic potential of HMGB1 neutralizing antibodies has been evaluated in the DMM mouse model, in which they showed cartilage protective effects[192].

Heat shock proteins. Heat-shock proteins (HSPs) are induced by cellular stress to protect and maintain cellular integrity and function[193]. In OA synovium, HSP60, HSP70 and HSP90 are the most abundant members of the HSP family[194]. HSP70 levels in serum and synovial fluid are higher in individuals with knee OA than in healthy controls and correlate with radiographic disease severity[195]. In the DMM mouse model, overexpression of the HSP70 family member HSPA1A abrogated cartilage erosion while having no effect on DMM-induced osteophyte formation or subchondral bone plate thickening[196].

S100 protein family members. S100 proteins are intracellular calcium-binding proteins that participate in regulating the cytoskeleton and cell migration[188]. S100A9 is strongly upregulated in inflamed synovial tissue[197] and is involved in cartilage matrix degradation and osteophyte formation[198]. A study in 141 individuals with clinical knee OA showed that serum levels of S100A8 or S100A9 correlated with total WOMAC scores ($P = 0.021$), weight-bearing pain ($P = 0.043$) and physical dysfunction ($P = 0.010$)[199]. Similar results were obtained in 294 patients with hand OA[200].

Other NLRP3 inflammasome activators

Evidence of the participation of the NLRP3 inflammasome, a protein complex involved in processing and maturation of IL-1β and IL-18, in OA onset and progression has led to this complex being proposed as a potential biomarker for OA diagnosis and patient classification[201–203]. Maturation of IL-1β and IL-18 is a two-step process. First, activation of NF-κB-dependent transcription of *NLRP3* and *IL1B*[204], *p62* (also known as *SQSTM1*)[205] and *SLC44A1* (REF.[206]) among other genes, and de novo synthesis of mtDNA[207]. Second, the assembly of the NLRP3 inflammasome, activation of caspase 1 and processing of pro-IL-1β and pro-IL-18 to mature cytokines[204]. In the OA synovium, ectopic deposition of hydroxyapatite crystals, calcium pyrophosphate dihydrate microcrystals and monosodium urate crystals, and ATP released from dying cells are detected by macrophages and trigger NLRP3 inflammasome activation and IL-1β and IL-18 production[208,209]. Of note, monosodium urate crystals in the joint are associated with increased synovial fluid levels of IL-1β ($r^2 = 0.34$, $P < 0.0001$) and IL-18 ($r^2 = 0.41$, $P < 0.0001$), OA severity and radiographic progression ($P = 0.001$), and osteophyte formation ($P < 0.0001$)[210]. IL-1β induced chondrocyte catabolism, increased MMP and ADAMTS5 activity and suppressed proteoglycan synthesis[183]. The pathogenetic role of IL-1β in OA synovium might also be due in part to the lack of production of the natural IL-1β antagonist IL-1 receptor antagonist (IL-1Ra) by OA chondrocytes[58]. Preclinical studies using recombinant IL-1Ra (anakinra) demonstrated a strong protection in OA animal models by improving lubricin expression, preserving cartilage integrity and reducing synovial hyperplasia and inflammatory cell infiltration[211]. Furthermore, in an exploratory analysis of a randomized controlled trial for the prevention of cardiovascular events, canakinumab (anti-IL-1β) treatment was associated with a lower incidence of hip and knee replacement than placebo[212,213], although in a randomized controlled trial, intra-articular injection of anakinra did not improve OA symptoms compared with placebo[214]. These disparate outcomes have resulted in a lack of consensus regarding the use of IL-1 signalling therapy for OA, concluding that inhibiting the actions of IL-1β alone is not enough to block OA pathogenesis. In this sense, NLRP3 inflammasome inhibitors, which block not only the production of IL-1β but also that of IL-18 and active caspase 1, might be a more potent intervention. In the OA synovium, IL-18 promotes chondrocyte proliferation and expression of COX2, iNOS and MMPs, and boosts IL-6 production, further supporting a pro-catabolic environment[215]. Importantly, active caspase 1 also cleaves gasdermin D, releasing the active amino terminal portion that has pyroptotic activity (by pore formation), which could contribute to maintaining persistent inflammation within the synovium[216].

Complement

Complement factors are highly expressed in the main tissues involved in OA, including cartilage, bone and synovium, in patients with OA compared with in healthy controls[217,218]. Furthermore, expression of complement effectors is higher and that of complement inhibitors is lower in the synovium of patients with OA than in healthy donors[217]. In addition, C5 and C6 deficiency are protective against the development of synovitis and cartilage damage in animal models of OA, and complement activation is associated with increased production of ECM-degrading proteins and inflammatory mediators[217]. These data suggest a role for complement activation not only in synovitis but also in the development and progression of OA, and it could therefore represent a potential therapeutic target.

Cellular interplay in OA synovium

Interactions between synoviocytes, chondrocytes and osteocytes

In a healthy joint, chondrocytes balance the synthesis and breakdown of the cartilaginous matrix to ensure correct distribution of load across the joint, thereby reducing friction. Both non-mechanical and mechanical factors contribute to OA development, which involves a shift in chondrocyte metabolism to increased proteolytic activity and inflammation and cartilage degradation. Direct and indirect communication between chondrocytes and synoviocytes is thought to contribute to maintaining anabolic and catabolic responses of each cell type[219] (FIG. 3). In the inflamed joint, chondrocytes and synoviocytes mutually induce alterations in their transcription programme to favour the production of MMPs[220]. Cartilage fragments, aggrecan, fibronectin and other DAMPs are sensed by synoviocytes to shift their transcriptomic profile towards chronic inflammatory responses, including cytokine, NLRP3 inflammasome,

hypoxia, scavenger receptor and TLR, and integrin pathways[221]. Importantly, the array of upregulated genes related to maintenance of an inflammatory phenotype are under control of transcription factors that support synovitis, including ATF2, STAT3 and NFKB1 (REF.[221]). Cartilage fragments can also induce inflammatory responses in synoviocytes by reorganization of the actin cytoskeleton, enhanced production of NO and PGE2 and increased deposition of collagen[222] (FIG. 4).

Synovitis can coexist with BML and both can precede the development of radiographic OA[223,224]. Although it is thought that BMLs result from excessive mechanical loading, it is not known whether synovitis contributes to subchondral bone pathology[225], other than by invasion of subchondral bone by pannus-like tissue in the medial compartment[226] without producing the marginal erosions typically seen in RA[227]. The temporal relationship between the synovitis and BMLs is still not known. In a study in patients with end-stage OA before joint replacement, histologically assessed synovitis correlated moderately with the presence of subchondral cysts by MRI ($r = 0.350$; $P = 0.03$)[228]. However, a subsequent analysis of the contribution of different OA pathological processes to pain found no association between subchondral pathology and synovitis, suggesting that subchondral pathology is associated with knee pain independently of cartilage and synovial pathology[229].

Synovitis is also associated with osteophyte formation. Hoffa synovitis is significantly associated with osteophyte development in both anterior ($P = 0.013$, adjusted OR 1.12, 95% CI 1.03–1.23) and medial ($P = 0.000$, adjusted OR 1.21, 95% CI 1.11–1.31) lesions of the tibia[230]. In support of the association between Hoffa synovitis and osteophyte development, depletion of macrophages in the collagenase-induced OA model strongly reduced the formation of osteophytes and fibrosis[231,232]. Macrophages, which together with FLS are the main source of TGFβ in the synovium, contribute to the stimulation of bone formation and production of proteoglycan and type II collagen, and enhance chondrogenesis. TGFβ and the related proteins BMP2 and BMP4 are essential growth factors implicated in osteophyte formation[233] and pathological type I collagen deposition during fibrosis[234].

Although the relationship between synovitis and OA structural progression is better defined, the role of synovial inflammation in OA pain is not completely understood (BOX 2). Treatment with antibodies against granulocyte–macrophage colony-stimulating factor (GM-CSF), which signals in both the immune and nervous systems, has an analgesic effect in OA without affecting synovitis scores[235]. Indeed, the number of GM-CSF- and GM-CSFRα-expressing cells per mm^2 synovial sub-lining correlated negatively with knee pain[235], reinforcing the idea that synovitis and pain are not always associated. Research in OA-related pain has also focused on nerve growth factor (NGF)[236], which is a neurotrophin that activates nociceptive neurons to transmit pain signals from the periphery to the central nervous system. NGF is expressed by FLS and macrophages in the synovium[237] and by subchondral mononuclear cells, osteoclasts and chondrocytes in the cartilage from patients with knee OA[229]. Other studies suggest that symptomatic OA is associated with upregulation of MMP1 in the synovium and downregulation of IL-1R1 and VEGF compared with the levels of these molecules in individuals with asymptomatic chondropathy with similar macroscopic joint surface appearances who did not seek total knee replacement[18]. IL-1β signals through its receptor IL-1R1 and induces the expression and release of MMP1 by FLS. After IL-1–IL-1R1 engagement, IL-1R1 is downregulated, which may explain why therapy with an IL-1R1 antagonist failed in clinical trials[238]. Angiogenesis in the subchondral bone has been postulated as the initial event in OA pain, as the blood vessels supply nutrients for axonal growth and neo-innervation of the osteochondral junction, likely driven by NGF released from basal articular chondrocytes[239]. The synovium then contributes to pain by secreting pro-inflammatory factors, such as TRKB (the receptor for brain-derived growth factor), CCL14 and ADAMTS15, angiotensinogen, angiotensin-converting enzyme, netrin 1, CCL2 and CCL8 (REFS[18,237,240]), either independently or amplifying the process already initiated by NGF.

Interactions between synovial tissue and the infrapatellar fat pad

Fibrosis may contribute to joint stiffness and pain, which are the main symptoms in OA, but most of the clinical studies relate to postoperative synovial fibrosis. Intra-articular fibrosis can be detected by using MRI scans with advanced metal suppression and with gadolinium contrast[241] in patients with stiff and painful arthroplasty. Patients with diagnosed fibrosis[242] exhibit thicker tissue (4.4 mm ± 0.2 mm) than patients with a non-fibrotic phenotype (2.5 mm ± 0.4 mm) after total knee arthroplasty (1.9 mm ± 0.2 mm; $P < 0.05$)[241]. A promising fibroblast radiotracer for PET, ^{18}F-labelled glycosylated FAPI, demonstrated highly specific uptake in bone structures and joints[243] and could aid in

Box 2 | **Gaps in and proposed agenda for synovitis research in OA**

Research gap
To date, the fundamental mechanisms underlying the crosstalk between synovitis and clinical symptoms of osteoarthritis (OA) are not completely identified. Current studies describe histological and molecular characteristics at end-stage OA using imaging but do not capture change over time.

Proposed research agenda
Longitudinal studies that combine cellular and molecular evaluation in combination with histology and imaging at different stages of OA to establish mechanistic links with clinical OA progression.

Research gap
Current imaging techniques cannot capture all histological features of the OA synovium.

Proposed research agenda
Optimization of imaging, including MRI, functional imaging and positron emission tomography to capture the different histological patterns and phenotype subsets in OA.

Research gap
Defining phenotypes that capture the heterogeneous features of OA synovitis.

Proposed research agenda
Personalized medicine for patients with OA by defining phenotypes of OA that capture the inflammatory subtypes through advanced imaging.

improving understanding of the role of fibroblasts and fibrosis in OA and whether or not fibrosis in the synovial tissue contributes to joint pain.

The exact mechanism by which fibrosis occurs in OA synovium is still not entirely clear. It is generally accepted that there is more inflammation, with an increased number of macrophages and T cells in the lining layer, in early OA than in advanced OA, in which inflammation and fibrosis coexist[48,244]. These observations suggest that the progression of OA is accompanied by a transition from an inflammatory phase to a fibrotic stage and that factors that initiate fibrosis might be induced during synovitis. Several factors that are increased in inflamed OA joints are associated with fibrosis, including TGFβ, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), tissue inhibitor of metalloproteinase 1 (TIMP1), connective tissue growth factor (CTGF), disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) and prostaglandin F2a. For example, ADAM12 mRNA and protein levels in synovium correlate with the severity of histological synovitis[244]. Both PLOD2 and TIMP1 have been directly implicated in synovial fibrosis and are elevated in the synovium of patients with end-stage OA and mice with experimental OA[245].

Another compartment that is involved in the development of synovial fibrosis is the IFP, which is located below the kneecap between the joint capsule and the synovial membrane, protects the knee from mechanical stress and provides a vascular supply. However, during OA development and progression the IFP also undergoes structural changes characterized by increased fibrosis and neovascularization, lymphocyte infiltration in the interlobular septum and smaller fat lobules[246–248]. Of note, individuals who develop AKOA are more likely to have altered signal intensity in the IFP (30%) than those with no knee OA at 2 years prior to the index visit (OR 2.07, 95% CI 1.14–3.78), and these odds increase twofold at 1 year prior to disease onset and for the next 3 years[249]. The infiltration of immune cells and increased fibrosis cause the disappearance of adipocytes in the parenchymal region of the IFP[250]. Part of the contribution of the IFP to synovial fibrosis is mediated by the activation of FLS: PLOD2 expression and collagen production by FLS increases sixfold and 1.8-fold respectively when FLS are co-cultured with fat-conditioned medium from the IFP of patients with OA[247]. Furthermore, collagen production by FLS correlated with increased levels of prostaglandin F2a in the fat-conditioned medium, whereas no correlation with TGFβ amounts was observed[247]. In addition, IFP tissue in obese individuals shows increased expression of genes associated with fibrosis and ECM production, but no change in adipocyte size, inflammatory cell infiltration, macrophage polarization, formation of crown-like structures, or expression of genes encoding inflammatory cytokines and chemokines[251]. In another study, IFP volume was not associated with BMI[252]. However, macrophages in the IFP from patients with obesity with knee OA were positive for the surface markers CD206 and CD163 (~80% and 40% of all $CD14^+$ macrophages, respectively), and these macrophages produce IL-6 and TNF but not much IL-10; of note, none of these features correlated with BMI[252]. Animal models of OA also showed an association between synovitis, changes in macrophage polarization (including enrichment of pro-inflammatory macrophage phenotype or increased crown-like structures), and fibrosis in both synovial tissue and the IFP, and treatment that decreased inflammation was associated with changes in macrophage phenotypes and attenuation of fibrosis[251–253]. Although all these studies showed some contribution of IFP dysfunction to joint inflammation and fibrosis, the mediators of these interactions remain unknown.

Of interest, TGFβ has been proposed as a nexus between fibrosis and pain in OA. In a study of patients with radiographic KL grade 3–4 after total knee arthroplasty, NGF expression in synovial tissue correlated positively with TGFβ expression ($P<0.001$) while showing no association with levels of the pro-inflammatory cytokines IL-1β and TNF ($P=0.576$ and $P=0.616$, respectively). Both TGFβ and NGF colocalized in the lining layer, mainly in the $CD45^-CD90^+$ fibroblast population (86.3% of analysed cells in the synovial tissue)[254]. Similar to findings in articular cartilage[255], TGFβ–ALK5 signalling mediates NGF production through the TAK1–p38 pathway in the synovium of patients with knee OA[254].

Conclusions

OA is a complex disease in which symptoms and joint function are often dissociated from structural damage. In an effort to identify pathobiological mechanisms in OA, the OA community is intensely investigating synovial inflammation. Consequently, we now know more about the cellular and molecular players in synovitis, although more in-depth studies are needed to evaluate the association of these factors with radiographic progression and contribution to OA symptoms at both early and late stages of disease. Hurdles to be overcome might include the heterogeneous nature of the OA synovium and the complex network of interactions that are involved in synovial inflammation, fibrosis and cartilage damage, processes that often cannot be completely dissociated and evaluated using current imaging techniques. Research advances in phenotype-specific treatment options have provided several novel therapies that could target the inflammatory component of OA[256,257]. However, further research is needed to determine whether synovial inflammation is relevant for diagnosis, risk stratification or identification of therapeutic targets.

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1. Hunter, D. J., March, L. & Chew, M., Lancet Commission on Osteoarthritis. Osteoarthritis in 2020 and beyond — Authors' reply. *Lancet* **397**, 1060 (2021).
2. Deshpande, B. R. et al. Number of persons with symptomatic knee osteoarthritis in the US: impact of race and ethnicity, age, sex, and obesity. *Arthritis Care Res.* **68**, 1743–1750 (2016).
3. Loeser, R. F., Collins, J. A. & Diekman, B. O. Ageing and the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol.* **12**, 412–420 (2016).
4. Felson, D. T. et al. Synovitis and the risk of knee osteoarthritis: the MOST Study. *Osteoarthritis Cartilage* **24**, 458–464 (2016).
5. Collins, J. E. et al. Semiquantitative imaging biomarkers of knee osteoarthritis progression: data from the foundation for the national institutes of health osteoarthritis biomarkers consortium. *Arthritis Rheumatol.* **68**, 2422–2431 (2016).
6. Wang, Y. et al. Knee effusion volume assessed by magnetic resonance imaging and progression of knee osteoarthritis: data from the Osteoarthritis Initiative. *Rheumatology* **58**, 246–253 (2019).

7. Atukorala, I. et al. Synovitis in knee osteoarthritis: a precursor of disease? *Ann. Rheum. Dis.* **75**, 390–395 (2016).
8. MacFarlane, L. A. et al. Association of changes in effusion-synovitis with progression of cartilage damage over eighteen months in patients with osteoarthritis and meniscal tear. *Arthritis Rheumatol.* **71**, 73–81 (2019).
9. Ayral, X., Pickering, E. H., Woodworth, T. G., Mackillop, N. & Dougados, M. Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis–results of a 1 year longitudinal arthroscopic study in 422 patients. *Osteoarthritis Cartilage* **13**, 361–367 (2005).
10. Roemer, F. W. et al. Can structural joint damage measured with MR imaging be used to predict knee replacement in the following year? *Radiology* **274**, 810–820 (2015).
11. Roemer, F. W. et al. Presence of MRI-detected joint effusion and synovitis increases the risk of cartilage loss in knees without osteoarthritis at 30-month follow-up: the MOST study. *Ann. Rheum. Dis.* **70**, 1804–1809 (2011).
12. Perry, T. A. et al. Association between bone marrow lesions & synovitis and symptoms in symptomatic knee osteoarthritis. *Osteoarthritis Cartilage* **28**, 316–323 (2020).
13. Haugen, I. K. et al. Synovitis and radiographic progression in non-erosive and erosive hand osteoarthritis: is erosive hand osteoarthritis a separate inflammatory phenotype? *Osteoarthritis Cartilage* **24**, 647–654 (2016).
14. Haugen, I. K. et al. MRI findings predict radiographic progression and development of erosions in hand osteoarthritis. *Ann. Rheum. Dis.* **75**, 117–123 (2016).
15. Mancarella, L., Addimanda, O., Cavallari, C. & Meliconi, R. Synovial inflammation drives structural damage in hand osteoarthritis: a narrative literature review. *Curr. Rheumatol. Rev.* **13**, 43–50 (2017).
16. Marshall, M., Watt, F. E., Vincent, T. L. & Dziedzic, K. Hand osteoarthritis: clinical phenotypes, molecular mechanisms and disease management. *Nat. Rev. Rheumatol.* **14**, 641–656 (2018).
17. Driban, J. B. et al. Risk factors and the natural history of accelerated knee osteoarthritis: a narrative review. *BMC Musculoskelet. Disord.* **21**, 332 (2020).
18. Wyatt, L. A. et al. Molecular expression patterns in the synovium and their association with advanced symptomatic knee osteoarthritis. *Osteoarthritis Cartilage* **27**, 667–675 (2019).
19. Sarmanova, A. et al. Association between ultrasound-detected synovitis and knee pain: a population-based case-control study with both cross-sectional and follow-up data. *Arthritis Res. Ther.* **19**, 281 (2017).
20. Hill, C. L. et al. Synovitis detected on magnetic resonance imaging and its relation to pain and cartilage loss in knee osteoarthritis. *Ann. Rheum. Dis.* **66**, 1599–1603 (2007).
21. Riis, R. G. et al. The association between histological, macroscopic and magnetic resonance imaging assessed synovitis in end-stage knee osteoarthritis: a cross-sectional study. *Osteoarthritis Cartilage* **25**, 272–280 (2017).
22. Abbasi, B., Pezeshki-Rad, M., Akhavan, R. & Sahebari, M. Association between clinical and sonographic synovitis in patients with painful knee osteoarthritis. *Int. J. Rheum. Dis.* **20**, 561–566 (2017).
23. Baker, K. et al. Relation of synovitis to knee pain using contrast-enhanced MRIs. *Ann. Rheum. Dis.* **69**, 1779–1783 (2010).
24. Ballegaard, C. et al. Knee pain and inflammation in the infrapatellar fat pad estimated by conventional and dynamic contrast-enhanced magnetic resonance imaging in obese patients with osteoarthritis: a cross-sectional study. *Osteoarthritis Cartilage* **22**, 933–940 (2014).
25. Kanthawang, T. et al. Obese and overweight individuals have greater knee synovial inflammation and associated structural and cartilage compositional degeneration: data from the osteoarthritis initiative. *Skeletal Radiol.* **50**, 217–229 (2021).
26. Guermazi, A. et al. Assessment of synovitis with contrast-enhanced MRI using a whole-joint semiquantitative scoring system in people with, or at high risk of, knee osteoarthritis: the MOST study. *Ann. Rheum. Dis.* **70**, 805–811 (2011).
27. Riis, R. G. et al. Synovitis assessed on static and dynamic contrast-enhanced magnetic resonance imaging and its association with pain in knee osteoarthritis: a cross-sectional study. *Eur. J. Radiol.* **85**, 1099–1108 (2016).
28. Bacon, K., LaValley, M. P., Jafarzadeh, S. R. & Felson, D. Does cartilage loss cause pain in osteoarthritis and if so, how much? *Ann. Rheum. Dis.* **79**, 1105–1110 (2020).
29. Chevalier, X., Eymard, F. & Richette, P. Biologic agents in osteoarthritis: hopes and disappointments. *Nat. Rev. Rheumatol.* **9**, 400–410 (2013).
30. Ansari, M. Y. et al. Genetic inactivation of ZCCHC6 suppresses interleukin-6 expression and reduces the severity of experimental osteoarthritis in mice. *Arthritis Rheumatol.* **71**, 583–593 (2019).
31. Attur, M. et al. Interleukin 1 receptor antagonist (IL1RN) gene variants predict radiographic severity of knee osteoarthritis and risk of incident disease. *Ann. Rheum. Dis.* **79**, 400–407 (2020).
32. Latourte, A. et al. Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. *Ann. Rheum. Dis.* **76**, 748–755 (2017).
33. Nasi, S., So, A., Combes, C., Daudon, M. & Busso, N. Interleukin-6 and chondrocyte mineralisation act in tandem to promote experimental osteoarthritis. *Ann. Rheum. Dis.* **75**, 1372–1379 (2016).
34. Kadri, A. et al. Osteoprotegerin inhibits cartilage degradation through an effect on trabecular bone in murine experimental osteoarthritis. *Arthritis Rheum.* **58**, 2379–2386 (2008).
35. Chevalier, X. et al. Adalimumab in patients with hand osteoarthritis refractory to analgesics and NSAIDs: a randomised, multicentre, double-blind, placebo-controlled trial. *Ann. Rheum. Dis.* **74**, 1697–1705 (2015).
36. Kloppenburg, M. et al. Phase IIa, placebo-controlled, randomised study of lutikizumab, an anti-interleukin-1alpha and anti-interleukin-1beta dual variable domain immunoglobulin, in patients with erosive hand osteoarthritis. *Ann. Rheum. Dis.* **78**, 413–420 (2019).
37. Persson, M. S. M., Sarmanova, A., Doherty, M. & Zhang, W. Conventional and biologic disease-modifying anti-rheumatic drugs for osteoarthritis: a meta-analysis of randomized controlled trials. *Rheumatology* **57**, 1830–1837 (2018).
38. Richette, P. et al. Efficacy of tocilizumab in patients with hand osteoarthritis: double blind, randomised, placebo-controlled, multicentre trial. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-218547 (2020).
39. Sellam, J. & Berenbaum, F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nat. Rev. Rheumatol.* **6**, 625–635 (2010).
40. Chao, J. et al. Inflammatory characteristics on ultrasound predict poorer longterm response to intraarticular corticosteroid injections in knee osteoarthritis. *J. Rheumatol.* **37**, 650–655 (2010).
41. de Lange-Brokaar, B. J. et al. Degree of synovitis on MRI by comprehensive whole knee semi-quantitative scoring method correlates with histologic and macroscopic features of synovial tissue inflammation in knee osteoarthritis. *Osteoarthritis Cartilage* **22**, 1606–1613 (2014).
42. Loeuille, D. et al. Macroscopic and microscopic features of synovial membrane inflammation in the osteoarthritic knee: correlating magnetic resonance imaging findings with disease severity. *Arthritis Rheum.* **52**, 3492–3501 (2005).
43. af Klint, E. et al. Evaluation of arthroscopy and macroscopic scoring. *Arthritis Res. Ther.* **11**, R81 (2009).
44. Krenn, V. et al. Grading of chronic synovitis — a histopathological grading system for molecular and diagnostic pathology. *Pathol. Res. Pract.* **198**, 317–325 (2002).
45. Krenn, V. et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* **49**, 358–364 (2006).
46. Slansky, E. et al. Quantitative determination of the diagnostic accuracy of the synovitis score and its components. *Histopathology* **57**, 436–443 (2010).
47. Krenn, V. et al. 15 years of the histopathological synovitis score, further development and review: a diagnostic score for rheumatology and orthopaedics. *Pathol. Res. Pract.* **213**, 874–881 (2017).
48. Oehler, S., Neureiter, D., Meyer-Scholten, C. & Aigner, T. Subtyping of osteoarthritic synoviopathy. *Clin. Exp. Rheumatol.* **20**, 633–640 (2002).
49. Mathiessen, A. & Conaghan, P. G. Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis Res. Ther.* **19**, 18 (2017).
50. Mussawy, H. et al. The histopathological synovitis score is influenced by biopsy location in patients with knee osteoarthritis. *Arch. Orthop. Trauma. Surg.* https://doi.org/10.1007/s00402-021-03889-x (2021).
51. de Lange-Brokaar, B. J. et al. Association of pain in knee osteoarthritis with distinct patterns of synovitis. *Arthritis Rheumatol.* **67**, 733–740 (2015).
52. Nanus, D. E. et al. Synovial tissue from sites of joint pain in knee osteoarthritis patients exhibits a differential phenotype with distinct fibroblast subsets. *EBioMedicine* https://doi.org/10.1016/j.ebiom.2021.103618 (2021).
53. Wyatt, L. A. et al. Histopathological subgroups in knee osteoarthritis. *Osteoarthritis Cartilage* **25**, 14–22 (2017).
54. Benito, M. J., Veale, D. J., FitzGerald, O., van den Berg, W. B. & Bresnihan, B. Synovial tissue inflammation in early and late osteoarthritis. *Ann. Rheum. Dis.* **64**, 1263–1267 (2005).
55. Livshits, G. et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford Study. *Arthritis Rheum.* **60**, 2037–2045 (2009).
56. Stannus, O. 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* **18**, 1441–1447 (2010).
57. Labinsky, H. et al. Multiparameter analysis identifies heterogeneity in knee osteoarthritis synovial responses. *Arthritis Rheumatol.* **72**, 598–608 (2020).
58. Chou, C. H. et al. Synovial cell cross-talk with cartilage plays a major role in the pathogenesis of osteoarthritis. *Sci. Rep.* **10**, 10868 (2020).
59. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
60. Klein-Wieringa, I. R. et al. Inflammatory cells in patients with endstage knee osteoarthritis: a comparison between the synovium and the infrapatellar fat pad. *J. Rheumatol.* **43**, 771–778 (2016).
61. Wood, M. J. et al. Macrophage proliferation distinguishes 2 subgroups of knee osteoarthritis patients. *JCI Insight* **4**, e125325 (2019).
62. Watanabe, S., Alexander, M., Misharin, A. V. & Budinger, G. R. S. The role of macrophages in the resolution of inflammation. *J. Clin. Invest.* **129**, 2619–2628 (2019).
63. Gomez-Aristizabal, A., Gandhi, R., Mahomed, N. N., Marshall, K. W. & Viswanathan, S. Synovial fluid monocyte/macrophage subsets and their correlation to patient-reported outcomes in osteoarthritic patients: a cohort study. *Arthritis Res. Ther.* **21**, 26 (2019).
64. Kraus, V. B. et al. Direct in vivo evidence of activated macrophages in human osteoarthritis. *Osteoarthritis Cartilage* **24**, 1613–1621 (2016).
65. Raghu, H. et al. CCL2/CCR2, but not CCL5/CCR5, mediates monocyte recruitment, inflammation and cartilage destruction in osteoarthritis. *Ann. Rheum. Dis.* **76**, 914–922 (2017).
66. Bondeson, J. et al. The role of synovial macrophages and macrophage-produced mediators in driving inflammatory and destructive responses in osteoarthritis. *Arthritis Rheum.* **62**, 647–657 (2010).
67. Bondeson, J., Wainwright, S. D., Lauder, S., Amos, N. & Hughes, C. E. The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. *Arthritis Res. Ther.* **8**, R187 (2006).
68. Wu, C. L. et al. Conditional macrophage depletion increases inflammation and does not inhibit the development of osteoarthritis in obese macrophage fas-induced apoptosis-transgenic mice. *Arthritis Rheumatol.* **69**, 1772–1783 (2017).
69. Orecchioni, M., Ghosheh, Y., Pramod, A. B. & Ley, K. Macrophage polarization: different gene signatures in M1(LPS+) vs. classically and M2(LPS-) vs. alternatively activated macrophages. *Front. Immunol.* **10**, 1084 (2019).
70. Viola, A., Munari, F., Sanchez-Rodriguez, R., Scolaro, T. & Castegna, A. The metabolic signature of macrophage responses. *Front. Immunol.* **10**, 1462 (2019).
71. Daghestani, H. N., Pieper, C. F. & Kraus, V. B. Soluble macrophage biomarkers indicate inflammatory phenotypes in patients with knee osteoarthritis. *Arthritis Rheumatol.* **67**, 956–965 (2015).
72. Liu, B., Zhang, M., Zhao, J., Zheng, M. & Yang, H. Imbalance of M1/M2 macrophages is linked to severity level of knee osteoarthritis. *Exp. Ther. Med.* **16**, 5009–5014 (2018).

73. Manferdini, C. et al. From osteoarthritic synovium to synovial-derived cells characterization: synovial macrophages are key effector cells. *Arthritis Res. Ther.* **18**, 83 (2016).
74. Fahy, N. et al. Human osteoarthritic synovium impacts chondrogenic differentiation of mesenchymal stem cells via macrophage polarisation state. *Osteoarthritis Cartilage* **22**, 1167–1175 (2014).
75. Saito, I., Koshino, T., Nakashima, K., Uesugi, M. & Saito, T. Increased cellular infiltrate in inflammatory synovia of osteoarthritic knees. *Osteoarthritis Cartilage* **10**, 156–162 (2002).
76. Madsen, D. H. et al. M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway. *J. Cell Biol.* **202**, 951–966 (2013).
77. Dai, M., Sui, B., Xue, Y., Liu, X. & Sun, J. Cartilage repair in degenerative osteoarthritis mediated by squid type II collagen via immunomodulating activation of M2 macrophages, inhibiting apoptosis and hypertrophy of chondrocytes. *Biomaterials* **180**, 91–103 (2018).
78. Hoeksema, M. A. & Glass, C. K. Nature and nurture of tissue-specific macrophage phenotypes. *Atherosclerosis* **281**, 159–167 (2019).
79. Young, L. et al. Effects of intraarticular glucocorticoids on macrophage infiltration and mediators of joint damage in osteoarthritis synovial membranes: findings in a double-blind, placebo-controlled study. *Arthritis Rheum.* **44**, 343–350 (2001).
80. Siebelt, M. et al. Triamcinolone acetonide activates an anti-inflammatory and folate receptor-positive macrophage that prevents osteophytosis in vivo. *Arthritis Res. Ther.* **17**, 352 (2015).
81. Ha, C. W. et al. A multicenter, single-blind, phase IIa clinical trial to evaluate the efficacy and safety of a cell-mediated gene therapy in degenerative knee arthritis patients. *Hum. Gene Ther. Clin. Dev.* **26**, 125–130 (2015).
82. Cherian, J. J. et al. Preliminary results of a phase II randomized study to determine the efficacy and safety of genetically engineered allogeneic human chondrocytes expressing TGF-beta1 in patients with grade 3 chronic degenerative joint disease of the knee. *Osteoarthritis Cartilage* **23**, 2109–2118 (2015).
83. Ting, R. et al. Fast 18F labeling of a near-infrared fluorophore enables positron emission tomography and optical imaging of sentinel lymph nodes. *Bioconjug. Chem.* **21**, 1811–1819 (2010).
84. Yang, X. et al. Detection of osteoarthritis inflammation by single-photon emission computed tomography based on an inflammation-targeting peptide cFLFLF. *Mol. Imaging Biol.* **23**, 895–904 (2021).
85. Meester, E. J. et al. Imaging inflammation in atherosclerotic plaques, targeting SST_2 with [^{111}In] In-DOTA-JR11. *J. Nucl. Cardiol.* **28**, 2506–2513 (2021).
86. Temple-Wong, M. M. et al. Hyaluronan concentration and size distribution in human knee synovial fluid: variations with age and cartilage degeneration. *Arthritis Res. Ther.* **18**, 18 (2016).
87. Balazs, E. A. Viscosupplementation for treatment of osteoarthritis: from initial discovery to current status and results. *Surg. Technol. Int.* **12**, 278–289 (2004).
88. Ni, S. et al. The involvement of follistatin-like protein 1 in osteoarthritis by elevating NF-kappaB-mediated inflammatory cytokines and enhancing fibroblast like synoviocyte proliferation. *Arthritis Res. Ther.* **17**, 91 (2015).
89. Kapoor, M., Martel-Pelletier, J., Lajeunesse, D., Pelletier, J. P. & Fahmi, H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat. Rev. Rheumatol.* **7**, 33–42 (2011).
90. Mizoguchi, F. et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat. Commun.* **9**, 789 (2018).
91. Hsueh, M. F., Zhang, X., Wellman, S. S., Bolognesi, M. P. & Kraus, V. B. Synergistic roles of macrophages and neutrophils in osteoarthritis progression. *Arthritis Rheumatol.* **73**, 89–99 (2021).
92. Gupta, K., Shukla, M., Cowland, J. B., Malemud, C. J. & Haqqi, T. M. Neutrophil gelatinase-associated lipocalin is expressed in osteoarthritis and forms a complex with matrix metalloproteinase 9. *Arthritis Rheum.* **56**, 3326–3335 (2007).
93. de Lange-Brokaar, B. J. et al. Characterization of synovial mast cells in knee osteoarthritis: association with clinical parameters. *Osteoarthritis Cartilage* **24**, 664–671 (2016).
94. Wang, Q. et al. IgE-mediated mast cell activation promotes inflammation and cartilage destruction in osteoarthritis. *eLife* **8**, e39905 (2019).
95. Sousa-Valente, J. et al. Role of TrkA signalling and mast cells in the initiation of osteoarthritis pain in the monoiodoacetate model. *Osteoarthritis Cartilage* **26**, 84–94 (2018).
96. Enomoto, H. et al. Vascular endothelial growth factor isoforms and their receptors are expressed in human osteoarthritic cartilage. *Am. J. Pathol.* **162**, 171–181 (2003).
97. Kennedy, A. et al. Angiogenesis and blood vessel stability in inflammatory arthritis. *Arthritis Rheum.* **62**, 711–721 (2010).
98. Kim, H. R., Lee, J. H., Kim, K. W., Kim, B. M. & Lee, S. H. The relationship between synovial fluid VEGF and serum leptin with ultrasonographic findings in knee osteoarthritis. *Int. J. Rheum. Dis.* **19**, 233–240 (2016).
99. Nagao, M. et al. Vascular endothelial growth factor in cartilage development and osteoarthritis. *Sci. Rep.* **7**, 13027 (2017).
100. Li, Y. S., Luo, W., Zhu, S. A. & Lei, G. H. T cells in osteoarthritis: alterations and beyond. *Front. Immunol.* **8**, 356 (2017).
101. Rosshirt, N. et al. Proinflammatory T cell polarization is already present in patients with early knee osteoarthritis. *Arthritis Res. Ther.* **23**, 37 (2021).
102. Nees, T. A. et al. T helper cell infiltration in osteoarthritis-related knee pain and disability. *J. Clin. Med.* **9**, 2423 (2020).
103. Thomas, A. C., Hubbard-Turner, T., Wikstrom, E. A. & Palmieri-Smith, R. M. Epidemiology of posttraumatic osteoarthritis. *J. Athl. Train.* **52**, 491–496 (2017).
104. Whittaker, J. L. et al. Association between MRI-defined osteoarthritis, pain, function and strength 3–10 years following knee joint injury in youth sport. *Br. J. Sports Med.* **52**, 934–939 (2018).
105. Scanzello, C. R. et al. Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. *Arthritis Rheum.* **63**, 391–400 (2011).
106. Pessler, F. et al. The synovitis of "non-inflammatory" orthopaedic arthropathies: a quantitative histological and immunohistochemical analysis. *Ann. Rheum. Dis.* **67**, 1184–1187 (2008).
107. Lieberthal, J., Sambamurthy, N. & Scanzello, C. R. Inflammation in joint injury and post-traumatic osteoarthritis. *Osteoarthritis Cartilage* **23**, 1825–1834 (2015).
108. Davis, J. E. et al. A single recent injury is a potent risk factor for the development of accelerated knee osteoarthritis: data from the osteoarthritis initiative. *Rheumatol. Int.* **37**, 1759–1764 (2017).
109. Driban, J. B. et al. Association of knee injuries with accelerated knee osteoarthritis progression: data from the Osteoarthritis Initiative. *Arthritis Care Res.* **66**, 1673–1679 (2014).
110. Gelber, A. C. et al. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. *Ann. Intern. Med.* **133**, 321–328 (2000).
111. Sward, P., Frobell, R., Englund, M., Roos, H. & Struglics, A. Cartilage and bone markers and inflammatory cytokines are increased in synovial fluid in the acute phase of knee injury (hemarthrosis) — a cross-sectional analysis. *Osteoarthritis Cartilage* **20**, 1302–1308 (2012).
112. Pham, T. M., Erichsen, J. L., Kowal, J. M., Overgaard, S. & Schmal, H. Elevation of pro-inflammatory cytokine levels following intra-articular fractures-a systematic review. *Cells* **10**, 902 (2021).
113. Pham, T. M., Frich, L. H., Lambertsen, K. L., Overgaard, S. & Schmal, H. Elevation of inflammatory cytokines and proteins after intra-articular ankle fracture: a cross-sectional study of 47 ankle fracture patients. *Mediators Inflamm.* **2021**, 8897440 (2021).
114. Jacobs, C. A. et al. Dysregulated inflammatory response related to cartilage degradation after ACL injury. *Med. Sci. Sports Exerc.* **52**, 535–541 (2020).
115. Adams, S. B. et al. Inflammatory microenvironment persists after bone healing in intra-articular ankle fractures. *Foot Ankle Int.* **38**, 479–484 (2017).
116. Roemer, F. W. 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.* **71**, 238–243 (2019).
117. Griffin, T. M. & Guilak, F. The role of mechanical loading in the onset and progression of osteoarthritis. *Exerc. Sport. Sci. Rev.* **33**, 195–200 (2005).
118. Fang, T., Zhou, X., Jin, M., Nie, J. & Li, X. Molecular mechanisms of mechanical load-induced osteoarthritis. *Int. Orthop.* **45**, 1125–1136 (2021).
119. Nazet, U. et al. Housekeeping gene validation for RT-qPCR studies on synovial fibroblasts derived from healthy and osteoarthritic patients with focus on mechanical loading. *PLoS One* **14**, e0225790 (2019).
120. Fahy, N., Menzel, U., Alini, M. & Stoddart, M. J. Shear and dynamic compression modulates the inflammatory phenotype of human monocytes in vitro. *Front. Immunol.* **10**, 383 (2019).
121. Ruffino, J. S. et al. Moderate-intensity exercise alters markers of alternative activation in circulating monocytes in females: a putative role for PPARgamma. *Eur. J. Appl. Physiol.* **116**, 1671–1682 (2016).
122. Helmark, I. C. et al. Exercise increases interleukin-10 levels both intraarticularly and peri-synovially in patients with knee osteoarthritis: a randomized controlled trial. *Arthritis Res. Ther.* **12**, R126 (2010).
123. Boonstra, A. et al. Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88- and TRIF-dependent TLR signals, and TLR-independent signals. *J. Immunol.* **177**, 7551–7558 (2006).
124. Yoshimura, N. et al. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthritis Cartilage* **20**, 1217–1226 (2012).
125. Batushansky, A. et al. Fundamentals of OA. An initiative of osteoarthritis and cartilage. Chapter 9: obesity and metabolic factors in OA. *Osteoarthritis Cartilage* https://doi.org/10.1016/j.joca.2021.06.013 (2021).
126. Prieto-Alhambra, D. et al. Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. *Ann. Rheum. Dis.* **73**, 1659–1664 (2014).
127. Losina, E. et al. Lifetime risk and age at diagnosis of symptomatic knee osteoarthritis in the US. *Arthritis Care Res.* **65**, 703–711 (2013).
128. Murphy, L. B. et al. One in four people may develop symptomatic hip osteoarthritis in his or her lifetime. *Osteoarthritis Cartilage* **18**, 1372–1379 (2010).
129. Berenbaum, F., Wallace, I. J., Lieberman, D. E. & Felson, D. T. Modern-day environmental factors in the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol.* **14**, 674–681 (2018).
130. Harasymowicz, N. S. et al. Regional differences between perisynovial and infrapatellar adipose tissue depots and their response to Class II and Class III obesity in patients with osteoarthritis. *Arthritis Rheumatol.* **69**, 1396–1406 (2017).
131. Duan, L. et al. Infrapatellar fat pads participate in the development of knee osteoarthritis in obese patients via the activation of the NFkappaB signaling pathway. *Int. J. Mol. Med.* **46**, 2260–2270 (2020).
132. Takata, K. et al. Increase in tryptase and its role in the synovial membrane of overweight and obese patients with osteoarthritis of the knee. *Diabetes Metab. Syndr. Obes.* **13**, 1491–1497 (2020).
133. Sun, A. R. et al. Obesity-associated metabolic syndrome spontaneously induces infiltration of pro-inflammatory macrophage in synovium and promotes osteoarthritis. *PLoS One* **12**, e0183693 (2017).
134. Larranaga-Vera, A. et al. Increased synovial lipodystrophy induced by high fat diet aggravates synovitis in experimental osteoarthritis. *Arthritis Res. Ther.* **19**, 264 (2017).
135. Yusuf, E. et al. Association between weight or body mass index and hand osteoarthritis: a systematic review. *Ann. Rheum. Dis.* **69**, 761–765 (2010).
136. Reyes, C. et al. Association between overweight and obesity and risk of clinically diagnosed knee, hip, and hand osteoarthritis: a population-based cohort study. *Arthritis Rheumatol.* **68**, 1869–1875 (2016).
137. Yang, W. H. et al. Leptin induces IL-6 expression through OBRI receptor signaling pathway in human synovial fibroblasts. *PLoS One* **8**, e75551 (2013).
138. Jafarzadeh, S. R. et al. Mediating role of bone marrow lesions, synovitis, pain sensitization, and depressive symptoms on knee pain improvement following substantial weight loss. *Arthritis Rheumatol.* **72**, 420–427 (2020).
139. Gudbergsen, H. et al. Changes in bone marrow lesions in response to weight-loss in obese knee osteoarthritis patients: a prospective cohort study. *BMC Musculoskelet. Disord.* **14**, 106 (2013).
140. Henriksen, M. et al. Structural changes in the knee during weight loss maintenance after a significant weight loss in obese patients with osteoarthritis: a report of secondary outcome analyses from a randomized controlled trial. *Osteoarthritis Cartilage* **22**, 639–646 (2014).

141. Daugaard, C. L. et al. The impact of a significant weight loss on inflammation assessed on DCE-MRI and static MRI in knee osteoarthritis: a prospective cohort study. *Osteoarthritis Cartilage* **28**, 766–773 (2020).
142. Piva, S. R. et al. Links between osteoarthritis and diabetes: implications for management from a physical activity perspective. *Clin. Geriatr. Med.* **31**, 67–87 (2015).
143. Schett, G. et al. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care* **36**, 403–409 (2013).
144. Louati, K., Vidal, C., Berenbaum, F. & Sellam, J. Association between diabetes mellitus and osteoarthritis: systematic literature review and meta-analysis. *RMD Open* **1**, e000077 (2015).
145. Niu, J., Clancy, M., Aliabadi, P., Vasan, R. & Felson, D. T. Metabolic syndrome, its components, and knee osteoarthritis: the Framingham Osteoarthritis Study. *Arthritis Rheumatol.* **69**, 1194–1203 (2017).
146. Monira Hussain, S. et al. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin. Arthritis Rheum.* **43**, 429–436 (2014).
147. Kuusalo, L. et al. Metabolic osteoarthritis — relation of diabetes and cardiovascular disease with knee osteoarthritis. *Osteoarthritis Cartilage* **29**, 230–234 (2021).
148. Rogers-Soeder, T. S. et al. Association of diabetes mellitus and biomarkers of abnormal glucose metabolism with incident radiographic knee osteoarthritis. *Arthritis Care Res.* **72**, 98–106 (2020).
149. Hamada, D. et al. Suppressive effects of insulin on tumor necrosis factor-dependent early osteoarthritic changes associated with obesity and type 2 diabetes mellitus. *Arthritis Rheumatol.* **68**, 1392–1402 (2016).
150. Griffin, T. M. & Huffman, K. M. Editorial: insulin resistance: releasing the brakes on synovial inflammation and osteoarthritis? *Arthritis Rheumatol.* **68**, 1330–1333 (2016).
151. Tsai, C. H. et al. High glucose induces vascular endothelial growth factor production in human synovial fibroblasts through reactive oxygen species generation. *Biochim. Biophys. Acta* **1830**, 2649–2658 (2013).
152. Steenvoorden, M. M. et al. Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes. *Arthritis Rheum.* **54**, 253–263 (2006).
153. Veronese, N. et al. The relationship between the dietary inflammatory index and prevalence of radiographic symptomatic osteoarthritis: data from the Osteoarthritis Initiative. *Eur. J. Nutr.* **58**, 253–260 (2019).
154. Wang, X. et al. Knee effusion-synovitis volume measurement and effects of vitamin D supplementation in patients with knee osteoarthritis. *Osteoarthritis Cartilage* **25**, 1304–1312 (2017).
155. Perry, T. A. et al. Effect of Vitamin D supplementation on synovial tissue volume and subchondral bone marrow lesion volume in symptomatic knee osteoarthritis. *BMC Musculoskelet. Disord.* **20**, 76 (2019).
156. Wang, Z. et al. Effectiveness of curcuma longa extract for the treatment of symptoms and effusion-synovitis of knee osteoarthritis: a randomized trial. *Ann. Intern. Med.* **173**, 861–869 (2020).
157. Veronese, N. et al. Mediterranean diet and knee osteoarthritis outcomes: a longitudinal cohort study. *Clin. Nutr.* **38**, 2735–2739 (2019).
158. Xu, C. et al. Dietary patterns and progression of knee osteoarthritis: data from the osteoarthritis initiative. *Am. J. Clin. Nutr.* **111**, 667–676 (2020).
159. Ulici, V. et al. Osteoarthritis induced by destabilization of the medial meniscus is reduced in germ-free mice. *Osteoarthritis Cartilage* **26**, 1098–1109 (2018).
160. Dunn, C. M. et al. Identification of cartilage microbial DNA signatures and associations with knee and hip osteoarthritis. *Arthritis Rheumatol.* **72**, 1111–1122 (2020).
161. Zhao, Y. et al. Detection and characterization of bacterial nucleic acids in culture-negative synovial tissue and fluid samples from rheumatoid arthritis or osteoarthritis patients. *Sci. Rep.* **8**, 14305 (2018).
162. Schott, E. M. et al. Targeting the gut microbiome to treat the osteoarthritis of obesity. *JCI Insight* **3**, e95997 (2018).
163. Huang, Z. Y., Stabler, T., Pei, F. X. & Kraus, V. B. Both systemic and local lipopolysaccharide (LPS) burden are associated with knee OA severity and inflammation. *Osteoarthritis Cartilage* **24**, 1769–1775 (2016).
164. Mohammad, S. & Thiemermann, C. Role of metabolic endotoxemia in systemic inflammation and potential interventions. *Front. Immunol.* **11**, 594150 (2020).
165. Fuke, N., Nagata, N., Suganuma, H. & Ota, T. Regulation of gut microbiota and metabolic endotoxemia with dietary factors. *Nutrients* **11**, 2277 (2019).
166. Boer, C. G. et al. Intestinal microbiome composition and its relation to joint pain and inflammation. *Nat. Commun.* **10**, 4881 (2019).
167. Mao, X., Fu, P., Wang, L. & Xiang, C. Mitochondria: potential targets for osteoarthritis. *Front. Med.* **7**, 581402 (2020).
168. Geurts, J. et al. Prematurely aging mitochondrial DNA mutator mice display subchondral osteopenia and chondrocyte hypertrophy without further osteoarthritis features. *Sci. Rep.* **10**, 1296 (2020).
169. Torroni, A. et al. Classification of European mtDNAs from an analysis of three European populations. *Genetics* **144**, 1835–1850 (1996).
170. Da Sylva, T. R., Connor, A., Mburu, Y., Keystone, E. & Wu, G. E. Somatic mutations in the mitochondria of rheumatoid arthritis synoviocytes. *Arthritis Res. Ther.* **7**, R844–R851 (2005).
171. Van den Bossche, J. et al. mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Rep.* **17**, 684–696 (2016).
172. Jeon, O. H. et al. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat. Med.* **23**, 775–781 (2017).
173. Yohn, C. O. B., et al. Senescent synoviocytes in knee osteoarthritis correlate with disease biomarkers, synovitis, and knee pain [abstract]. *Arthritis Rheumatol*. https://acrabstracts.org/abstract/senescent-synoviocytes-in-knee-osteoarthritis-correlate-with-disease-biomarkers-synovitis-and-knee-pain/ (2019).
174. Anderson, J. R. et al. ^{1}H NMR metabolomics identifies underlying inflammatory pathology in osteoarthritis and rheumatoid arthritis synovial joints. *J. Proteome Res.* **17**, 3780–3790 (2018).
175. Farrell, A. J., Blake, D. R., Palmer, R. M. & Moncada, S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Ann. Rheum. Dis.* **51**, 1219–1222 (1992).
176. Melchiorri, C. et al. Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. *Arthritis Rheum.* **41**, 2165–2174 (1998).
177. Abramson, S. B. Nitric oxide in inflammation and pain associated with osteoarthritis. *Arthritis Res. Ther.* **10**, S2 (2008).
178. Pelletier, J. P. et al. Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. *J. Rheumatol.* **26**, 2002–2014 (1999).
179. Hellio le Graverand, M. P. et al. A 2-year randomised, double-blind, placebo-controlled, multicentre study of oral selective iNOS inhibitor, cindunistat (SD-6010), in patients with symptomatic osteoarthritis of the knee. *Ann. Rheum. Dis.* **72**, 187–195 (2013).
180. Tannahill, G. M. et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* **496**, 238–242 (2013).
181. Keiran, N. et al. SUCNR1 controls an anti-inflammatory program in macrophages to regulate the metabolic response to obesity. *Nat. Immunol.* **20**, 581–592 (2019).
182. Li, Y. et al. Succinate induces synovial angiogenesis in rheumatoid arthritis through metabolic remodeling and HIF-1alpha/VEGF axis. *Free Radic. Biol. Med.* **126**, 1–14 (2018).
183. Wojdasiewicz, P., Poniatowski, L. A. & Szukiewicz, D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm.* **2014**, 561459 (2014).
184. Futani, H. et al. Relation between interleukin-18 and PGE2 in synovial fluid of osteoarthritis: a potential therapeutic target of cartilage degradation. *J. Immunother.* **25**, S61–S64 (2002).
185. Attur, M. et al. Prostaglandin E2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the EP4 receptor. *J. Immunol.* **181**, 5082–5088 (2008).
186. Valdes, A. M. et al. Omega-6 oxylipins generated by soluble epoxide hydrolase are associated with knee osteoarthritis. *J. Lipid Res.* **59**, 1763–1770 (2018).
187. Lambert, C. et al. The damage-associated molecular patterns (DAMPs) as potential targets to treat osteoarthritis: perspectives from a review of the literature. *Front. Med.* **7**, 607186 (2020).
188. Millerand, M., Berenbaum, F. & Jacques, C. Danger signals and inflammaging in osteoarthritis. *Clin. Exp. Rheumatol.* **37**, 48–56 (2019).
189. Heinola, T. et al. High mobility group box-1 (HMGB-1) in osteoarthritic cartilage. *Clin. Exp. Rheumatol.* **28**, 511–518 (2010).
190. Li, Z. C. et al. Correlation of synovial fluid HMGB-1 levels with radiographic severity of knee osteoarthritis. *Clin. Invest. Med.* **34**, E298 (2011).
191. Ke, X. et al. Synovial fluid HMGB-1 levels are associated with osteoarthritis severity. *Clin. Lab.* **61**, 809–818 (2015).
192. Aulin, C., Lassacher, T., Palmblad, K. & Erlandsson Harris, H. Early stage blockade of the alarmin HMGB1 reduces cartilage destruction in experimental OA. *Osteoarthritis Cartilage* **28**, 698–707 (2020).
193. Lambrecht, S., Juchtmans, N. & Elewaut, D. Heat-shock proteins in stromal joint tissues: innocent bystanders or disease-initiating proteins? *Rheumatology* **53**, 223–232 (2014).
194. Takahashi, K. et al. Localization of heat shock protein in osteoarthritic cartilage. *Scand. J. Rheumatol.* **26**, 368–375 (1997).
195. Ngarmukos, S., Scaramuzza, S., Theerawattanapong, N., Tanavalee, A. & Honsawek, S. Circulating and synovial fluid heat shock protein 70 are correlated with severity in knee osteoarthritis. *Cartilage* **11**, 323–328 (2020).
196. Son, Y. O., Kim, H. E., Choi, W. S., Chun, C. H. & Chun, J. S. RNA-binding protein ZFP36L1 regulates osteoarthritis by modulating members of the heat shock protein 70 family. *Nat. Commun.* **10**, 77 (2019).
197. Lambert, C. et al. Gene expression pattern of cells from inflamed and normal areas of osteoarthritis synovial membrane. *Arthritis Rheumatol.* **66**, 960–968 (2014).
198. Schelbergen, R. F. et al. Alarmins S100A8/S100A9 aggravate osteophyte formation in experimental osteoarthritis and predict osteophyte progression in early human symptomatic osteoarthritis. *Ann. Rheum. Dis.* **75**, 218–225 (2016).
199. Ruan, G. et al. Associations between serum S100A8/S100A9 and knee symptoms, joint structures and cartilage enzymes in patients with knee osteoarthritis. *Osteoarthritis Cartilage* **27**, 99–105 (2019).
200. Addimanda, O. et al. Elevated serum levels of alarmin S100A8/A9 in patients with hand osteoarthritis. *Clin. Exp. Rheumatol.* **37**, 885 (2019).
201. Daheshia, M. & Yao, J. Q. The interleukin 1beta pathway in the pathogenesis of osteoarthritis. *J. Rheumatol.* **35**, 2306–2312 (2008).
202. Inoue, H. et al. High levels of serum IL-18 promote cartilage loss through suppression of aggrecan synthesis. *Bone* **42**, 1102–1110 (2008).
203. McAllister, M. J., Chemaly, M., Eakin, A. J., Gibson, D. S. & McGilligan, V. E. NLRP3 as a potentially novel biomarker for the management of osteoarthritis. *Osteoarthritis Cartilage* **26**, 612–619 (2018).
204. Schroder, K., Zhou, R. & Tschopp, J. The NLRP3 inflammasome: a sensor for metabolic danger? *Science* **327**, 296–300 (2010).
205. Zhong, Z. et al. NF-kappaB restricts inflammasome activation via elimination of damaged mitochondria. *Cell* **164**, 896–910 (2016).
206. Sanchez-Lopez, E. et al. Choline uptake and metabolism modulate macrophage IL-1beta and IL-18 production. *Cell Metab.* **29**, 1350–1362.e7 (2019).
207. Zhong, Z. et al. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature* **560**, 198–203 (2018).
208. Jin, C. et al. NLRP3 inflammasome plays a critical role in the pathogenesis of hydroxyapatite-associated arthropathy. *Proc. Natl Acad. Sci. USA* **108**, 14867–14872 (2011).
209. Martinon, F., Petrilli, V., Mayor, A., Tardivel, A. & Tschopp, J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **440**, 237–241 (2006).
210. Denoble, A. E. et al. Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation. *Proc. Natl Acad. Sci. USA* **108**, 2088–2093 (2011).
211. Elsaid, K. A. et al. The impact of early intra-articular administration of interleukin-1 receptor antagonist on lubricin metabolism and cartilage degeneration in an anterior cruciate ligament transection model. *Osteoarthritis Cartilage* **23**, 114–121 (2015).

212. Schieker, M. et al. Effects of interleukin-1beta inhibition on incident hip and knee replacement: exploratory analyses from a randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* **173**, 509–515 (2020).
213. Lane, N. & Felson, D. A promising treatment for osteoarthritis? *Ann. Intern. Med.* **173**, 580–581 (2020).
214. Chevalier, X. et al. Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum.* **61**, 344–352 (2009).
215. Fu, Z. et al. Interleukin-18-induced inflammatory responses in synoviocytes and chondrocytes from osteoarthritic patients. *Int. J. Mol. Med.* **30**, 805–810 (2012).
216. Shi, J., Gao, W. & Shao, F. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem. Sci.* **42**, 245–254 (2017).
217. Wang, Q. et al. Identification of a central role for complement in osteoarthritis. *Nat. Med.* **17**, 1674–1679 (2011).
218. Assirelli, E. et al. Complement expression and activation in osteoarthritis joint compartments. *Front. Immunol.* **11**, 535010 (2020).
219. Li, Z., Huang, Z. & Bai, L. Cell interplay in osteoarthritis. *Front. Cell Dev. Biol.* **9**, 720477 (2021).
220. Dreier, R., Wallace, S., Fuchs, S., Bruckner, P. & Grassel, S. Paracrine interactions of chondrocytes and macrophages in cartilage degradation: articular chondrocytes provide factors that activate macrophage-derived pro-gelatinase B (pro-MMP-9). *J. Cell Sci.* **114**, 3813–3822 (2001).
221. Hamasaki, M. et al. Transcriptional profiling of murine macrophages stimulated with cartilage fragments revealed a strategy for treatment of progressive osteoarthritis. *Sci. Rep.* **10**, 7558 (2020).
222. Silverstein, A. M. et al. Toward understanding the role of cartilage particulates in synovial inflammation. *Osteoarthritis Cartilage* **25**, 1353–1361 (2017).
223. Roemer, F. W. et al. What comes first? Multitissue involvement leading to radiographic osteoarthritis: magnetic resonance imaging-based trajectory analysis over four years in the osteoarthritis initiative. *Arthritis Rheumatol.* **67**, 2085–2096 (2015).
224. Hugle, T. & Geurts, J. What drives osteoarthritis?-synovial versus subchondral bone pathology. *Rheumatology* **56**, 1461–1471 (2017).
225. Hu, W., Chen, Y., Dou, C. & Dong, S. Microenvironment in subchondral bone: predominant regulator for the treatment of osteoarthritis. *Ann. Rheum. Dis.* **80**, 413–422 (2020).
226. Yang, C. C., Lin, C. Y., Wang, H. S. & Lyu, S. R. Matrix metalloproteases and tissue inhibitors of metalloproteinases in medial plica and pannus-like tissue contribute to knee osteoarthritis progression. *PLoS One* **8**, e79662 (2013).
227. Furuzawa-Carballeda, J., Macip-Rodriguez, P. M. & Cabral, A. R. Osteoarthritis and rheumatoid arthritis pannus have similar qualitative metabolic characteristics and pro-inflammatory cytokine response. *Clin. Exp. Rheumatol.* **26**, 554–560 (2008).
228. Yusup, A. et al. Bone marrow lesions, subchondral bone cysts and subchondral bone attrition are associated with histological synovitis in patients with end-stage knee osteoarthritis: a cross-sectional study. *Osteoarthritis Cartilage* **23**, 1858–1864 (2015).
229. Aso, K. et al. Associations of symptomatic knee osteoarthritis with histopathologic features in subchondral bone. *Arthritis Rheumatol.* **71**, 916–924 (2019).
230. Arepati, A. I. et al. Osteophyte formation is associated with synovitis in osteoarthritis — the Bunkyo Health Study. *Osteoarthritis Cartilage* **28**, S86–S527 (2020).
231. Blom, A. B. et al. Synovial lining macrophages mediate osteophyte formation during experimental osteoarthritis. *Osteoarthritis Cartilage* **12**, 627–635 (2004).
232. van Lent, P. L. et al. Crucial role of synovial lining macrophages in the promotion of transforming growth factor beta-mediated osteophyte formation. *Arthritis Rheum.* **50**, 103–111 (2004).
233. Blaney Davidson, E. N. et al. Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. *Arthritis Rheum.* **56**, 4065–4073 (2007).
234. Remst, D. F., Blaney Davidson, E. N. & van der Kraan, P. M. Unravelling osteoarthritis-related synovial fibrosis: a step closer to solving joint stiffness. *Rheumatology* **54**, 1954–1963 (2015).
235. van Helvoort, E. M., Eijkelkamp, N., Lafeber, F. & Mastbergen, S. C. Expression of granulocyte macrophage-colony stimulating factor and its receptor in the synovium of osteoarthritis patients is negatively correlated with pain. *Rheumatology* **59**, 3452–3457 (2020).
236. Wise, B. L., Seidel, M. F. & Lane, N. E. The evolution of nerve growth factor inhibition in clinical medicine. *Nat. Rev. Rheumatol.* **17**, 34–46 (2021).
237. Stoppiello, L. A. et al. Structural associations of symptomatic knee osteoarthritis. *Arthritis Rheumatol.* **66**, 3018–3027 (2014).
238. Cohen, S. B. et al. A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to IL-1R1) in patients with osteoarthritis of the knee. *Arthritis Res. Ther.* **13**, R125 (2011).
239. Vincent, T. L. Peripheral pain mechanisms in osteoarthritis. *Pain* **161**, S138–S146 (2020).
240. Bratus-Neuenschwander, A. et al. Pain-associated transcriptome changes in synovium of knee osteoarthritis patients. *Genes (Basel)* **9**, 338 (2018).
241. Attard, V. et al. Quantification of intra-articular fibrosis in patients with stiff knee arthroplasties using metal-reduction MRI. *Bone Jt. J.* **102-B**, 1331–1340 (2020).
242. Kalson, N. S. et al. International consensus on the definition and classification of fibrosis of the knee joint. *Bone Jt. J.* **98-B**, 1479–1488 (2016).
243. Toms, J. et al. Targeting fibroblast activation protein: radiosynthesis and preclinical evaluation of an ^{18}F-labeled FAP inhibitor. *J. Nucl. Med.* **61**, 1806–1813 (2020).
244. Kerna, I. et al. The ADAM12 is upregulated in synovitis and postinflammatory fibrosis of the synovial membrane in patients with early radiographic osteoarthritis. *Jt. Bone Spine* **81**, 51–56 (2014).
245. Remst, D. F. et al. Gene expression analysis of murine and human osteoarthritis synovium reveals elevation of transforming growth factor beta-responsive genes in osteoarthritis-related fibrosis. *Arthritis Rheumatol.* **66**, 647–656 (2014).
246. Rim, Y. A. & Ju, J. H. The role of fibrosis in osteoarthritis progression. *Life (Basel)* **11**, 3 (2020).
247. Bastiaansen-Jenniskens, Y. M. 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.* **65**, 2070–2080 (2013).
248. Eymard, F. 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.* **76**, 1142–1148 (2017).
249. Davis, J. E. et al. Effusion-synovitis and infrapatellar fat pad signal intensity alteration differentiate accelerated knee osteoarthritis. *Rheumatology* **58**, 418–426 (2019).
250. Inomata, K. 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.* **20**, 8 (2019).
251. Barboza, E. et al. Profibrotic infrapatellar fat pad remodeling without M1 macrophage polarization precedes knee osteoarthritis in mice with diet-induced obesity. *Arthritis Rheumatol.* **69**, 1221–1232 (2017).
252. de Jong, A. J. et al. Lack of high BMI-related features in adipocytes and inflammatory cells in the infrapatellar fat pad (IFP). *Arthritis Res. Ther.* **19**, 186 (2017).
253. Warmink, K. 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* **28**, 593–602 (2020).
254. Takano, S. et al. Transforming growth factor-beta stimulates nerve growth factor production in osteoarthritic synovium. *BMC Musculoskelet. Disord.* **20**, 204 (2019).
255. Blaney Davidson, E. N. et al. TGF-beta is a potent inducer of nerve growth factor in articular cartilage via the ALK5-Smad2/3 pathway. Potential role in OA related pain? *Osteoarthritis Cartilage* **3**, 478–486 (2015).
256. Grässel, S. & Muschter, D. Recent advances in the treatment of osteoarthritis. *F1000Res.* **9**, F1000 (2020).
257. Vincent, T. L. Of mice and men: converging on a common molecular understanding of osteoarthritis. *Lancet Rheumatol.* **2**, e633–e645 (2020).
258. Wang, X., Hunter, D. J., Jin, X. & Ding, C. The importance of synovial inflammation in osteoarthritis: current evidence from imaging assessments and clinical trials. *Osteoarthritis Cartilage* **26**, 165–174 (2018).
259. Roemer, F. W. et al. Anatomical distribution of synovitis in knee osteoarthritis and its association with joint effusion assessed on non-enhanced and contrast-enhanced MRI. *Osteoarthritis Cartilage* **18**, 1269–1274 (2010).
260. Shakoor, D. et al. Are contrast-enhanced and non-contrast MRI findings reflecting synovial inflammation in knee osteoarthritis: a meta-analysis of observational studies. *Osteoarthritis Cartilage* **28**, 126–136 (2020).
261. Takase, K. et al. Simultaneous evaluation of long-lasting knee synovitis in patients undergoing arthroplasty by power Doppler ultrasonography and contrast-enhanced MRI in comparison with histopathology. *Clin. Exp. Rheumatol.* **30**, 85–92 (2012).
262. Walther, M. et al. Correlation of power Doppler sonography with vascularity of the synovial tissue of the knee joint in patients with osteoarthritis and rheumatoid arthritis. *Arthritis Rheum.* **44**, 331–338 (2001).
263. Tarhan, S. & Unlu, Z. Magnetic resonance imaging and ultrasonographic evaluation of the patients with knee osteoarthritis: a comparative study. *Clin. Rheumatol.* **22**, 181–188 (2003).

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Skin involvement in early diffuse cutaneous systemic sclerosis: an unmet clinical need

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Abstract | Diffuse cutaneous systemic sclerosis (dcSSc) is associated with high mortality resulting from early internal-organ involvement. Clinicians therefore tend to focus on early diagnosis and treatment of potentially life-threatening cardiorespiratory and renal disease. However, the rapidly progressive painful, itchy skin tightening that characterizes dcSSc is the symptom that has the greatest effect on patients' quality of life, and there is currently no effective disease-modifying treatment for it. Considerable advances have been made in predicting the extent and rate of skin-disease progression (which vary between patients), including the development of techniques such as molecular analysis of skin biopsy samples. Risk stratification for progressive skin disease is especially relevant now that haematopoietic stem-cell transplantation is a treatment option, because stratification will inform the balance of risk versus benefit for each patient. Measurement of skin disease is a major challenge. Results from clinical trials have highlighted limitations of the modified Rodnan skin score (the current gold standard). Alternative patient-reported and other potential outcome measures have been and are being developed. Patients with early dcSSc should be referred to specialist centres to ensure best-practice management, including the management of their skin disease, and to maximize opportunities for inclusion in clinical trials.

Contractures
Deformities resulting from tissue shortening or hardening; in patients with SSc contracture is caused by tightening of the skin.

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Systemic sclerosis (SSc) is a multisystem connective-tissue disease characterized by fibrosis, and by vascular and immunological abnormalities. The two main subtypes of SSc, defined according to the extent of skin involvement (scleroderma, meaning 'hard skin'), are diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc[1]. dcSSc is the subtype of greater concern, because it is characterized by rapid progression and a high prevalence of early internal-organ involvement (including lung, heart and kidney), which can be life-threatening. dcSSc is therefore associated with high mortality[2–4], with a 5-year survival rate of around 70%, and clinicians understandably tend to focus their attention on early identification and treatment of internal-organ disease. However, on a day-to-day basis, in patients with early dcSSc (those within the first 3–5 years of the onset of symptoms), it is skin thickening that has the greatest impact on quality of life, causing pain, intractable itching and functional limitation.

Skin involvement in early dcSSc is an important topic, not only because of the effects of skin disease on the patient, but also because the skin is a very visible and accessible 'window' into the dcSSc disease process. Therefore, examining the skin enables the prediction and monitoring of disease progression and of treatment response. A Review of this topic is timely because of developments over the past 5 years in benchmarking of the burden of skin disease in patients with dcSSc and in understanding of how to identify 'progressors' (patients with progressive disease), not only on the basis of clinical features, but also through advances in molecular technologies applied to skin biopsy samples. In addition, controversies exist with regard to how best to measure the extent and consequences of skin disease, as highlighted by results from clinical trials, and there is an ongoing need to promote best-practice management of skin disease, as well as of internal-organ disease.

The aim of this Review is to provide a comprehensive description of the clinical and scientific implications of skin involvement in dcSSc. First, we describe skin involvement, patterns of progression and the associated clinical burden, including contractures and ulceration. Second, we outline how skin-disease progression can be predicted by consideration of clinical features (including disease duration, extent of skin disease and autoantibody status) and potentially by gene-expression profiling of biopsied skin. Identifying progressors is especially relevant now that autologous haematopoietic stem-cell transplantation (HSCT) is an option for patients at high risk of progression, so that only those patients most in

Key points

- Much of the pain and disability of early diffuse cutaneous systemic sclerosis (dcSSc) results from skin thickening (scleroderma), which can be rapidly progressive, commencing distally then extending proximally.
- 'Progressors' in terms of skin disease can now be identified by considering disease duration, extent of skin disease, autoantibody status and (potentially) gene-expression profiling of skin biopsy specimens.
- Improvement in the ability to predict progressive skin disease will inform the selection of patients for haematopoietic stem-cell transplantation, as well as more targeted inclusion of patients in clinical trials.
- Limitations of the modified Rodnan skin score are stimulating development of other outcome measures of skin disease, including patient-reported outcome measures, non-invasive imaging methods and composite scores.
- Best-practice management of early dcSSc includes early referral to a specialist centre, pain management, multidisciplinary input, immunosuppressive therapy and, when at all possible, inclusion in a clinical trial.

need are exposed to the potential toxicity (and even lethality) of HSCT. Third, we discuss outcome measures of skin disease, specifically the modified Rodnan skin score (mRSS), but also patient-reported outcome measures and non-invasive imaging techniques. Fourth, we describe best-practice management, including general measures, immunosuppressant treatment and HSCT, and discuss the controversial topic of whether or not glucocorticoids should be prescribed. We do not discuss recent, ongoing or proposed studies of new targeted therapies (including biologic agents such as tocilizumab and rituximab), as these have been reviewed elsewhere[5]. However, the information we present reinforces that patients with early dcSSc should, whenever possible, be recruited into clinical trials, to maximize the chances of identifying an effective disease-modifying therapy for this currently incurable disease.

Clinical features and disease burden

Clinical features

In patients with early dcSSc, skin involvement commences distally, usually first affecting the fingers, which often become swollen and painful. This early oedematous phase is sometimes misdiagnosed as inflammatory arthritis and can be associated with carpal tunnel syndrome, but over a few weeks the skin hardens and the diagnosis of SSc usually becomes obvious. A defining feature of the dcSSc subtype is the (often rapid) progression of skin involvement to proximal to the elbow or knee and/or involving the trunk. Conversely, in limited cutaneous SSc, skin involvement is confined to the extremities (distal to the elbows and knees) and to the face and neck[6].

During the early (inflammatory) phase of dcSSc, when the skin disease is progressing, the skin is often itchy and painful. Pigmentary change can occur[7,8] and can be distressing to patients, especially those with darker skins. Skin tightening commonly leads to contractures, particularly fixed flexion deformities of the fingers[9] (FIG. 1a), but also of the elbows and sometimes knees. Range of movement is often substantially reduced, for example, at the shoulder or at the ankle, subtalar and mid-tarsal joints. The flexion contractures predispose to overlying ulcers, which can be refractory to treatment and which can lead to underlying osteomyelitis. Rarely, the skin is so tightened that small superficial ulcers appear, unrelated to pressure points (FIG. 1b).

Itch, which is often described as the most troublesome skin symptom of early dcSSc, resolves when the early inflammatory phase subsides. In those patients who survive, the severity of the skin disease (as assessed by the mRSS) will generally plateau (usually within 3–5 years of onset)[10], followed by gradual softening and atrophying of the skin, to the extent that years later, there might no longer be any skin thickening. The contractures, however, persist and are usually irreversible[9] (FIG. 1c).

Associated morbidity

Although it has long been recognized that the skin involvement in early dcSSc is painful, disabling and disfiguring, these elements of the disease burden have only been quantified in the past few years. The European Scleroderma Observational Study (ESOS)[11] involved 326 patients with early dcSSc from 19 countries (with a median disease duration from onset of skin thickening of 11.9 months), and although the main aim was to assess treatment outcomes, ESOS also provided the opportunity to examine associations between severity of skin involvement and both functional ability and quality of life. Severity of skin involvement was measured with the mRSS. At the baseline visit, high mRSS was associated with high levels of disability (with 'grip' and 'activity' being most affected) as assessed by the Health Assessment Questionnaire disability index (HAQ-DI) (Spearman's $\rho = 0.34$, $P < 0.0001$), and specifically with high levels of hand disability, as assessed by the Cochin Hand Function Scale ($\rho = 0.35$, $P < 0.0001$)[12]. Fine finger movements were particularly affected. mRSS was also associated with severity of pain, as assessed on a 0–100 visual analogue scale ($\rho = 0.17$, $P = 0.002$), and severity of fatigue, as assessed by the Functional Assessment of Chronic Illness Therapy fatigue score ($\rho = -0.20$, $P = 0.0005$). Examining changes over 12 months, increases in the mRSS were associated with worsening disability as measured by HAQ-DI ($\rho = 0.40$, $P < 0.0001$). In summary, ESOS demonstrated that the greater the degree of skin thickening, the greater the disability (with an emphasis on hand disability), pain and fatigue, and that if skin thickening progresses then so too does disability. This association in early dcSSc has since been confirmed in other studies: in a single-centre retrospective study[13], an increase in mRSS was associated with worsening disability as measured by HAQ-DI in the subgroup of patients with early dcSSc ($\rho = 0.36$, $P = 0.004$), and in a study of 154 patients from Canada with early dcSSc[14], changes in mRSS correlated with changes in HAQ-DI (Pearson's $r = 0.43$ for 1-year data, $r = 0.41$ for 2-year data).

Predicting progression of skin disease

Associations with skin-disease severity

Among patients with early dcSSc, various trajectories of skin involvement are observed: skin score can progress (sometimes rapidly), stabilize or improve. An important aim is to identify those patients with progressive skin

Ulcers
Skin lesions with discernible depth and loss of the epithelium.

Scleroderma renal crisis
A complication of SSc that involves sudden onset of hypertension accompanied by renal failure.

Tendon friction rubs
Palpable rubs that are found, for example, over wrists, ankles and knees, and are thought to result from inflammatory change in the tenosynovium.

involvement, not only because it is painful and disabling, but also because extensive and/or progressive skin disease portends a poor outcome. Survival is reduced in patients with high skin scores[15–17]. A high 'skin-thickness progression rate' (the mRSS at first visit divided by patient-reported duration of skin thickening) is a predictor of early mortality and of scleroderma renal crisis[18]. Researchers who conducted an analysis of the European Scleroderma Trials and Research (EUSTAR) database identified reduced survival of progressors among patients with dcSSc: a group of 78 'skin progressors' had lower survival (and more decline in lung function) than 943 'non-progressors'[19]. Conversely, a reduction in skin thickening is reassuring, because it is associated with improvement in survival[20] and reduction of internal-organ involvement[21].

Predictors of progression

Accurate prediction of progressive skin involvement would enable clinicians to make informed decisions regarding whether or not to initiate potentially toxic treatments, usually an immunosuppressant but potentially (in highly selected patients) HSCT. Although treatment-related mortality with HSCT has fallen considerably since the introduction of the technique, it remains a concern, so the procedure should only be carried out in those at highest risk. Prediction of progressive skin disease is also important for researchers designing clinical trials of potential disease-modifying therapies; inclusion and exclusion criteria should be selected to include progressors rather than non-progressors, who are less likely to benefit from treatment. Progressors are often defined as those experiencing a 5-unit and 25% increase in mRSS over 12 months[22–24].

Tendon friction rubs are an indicator of disease that is very likely to progress[25,26]. In a study of an inception cohort from the University of Pittsburgh (reported in 2011)[18], anti-RNA polymerase III antibody positivity was associated with rapid skin-disease progression. More recently, several groups have investigated other predictors of progressive skin disease. Low mRSS, short disease duration and joint synovitis were predictors of disease progression in an analysis from the EUSTAR database[22], whereas a high baseline mRSS (and absence of friction rubs) predicted improvement[27]. These results led to the suggestion that only patients with an mRSS of ≤22 should be included in clinical trials of early dcSSc, because patients with higher scores are unlikely to have progressive skin disease[22]. This fairly stringent cut-off excludes many patients. An analysis of the ESOS cohort[23], in whom mRSS was assessed at 3-month intervals (enabling detailed assessment of disease trajectory), demonstrated that patients with higher skin scores could reasonably be included in clinical trials if their disease duration was short. Among the 293 patients with sufficient data to assess their status, the 66 progressors had shorter disease duration than the 227 non-progressors (median 8.1 months versus 12.6 months, $P = 0.001$), as well as lower mRSS (median 19 units versus 21 units, $P = 0.030$), with those patients who were anti-RNA polymerase III antibody positive going on to have the highest skin scores and peaking earliest. Two predictive models were derived for progressive skin thickening[23]: the first included mRSS, duration of skin thickening and their interaction, and the second added anti-RNA polymerase III antibody positivity. Both models were more accurate than a model with an mRSS cut-off of 22, and for a given skin score were more flexible, enabling a higher baseline skin score to be compensated for by a shorter disease duration[23]. Application of these models should maximize numbers of the most informative patients (progressors) to be included in clinical trials. Subsequently, results from other studies have confirmed the role of skin score and disease duration as predictors of progression. A 2021 analysis from the Pittsburgh cohort[28] led to the conclusion that ideally only patients with a disease duration of <18 months should be included in clinical trials, although the findings from ESOS[23] suggest that some flexibility in disease duration could be permitted in the

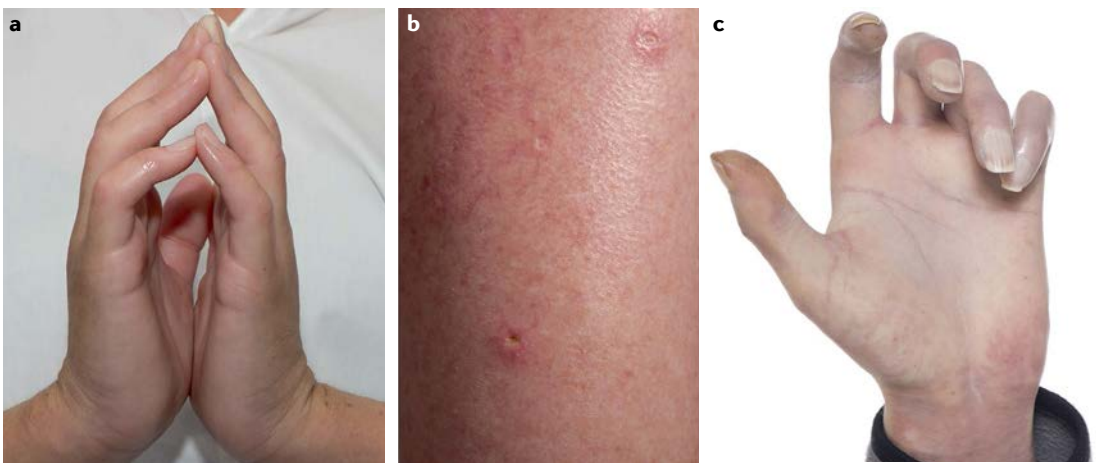

Fig. 1 | **Skin involvement in diffuse cutaneous systemic sclerosis. a** | Flexion contractures of the fingers in early diffuse cutaneous systemic sclerosis (dcSSc). **b** | Superficial cutaneous ulceration in early dcSSc. **c** | Late-stage dcSSc with persisting contracture (note the scar from carpal tunnel decompression, performed soon after the onset of symptoms of dcSSc). Images copyright of Northern Care Alliance NHS Foundation Trust.

presence of low skin scores. Findings from the Genetics versus Environment in Scleroderma Outcomes Study (GENISOS)[24] cohort suggested that an mRSS of ≤27 was predictive of progression, despite a mean disease duration of 2.4 ± 1.5 years (which is longer than the disease duration of the ESOS cohort[23]). In a Japanese multicentre prospective cohort study[29], disease duration of ≤12 months and an mRSS of ≤19 predicted progression (sensitivity 73.9%, specificity 81.1%), which is consistent with the findings from ESOS[23].

Results from skin global gene-expression studies indicate that SSc skin has a distinct transcript profile (although considerable heterogeneity exists). Although these results demonstrate the presence of prominent fibrotic and inflammatory signatures (which can co-occur in individual patients), a subgroup of SSc skin samples has a gene-expression pattern that resembles the transcript profile of healthy individuals (a 'normal-like' pattern)[30–32]. In addition, evidence increasingly indicates that the skin gene-expression profile of a patient with SSc changes over time, in parallel with the clinical course of skin involvement[31,33]. SSc skin gene-expression signatures might help to predict outcomes of dcSSc. Higher 'fibroinflammatory' scores are associated with higher skin scores (both mRSS and locally at the biopsy site)[30]. Results from a study of the Prospective Registry of Early Systemic Sclerosis (PRESS) cohort, published in 2020, suggest that gene-expression profiles in samples from forearm skin biopsies of patients with early dcSSc are associated with prior skin-disease progression, but are not predictive of future progression[31]. These findings contrast with those from a phase 2 trial of tocilizumab, in which expression of five fibrotic and inflammatory genes in forearm skin biopsy samples from patients treated with a placebo was associated with mRSS progression[34]. Inflammatory, fibroproliferative and normal-like skin gene-expression subsets were identified using a machine-learning approach[32], and might help to explain the variable response to immunomodulatory therapies. In a randomized controlled study of treatment with abatacept in dcSSc, the results of which were published in 2020, patients with the inflammatory or normal-like expression profiles responded to treatment, whereas no statistically significant treatment effect occurred in the overall study population[35]. Results from other studies (published from 2018 to 2021) have indicated that patients with an inflammatory skin gene-expression profile have shorter disease duration and higher skin score than individuals with other expression profiles[31,36,37]. Consistent with these findings, results published in 2021 from a longitudinal study indicated that immune cell and fibroblast signatures decline over time, and overall skin gene expression trends towards normalization in patients with early diffuse SSc[33]. Currently, it is not known to what extent skin gene-expression profiling can help to predict response to treatment beyond the information provided by easily obtained clinical predictors such as disease duration, baseline skin score and anti-RNA polymerase III antibody positivity status. Anti-RNA polymerase III antibody is one of the SSc-specific autoantibodies that are associated with the diffuse cutaneous subtype of SSc, another is anti-topoisomerase I antibody[11]. As mentioned above, patients with dcSSc with anti-RNA polymerase III positivity experience more rapidly progressive skin involvement than the overall population of patients with dcSSc[11]. Notably, differences in gene expression and pathway enrichment between major autoantibody subgroups in early dcSSc[38] might reflect both distinct and overlapping biological mechanisms determining progression and regression of skin disease at the patient level. Integration of high-dimensional gene and protein expression data by weighted gene co-expression network analysis (WGCNA) elucidates likely pathogenic mechanisms[39] and points towards the potential to better define longitudinal differences to link gene[33] and protein expression to clinical changes (FIG. 2). This analysis should provide additional insights into local pathogenesis of skin fibrosis[39], and might help to identify candidate biomarkers that can be used for inpatient stratification or assessment of outcome, building upon results from studies of biomarkers validated in conditions such as liver cirrhosis, including the enhanced liver fibrosis score, which correlates with skin severity and progression[40,41].

In summary, we now have a much better insight than 5 years ago into the factors that predict disease progression, and progress is being made towards a stratified approach to therapy. As we continue to advance our knowledge, it will be possible to build upon the conceptual framework for the association between skin-score trajectory and the biology of progression and regression, as outlined in FIG. 2.

Outcome measures

Reliable outcome measures that are sensitive to change are a prerequisite to monitoring both disease progression and the response to treatment. However, identification and/or development of reliable outcome measures for SSc skin disease has proved to be a major challenge, leading to much discussion between clinicians and industry partners, and demonstrating the need for further research. Here, we describe the main outcome measures used for the assessment of skin involvement[42]. The current outcome measures are not ideal, but efforts are ongoing to improve them through modification of existing tools and development of new measures, including (at least for early-phase studies) non-invasive imaging techniques.

The mRSS

Measurement of the extent of skin involvement is complex, and needs to take into account the surface area affected and the degree of involvement at various body sites. The mRSS[43], which involves skin palpation at 17 sites, has been fully validated as per OMERACT principles[44], but presents challenges. The mRSS is described in detail elsewhere[43], and key points relating to its use and limitations are presented in BOX 1.

Self-assessment of skin involvement

The 'hands on' nature of the mRSS has implications for both clinical practice and clinical trials in the era of COVID-19, when patient visits to hospital are being minimized. Therefore, patient self-assessment of skin

involvement, which was previously proposed[45,46], but not widely applied, is now an attractive option. An exciting development is the Patient Self-Assessment of Skin Thickness in Upper Limb (PASTUL) questionnaire[47]. In an initial study of 104 patients with SSc, 78 (75%) of whom also had an mRSS assessment, there was moderate correlation between PASTUL scores and both total mRSS ($r = 0.56$) and upper-limb mRSS ($r = 0.58$). PASTUL scores also strongly correlated with results from the Scleroderma Skin Patient-Reported Outcome (SSPRO)[48]. Once fully validated, PASTUL could be an important addition to clinical trials, bringing the possibility of more-frequent skin scoring during trial treatment than has previously been possible (and in the patient's own home).

Other outcome measures

The limitations of the mRSS have resulted in exploration of the use of other outcome measures of skin involvement, including composite measures. These measures are attracting increasing interest for application in trials of early dcSSc.

Patient-reported outcomes. The SSPRO[48] is an 18-item questionnaire for the assessment of skin-related quality of life in patients with SSc. Researchers have already applied the SSPRO in clinical trials[49], and its further use is likely. The HAQ-DI, although not specific to the skin involvement of early dcSSc, captures much of the associated disability and has the advantage that most clinicians are familiar with it. In the past 5 years, several trials have included the HAQ-DI as an outcome measure[35,49–55]. Because itch can be a very prominent feature in early dcSSc, itch assessment should also be considered, for example, with the 5-D itch scale[56], which researchers included in a 2020 phase 2 study of the safety and efficacy of the cannabinoid receptor 2 agonist lenabasum for the treatment of patients with SSc[49].

Non-invasive imaging methods. The two main methods in this category are high-frequency ultrasonography and optical-coherence tomography (OCT). Ultrasonography reliably measures skin thickness, according to results from several cross-sectional studies[57–60], and a 2021 study advocated ultrasonography as an outcome measure[61].

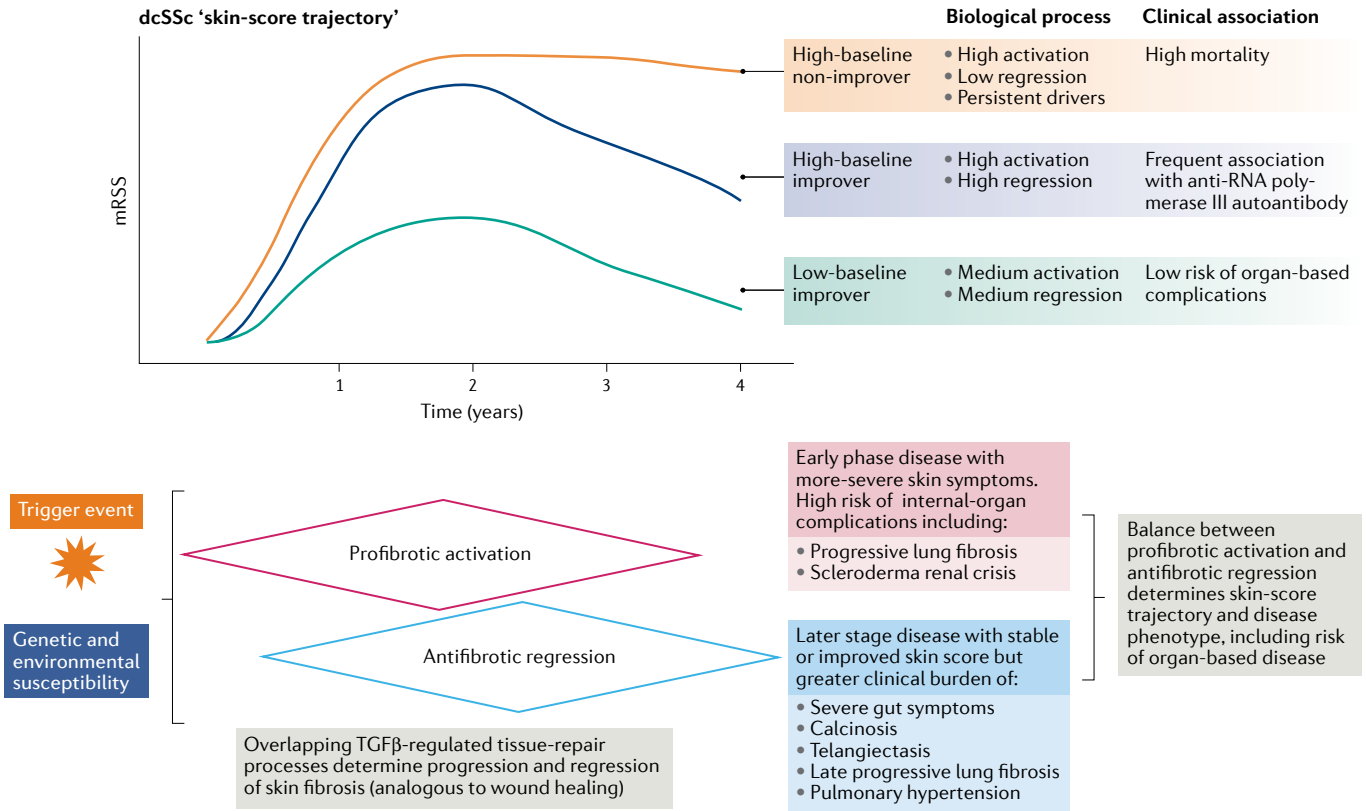

Fig. 2 | **Conceptual framework for skin-score trajectory and clinical diversity in diffuse cutaneous systemic sclerosis.** Although at a group level, cohort studies and clinical trials of systemic sclerosis (SSc) almost always show improvement in average skin score over 1–3 years, this group-level behaviour does not reflect differences in modified Rodnan skin score (mRSS) change over time for individual patients. Operationally, SSc can be differentiated into three subgroups, characterized by high peak mRSS followed by regression, high peak mRSS without disease regression or lower peak mRSS tending to improve over 2–5 years of follow-up. This pattern of subgroups is likely to reflect interplay between the effectors of progression and fibrosis and the counteracting influence of the mechanisms that determine spontaneous regression, which is a hallmark of normal skin wound healing. Molecular and cellular determinants of these processes are likely to interact and to underlie the distinct patterns of skin disease, and might also determine the development and severity of internal-organ complications in SSc. Greater understanding of the biological basis of heterogeneity in skin-score change could facilitate clinical trial design and a more stratified approach to patient care. Notably, in normal skin, wound-healing mediators such as TGFβ regulate both profibrotic mechanisms and processes involved in regression of fibrosis, such as induction of matrix-degrading metalloproteinases. The balance between these processes of activation and regression and the persistence of local mediators of fibrosis might underlie the distinct skin-score trajectories observed for individual patients with SSc[16,23,38].

Box 1 | **Modified Rodnan skin score**

What is the modified Rodnan skin score?

To determine the modified Rodnan skin score (mRSS), skin is assessed by palpation at 17 sites and scored on a 0–3 scale (0 = uninvolved, 1 = mild involvement, 2 = moderate involvement, 3 = so severely affected that the skin can hardly be moved), giving a total score of 0–51. The minimal clinically important difference for improvement at 12 months, in the context of a clinical trial, is 5 units[106].

Limitations

- Substantial inter-observer variability occurs with the mRSS[107], although in a study in which ten rheumatologists assessed seven patients, inter-observer and intra-observer reliability were high (0.81 and 0.94, respectively)[108]. A major contributor to inter-observer variability is that some raters tend to 'maximize' (select a score based on the most severely affected area), some choose a 'representative' score (select the score that seems more representative) and some choose an 'average' score[43,109]. Standardized training can reduce variability in skin scoring[110,111].
- With the mRSS, the skin is very difficult to assess in later-stage disease[112], because although the skin is then softening it can remain tethered, making it impossible to pinch.

Applicability

In clinical practice

- Without doubt, the mRSS is useful in the outpatient clinic, because it is quick and easy to perform and will help the clinician to decide whether to intensify or to begin withdrawing immunosuppressant treatment. The mRSS associates with patient-reported worsening of skin involvement[113].

In clinical trials

- The mRSS has tended to be the primary outcome in clinical trials of potential disease-modifying therapies in patients with early dcSSc, given that the degree of skin involvement reflects the 'overall' early dcSSc disease process. Several of these trials[35,49–53] have failed to meet their primary end points, although signs of efficacy have come from secondary end points. For example, in the FocuSSed phase 3 randomized placebo-controlled trial of tocilizumab[51], patients on active treatment showed no improvement in mRSS, but lung function did improve. In a randomized controlled trial of abatacept[35], active treatment resulted in improvement of scores for the Health Assessment Questionnaire-Disability Index (HAQ-DI)[114] and ACR Composite Response Index in dcSSc (a composite measure including the mRSS)[71], but not for mRSS alone. Experience in these and other studies raises the question of whether improvement in skin disease was 'missed' because of the limitations of the mRSS.

Ultrasonographic measurement of skin thickness with a 4–15 MHz linear probe correlated well with histological assessment ($r = 0.6926$, $P = 0.009$) and with local (forearm) mRSS ($r = 0.7961$, $P = 0.001$) in 13 patients with SSc (nine of whom had dcSSc) who underwent forearm skin biopsy[62]. As the imaging resolution with ultrasonographic devices improves and ultrasonography-based elastography becomes available in a clinical setting, additional studies will be needed to assess the reliability and validity of improved ultrasonographic skin-thickness measurement modalities in SSc[63]. Moreover, accurate measurement by ultrasonography requires training and is time-consuming if performed at multiple body sites in individual patients, which probably explains why ultrasonography has not been adopted as an outcome measure in later-phase multicentre studies.

The technical challenges associated with ultrasonography will most likely also apply to OCT, which is another promising tool for the assessment of skin thickness that is currently in early-phase proof-of-concept studies. OCT essentially takes in vivo 'optical biopsy' images of the skin[64] to visualize skin structure. In this way, epidermal thickness can be measured at high resolution (<10 μm). Very few studies have so far examined the use of OCT in patients with SSc[65,66]. Although OCT can provide higher imaging resolution than ultrasonography-based techniques, currently it has limited imaging depth, which complicates assessment of lower layers of dermis in certain body areas, underscoring the need for further development in this area. Polarization-sensitive OCT (PS-OCT)[67] is an extension to OCT that involves the measurement of birefringence (an optical property of collagen) in addition to skin thickness. Birefringence can be considered a measure of skin 'heterogeneity' and, therefore, potentially a measure of fibrosis. Epidermal thickness measured by PS-OCT correlated with histological thickness in a study that involved ten patients with SSc and ten healthy individuals[68]. Larger prospective studies that examine change over time are required to validate both ultrasonography and OCT as possible outcome measures.

Durometry. As a measure of skin hardness, durometry has long been advocated as a possible outcome measure in clinical trials of early dcSSc[69], but not widely adopted. However, in 2020 durometry was revisited[70], and it deserves further investigation, including in longitudinal studies with examination of sensitivity to change. A durometer is hand-held, portable and relatively easy to use, making durometry a potentially useful additional outcome measure in multicentre studies.

Composite scores. Composite scores incorporate multiple elements and might therefore be more representative of disease status than individual measures. At present there are no composite scoring systems specifically for skin disease in patients with SSc. However, the ACR provisional composite response index in dcSSc (CRISS)[71,72], which is heavily weighted by the mRSS, was used in patients with dcSSc in several studies that had results published in 2020 (REFS[35,49,51–53]). The ACR-CRISS includes five measures: the mRSS, percentage predicted forced vital capacity, the HAQ-DI, and patient and clinician global assessments.

Dynamic biomarkers. Longitudinal measurements of expression in skin of two genes, *THBS1* and *MS4A4A*, correlate with mRSS measurements[73]. However, no studies have yet produced evidence of changes in skin gene expression that correlate with how patients with dcSSc 'feel, function and survive', to establish them as surrogate outcome measures.

Serum is another possible source of composite biomarkers, such as those used for the enhanced liver fibrosis score[38,41], as well as novel proteomic markers that are currently being explored as candidates for the assessment of treatment response[74]. However, evidence suggests that substantial heterogeneity could exist in the longitudinal relationships between serum markers and mRSS[38].

Best-practice management

Although there is currently no cure for SSc (so it is important that whenever possible patients are recruited into clinical trials), there is no room for nihilism, as much can be done to support patients through the worrying phase of early dcSSc. Management options include

Elastography
Assessment of the elasticity and stiffness of soft tissues, usually by ultrasonography.

Raynaud phenomenon
Colour change of the fingers on exposure to cold or to emotional stress: the classic triphasic change is white to blue to red.

symptomatic treatment for progressive skin disease and (in most patients) immunosuppression. Notably, the evidence base in favour of immunosuppression is weak[11]. In addition, a small minority of patients are candidates for HSCT[6]. Despite recent interest in the tyrosine kinase inhibitor nintedanib as a treatment for SSc-related interstitial lung disease, the SENSCIS trial provided no evidence of an improvement in skin score[75], although it was primarily a trial investigating lung disease rather than a study of patients with early dcSSc.

Here, we describe aspects of best-practice management of skin thickening in early dcSSc, as shown in FIG. 3. Decisions on treatment (particularly on the choice of immunosuppressant) are influenced by the presence or absence of other SSc 'complications', such as concomitant myositis or interstitial lung disease[76].

Early recognition

Diagnosis of early dcSSc is often delayed[77], which prevents timely identification and early treatment of (for example) internal-organ involvement and delays patient education. These delays can be addressed by raising physicians' awareness of the signs and symptoms of dcSSc. Any patient with new onset of skin thickening that could indicate early dcSSc should be referred to a specialist centre, especially if the skin thickening has rapidly progressed. Although Raynaud phenomenon is a symptom in most patients with early dcSSc, in some individuals it develops only after skin thickening, so the use of Raynaud phenomenon as a 'red flag'[78] does not always apply to dcSSc, in contrast to the situation in limited cutaneous SSc, in which the onset of Raynaud phenomenon usually precedes the diagnosis of SSc by many years[6].

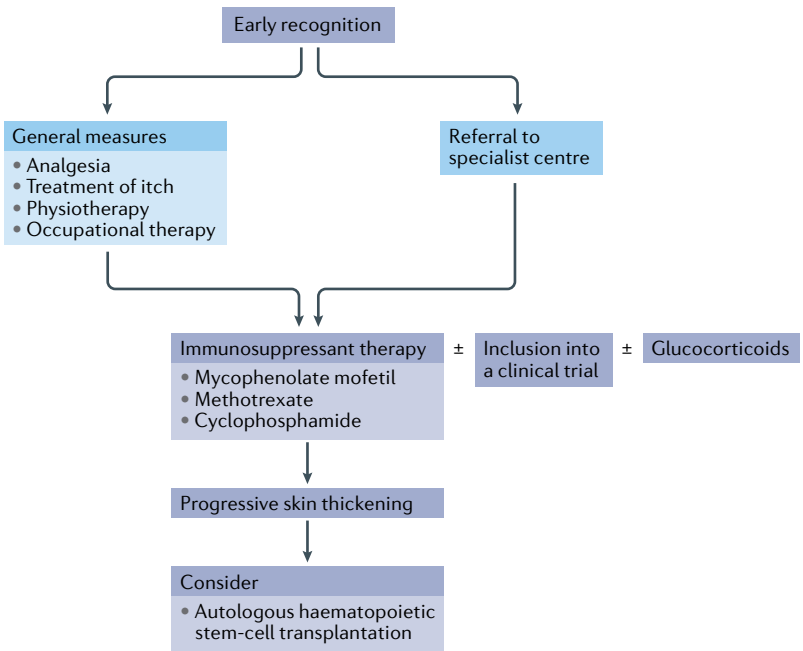

Fig. 3 | **Management of skin disease in patients with early diffuse cutaneous systemic sclerosis.** Early recognition and referral to a specialist centre are the first principles of management. Pending specialist review, 'general measures' should be initiated. In most patients, immunosuppressant therapy should be prescribed. If at all possible, patients should be offered the opportunity to participate in a clinical trial. For patients who continue to progress, haematopoietic stem-cell transplantation should be considered.

General measures

The four main general measures for the management of skin involvement in early dcSSc are analgesia, treatment of itch, physiotherapy and occupational therapy. Clinical psychology input is an additional consideration.

Analgesia. The pain of skin disease in early dcSSc is often insufficiently recognized, even though it has a considerable effect on quality of life. Among the 326 patients recruited into ESOS[12], the mean and median scores for the sHAQ pain scale (which has a range of 0–100, with 100 indicating the greatest disability) were 32.9 (standard deviation 26.9) and 29.0 (interquartile range 8.7–52.7), and skin thickening correlated with pain ($\rho = 0.17$, $P = 0.002$). Development of contractures and ulcers further contributes to pain. Analgesia is therefore a key aspect of management. The pain might have a neurogenic component[79], so treatment with gabapentin or pregabalin can be considered. Some patients will benefit from referral to a pain-management clinic.

Management of itch. Management of this symptom is very challenging. Antihistamines can be tried, but seldom seem to be helpful. Some patients find benefits with 1% menthol in aqueous cream. Anecdotally (A.H., unpublished observations), low-dose prednisolone can relieve itch. Prednisolone is, however, a risk factor for scleroderma renal crisis, as discussed below.

Physiotherapy and occupational therapy. Researchers have given little attention to the roles of physiotherapy and occupational therapy in early dcSSc, even though it seems logical that these approaches could be helpful to maintain range of movement and maximize function. Anecdotally, patients benefit from stretching exercises to maintain range of movement, and many enjoy hydrotherapy (A.H., unpublished observations). In a 2021 study that included 34 patients with dcSSc, but with unspecified disease duration, results suggested a benefit from hand exercises[80]. Ideally, all patients with early dcSSc should be assessed by an occupational therapist, as almost all patients have considerable functional disability, including impairment of hand function[12]. 'Remote' occupational therapy via a mobile app[81] could be a way forward, at least in some patients.

Clinical psychology input. Patients with early dcSSc report feeling overwhelmed by their disease, with loss of control. This feeling relates in large part to the disability, pain and fatigue that are directly or indirectly related to skin disease. Clinical psychology referral should be considered.

Immunosuppressant therapy

Both the British Society for Rheumatology (BSR)–British Health Professionals in Rheumatology (BHPR)[82] and EULAR[83] recommend immunosuppressant therapy for the skin disease of SSc. The BSR–BHPR guidelines suggest the use of mycophenolate mofetil (MMF),

methotrexate or cyclophosphamide, whereas the EULAR recommendation is for methotrexate. Among the few clinical trials of immunosuppressants that have specifically examined skin disease primarily in early dcSSc, two used methotrexate[84,85], none used MMF (despite results from several early retrospective and prospective observational studies that suggest benefit[86–88]) and none used cyclophosphamide. In ESOS[11], the researchers examined the relative effectiveness of commonly used immunosuppressants in patients with early dcSSc. The treatment options in this observational study were methotrexate (oral or subcutaneous at a target dose of 20–25 mg weekly), MMF (target dose 1 g twice daily), cyclophosphamide (intravenous or oral) or no immunosuppressant. A trend in favour of immunosuppression was seen, as after 12 months, mRSS fell in all groups, but more so in the immunosuppressant groups: for methotrexate ($n = 65$) −4.0 units (95% CI −5.2 units to −2.7 units), for MMF ($n = 118$) −4.1 units (95% CI −5.3 units to −2.9 units), for cyclophosphamide ($n = 87$) −3.3 units (95% CI −4.9 units to −1.7 units) and for no immunosuppressant ($n = 56$) −2.2 units (95% CI −4.0 units to −0.3 units) (P-value for between-group differences = 0.346). The conclusion from ESOS was that immunosuppression conferred benefit, but that this benefit was modest. Improvements in mRSS in patients with dcSSc (although not specifically early dcSSc) also occurred in the Scleroderma Lung Study I (cyclophosphamide compared with placebo) and the Scleroderma Lung Study II (cyclophosphamide and MMF compared with patients treated with placebo in Scleroderma Lung Study I) at 12, 18 and 24 months ($P < 0.05$)[89]. Further support for the use of MMF comes from the results of an Australian observational study[90] and from a report of five patients with recurrence of progressive skin involvement after either discontinuation or dose reduction of MMF[91].

Glucocorticoids

The use of glucocorticoids in early dcSSc is highly controversial[92], and although some clinicians prescribe them, others do not, as demonstrated by the observation that 44% of patients who were recruited into ESOS had been prescribed them[11]. Glucocorticoids are likely to reduce the itch and pain (from the skin) that occur in patients with early dcSSc because these symptoms are thought to result from skin inflammation. However, glucocorticoids are a risk factor for renal crisis, especially when used in high doses[93–95]. Many clinicians are, therefore, understandably reluctant to prescribe glucocorticoids for patients with early progressive dcSSc, who are already at high risk of renal crisis, a risk that is further increased with anti-RNA polymerase III antibody positivity[96,97]. Notably, patients who are anti-RNA polymerase III antibody positive often have rapidly progressive disease[23] and are therefore particularly likely to have itchy, painful skin that might benefit from glucocorticoid treatment. This controversial issue is currently being investigated in a randomized placebo-controlled trial of the use of prednisolone in patients with early dcSSc (ClinicalTrials.gov identifier: NCT03708718)[55].

Intravenous iloprost

Intravenous iloprost is widely used in the treatment of SSc-related digital vasculopathy, but might have other beneficial effects, such as the downregulation of expression of connective-tissue growth factor[98]. In our experience (C.D. and A.H., unpublished observations), intravenous iloprost can help to heal the superficial ulcers that can occur in patients with very tightened skin (FIG. 1b), suggesting that there is an ischaemic element to these ulcers.

Autologous HSCT

HSCT should be considered in highly selected patients with rapidly progressive dcSSc. In all three trials that provided the evidence base for this recommendation (ASSIST[99], ASTIS[100] and SCOT[101]), patients who underwent HSCT demonstrated benefit in terms of mRSS compared with patients treated with cyclophosphamide, although mRSS was not the primary end point (mRSS was, however, part of the composite primary end point in the ASSIST study[99]). Improvement in mRSS was also reported in a prospective 'real-world' study of 80 patients who underwent HSCT[102]. The treatment-related mortality of HSCT in the SCOT study was 3% at 54 months and 6% at 72 months[101], and therefore lower than previously reported (a 2001 phase 1/2 trial reported a procedure-related mortality of 17%)[103], most likely reflecting careful patient selection and adjustments to the transplantation regime. A key question that is currently being addressed[104] is whether HSCT should be recommended as a first-line therapy as opposed to being reserved for patients who do not respond to immunosuppressant therapies. This difficult decision will be informed by the stratified medicine approach referred to earlier (taking into account advances in our ability to predict those patients most likely to have progressive disease), and by ensuring that individualized care is tailored to patients' needs and expectations[105].

Conclusions

The past 5 years have provided new insights into the most visible and characteristic manifestation of early dcSSc — skin thickening (scleroderma) — which is often rapidly progressive. Importantly, we now recognize the burden of skin disease, which has a very considerable effect on quality of life; previously, it was often overlooked. We are now in a good position to predict which patients will develop rapid progression of skin thickening, thereby enabling early intervention with immunosuppressive therapies or with HSCT, and/or inclusion into clinical trials. The lack of reliable outcome measures of skin disease represents a major unmet need. However, the challenges of monitoring skin disease, both in the clinic and in the setting of clinical trials, are now better understood, and research is ongoing. Better outcome measures (and improved identification of progressors) will maximize the efficiency of future clinical trials of the many promising new targeted therapies. Pending identification of a safe and effective treatment, clinicians should not forget current best-practice guidelines, which can provide at the very least some symptomatic relief from painful, disabling skin disease.

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1. LeRoy, E. C. et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J. Rheumatol.* **15**, 202–205 (1988).
2. Domsic, R. T. et al. Derivation and external validation of a prediction rule for five-year mortality in patients with early diffuse cutaneous systemic sclerosis. *Arthritis Rheumatol.* **68**, 993–1003 (2016).
3. Pokeerbux, M. R. et al. Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. *Arthritis Res. Ther.* **21**, 86 (2019).
4. Jaafar, S. et al. Clinical characteristics, visceral involvement, and mortality in at-risk or early diffuse systemic sclerosis: a longitudinal analysis of an observational prospective multicenter US cohort. *Arthritis Res. Ther.* **23**, 170 (2021).
5. Campochiaro, C. & Allanore, Y. An update on targeted therapies in systemic sclerosis based on a systematic review from the last 3 years. *Arthritis Res. Ther.* **23**, 155 (2021).
6. Denton, C. P. & Khanna, D. Systemic sclerosis. *Lancet* **390**, 1685–1699 (2017).
7. Solanki, K. K., Hor, C., Chang, W. S. J., Frampton, C. & White, D. H. N. Clinical utility of hypo- and hyperpigmentation of skin in diffuse cutaneous systemic sclerosis. *Int. J. Rheum. Dis.* **20**, 767–773 (2017).
8. Leroy, V. et al. Association of skin hyperpigmentation disorders with digital ulcers in systemic sclerosis: analysis of a cohort of 239 patients. *J. Am. Acad. Dermatol.* **80**, 478–484 (2019).
9. Buni, M. et al. Predictors of hand contracture in early systemic sclerosis and the effect on function: a prospective study of the GENISOS cohort. *J. Rheumatol.* **46**, 1597–1604 (2019).
10. Amjadi, S. et al. Course of the modified Rodnan skin thickness score in systemic sclerosis clinical trials: analysis of three large multicenter, double-blind, randomized clinical trials. *Arthritis Rheum.* **60**, 2490–2498 (2009).
11. Herrick, A. L. et al. Treatment outcome in early diffuse cutaneous systemic sclerosis: the European Scleroderma Observational Study (ESOS). *Ann. Rheum. Dis.* **76**, 1207–1218 (2017).
12. Peytrignet, S. et al. Disability, fatigue, pain and their associates in early diffuse cutaneous systemic sclerosis: the European Scleroderma Observational Study. *Rheumatology* **57**, 370–381 (2018).
13. Peytrignet, S. et al. Changes in disability and their relationship with skin thickening, in diffuse and limited cutaneous systemic sclerosis: a retrospective cohort study. *Scand. J. Rheumatol.* **48**, 230–234 (2019).
14. Zheng, B. et al. Changes in skin score in early diffuse cutaneous systemic sclerosis are associated with changes in global disease severity. *Rheumatology* **59**, 398–406 (2020).
15. Clements, P. J. et al. Skin thickness score as a predictor and correlate of outcome in systemic sclerosis. *Arthritis Rheum.* **43**, 2445–2454 (2000).
16. Shand, L. et al. Relationship between change in skin score and disease outcome in diffuse cutaneous systemic sclerosis. *Arthritis Rheum.* **56**, 2422–2431 (2007).
17. Ledoult, E. et al. Early trajectories of skin thickening are associated with severity and mortality in systemic sclerosis. *Arthritis Res. Ther.* **22**, 30 (2020).
18. Domsic, R. T., Rodriguez-Reyna, T., Lucas, M., Fertig, N. & Medsger, T. A. Skin thickness progression rate: a predictor of mortality and early internal organ involvement in diffuse scleroderma. *Ann. Rheum. Dis.* **70**, 104–109 (2011).
19. Wu, W. et al. Progressive skin fibrosis is associated with a decline in lung function and worse survival in patients with diffuse cutaneous systemic sclerosis in the European Scleroderma Trials and Research (EUSTAR) cohort. *Ann. Rheum. Dis.* **78**, 648–656 (2019).
20. Steen, V. D. & Medsger, T. A. Improvement in skin thickening in systemic sclerosis associated with improved survival. *Arthritis Rheum.* **44**, 2828–2835 (2001).
21. Nevskaya, T. et al. Skin improvement is a surrogate for favourable changes in other organ systems in early diffuse cutaneous systemic sclerosis. *Rheumatology* **59**, 1715–1724 (2020).
22. Maurer, B. et al. Prediction of worsening of skin fibrosis in patients with diffuse cutaneous systemic sclerosis using the EUSTAR database. *Ann. Rheum. Dis.* **74**, 1124–1131 (2015).
23. Herrick, A. L. et al. Patterns and predictors of skin score change in early diffuse systemic sclerosis from the European Scleroderma Observational Study. *Ann. Rheum. Dis.* **77**, 563–570 (2018).
24. Mihai, C., Dobrota, R., Assassi, S., Mayes, M. D. & Distler, O. Enrichment strategy for systemic sclerosis clinical trials targeting skin fibrosis: a prospective, multiethnic cohort study. *ACR Open Rheumatol.* **2**, 496–502 (2020).
25. Khanna, P. P. et al. Tendon friction rubs in early diffuse systemic sclerosis: prevalence, characteristics and longitudinal changes in a randomized controlled trial. *Rheumatology* **49**, 955–959 (2010).
26. Avouac, J. et al. Joint and tendon involvement predict disease progression in systemic sclerosis: a EUSTAR prospective study. *Ann. Rheum. Dis.* **75**, 103–109 (2016).
27. Dobrota, R. et al. Prediction of improvement in skin fibrosis in diffuse cutaneous systemic sclerosis: a EUSTAR analysis. *Ann. Rheum. Dis.* **75**, 1743–1748 (2016).
28. Domsic, R. T. et al. Defining the optimal disease duration of early diffuse systemic sclerosis for clinical trial design. *Rheumatology* **60**, 4662–4670 (2021).
29. Kuwana, M. et al. Initial predictors of skin thickness progression in patients with diffuse cutaneous systemic sclerosis: results from a multicentre prospective cohort in Japan. *Mod. Rheumatol.* **31**, 386–393 (2021).
30. Assassi, S. et al. Dissecting the heterogeneity of skin gene expression patterns in systemic sclerosis. *Arthritis Rheumatol.* **67**, 3016–3026 (2015).
31. Skaug, B. et al. Global skin gene expression analysis of early diffuse cutaneous systemic sclerosis shows a prominent innate and adaptive inflammatory profile. *Ann. Rheum. Dis.* **79**, 379–386 (2020).
32. Franks, J. M. et al. A machine learning classifier for assigning individual patients with systemic sclerosis to intrinsic molecular subsets. *Arthritis Rheumatol.* **71**, 1701–1710 (2019).
33. Skaug, B. et al. Large-scale analysis of longitudinal skin gene expression in systemic sclerosis reveals relationships of immune cell and fibroblast activity with skin thickness and a trend towards normalisation over time. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-221352 (2021).
34. Stifano, G. et al. Skin gene expression is prognostic for the trajectory of skin disease in patients with diffuse cutaneous systemic sclerosis. *Arthritis Rheumatol.* **70**, 912–919 (2018).
35. Khanna, D. et al. Abatacept in early diffuse cutaneous systemic sclerosis: results of a phase II investigator-initiated, multicenter, double-blind, randomized, placebo-controlled trial. *Arthritis Rheumatol.* **72**, 125–136 (2020).
36. Hinchcliff, M. et al. Mycophenolate mofetil treatment of systemic sclerosis reduces myeloid cell numbers and attenuates the inflammatory gene signature in skin. *J. Invest. Dermatol.* **138**, 1301–1310 (2018).
37. Showalter, K. et al. Machine learning integration of scleroderma histology and gene expression identifies fibroblast polarisation as a hallmark of clinical severity and improvement. *Ann. Rheum. Dis.* **80**, 228–237 (2021).
38. Clark, K. E. N. et al. Molecular basis for clinical diversity between autoantibody subsets in diffuse cutaneous systemic sclerosis. *Ann. Rheum. Dis.* **80**, 1584–1593 (2021).
39. Clark, K. et al. High-density proteomic analysis of skin blister fluid and plasma in systemic sclerosis identifies local and systemic differences for key proteins [abstract]. *Arthritis Rheumatol.* **72**, https://acrabstracts.org/abstract/high-density-proteomic-analysis-of-skin-blister-fluid-and-plasma-in-systemic-sclerosis-identifies-local-and-systemic-differences-for-key-proteins/ (2020).
40. Abignano, G. et al. The enhanced liver fibrosis test: a clinical grade, validated serum test, biomarker of overall fibrosis in systemic sclerosis. *Ann. Rheum. Dis.* **73**, 420–427 (2014).
41. Abignano, G. et al. European multicentre study validates enhanced liver fibrosis test as biomarker of fibrosis in systemic sclerosis. *Rheumatology* **58**, 254–259 (2019).
42. Sumpton, D. et al. Scope and consistency of outcomes reported in trials of patients with systemic sclerosis. *Arthritis Care Res.* **72**, 1449–1458 (2020).
43. Khanna, D. et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. *J. Scleroderma Relat. Disord.* **2**, 11–18 (2017).
44. Merkel, P. A. et al. Current status of outcome measure development for clinical trials in systemic sclerosis. Report from OMERACT 6. *J. Rheumatol.* **30**, 1630–1647 (2003).
45. Nagy, Z. et al. Establishment and partial validation of a patient skin self-assessment questionnaire in systemic sclerosis. *Rheumatology* **48**, 309–314 (2009).
46. Daungkum, K. et al. Self-assessment of skin tightness severity by scleroderma patients. *Int. J. Rheum. Dis.* **19**, 989–995 (2016).
47. Spierings, J., Ong, V. & Denton, C. P. PASTUL questionnaire: a tool for self-assessment of scleroderma skin during the COVID-19 pandemic. *Ann. Rheum. Dis.* **80**, 819–820 (2021).
48. Man, A. et al. Development and validation of a patient-reported outcome instrument for skin involvement in patients with systemic sclerosis. *Ann. Rheum. Dis.* **76**, 1374–1380 (2017).
49. Spiera, R. et al. Safety and efficacy of lenabasum in a phase II, randomized, placebo-controlled trial in adults with systemic sclerosis. *Arthritis Rheumatol.* **72**, 1350–1360 (2020).
50. Khanna, D. et al. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet* **387**, 2630–2640 (2016).
51. Khanna, D. et al. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Resp. Med.* **8**, 963–974 (2020).
52. Khanna, D. et al. Riociguat in patients with early diffuse cutaneous systemic sclerosis (RISE-SSc): randomised, double-blind, placebo-controlled multicentre trial. *Ann. Rheum. Dis.* **79**, 618–625 (2020).
53. Allanore, Y. et al. A randomised, double-blind, placebo-controlled, 24-week, phase II, proof-of-concept study of romilkimab (SAR156597) in early diffuse cutaneous systemic sclerosis. *Ann. Rheum. Dis.* **79**, 1600–1607 (2020).
54. Allanore, Y. et al. Lysophosphatidic acid receptor 1 antagonist SAR100842 for patients with diffuse cutaneous systemic sclerosis: a double-blind, randomized, eight-week placebo-controlled study followed by a sixteen-week open-label extension study. *Arthritis Rheumatol.* **70**, 1634–1643 (2018).
55. Herrick, A. L., Griffiths-Jones, D. J., Ryder, W. D., Mason, J. C. & Denton, C. P. Clinical trial protocol: PRednisolone in early diffuse cutaneous Systemic Sclerosis (PRedSS). *J. Scleroderma Relat. Disord.* **6**, 146–153 (2021).
56. Elman, S., Hynan, L. S., Gabriel, V. & Mayo, M. J. The 5-D itch scale: a new measure of pruritus. *Br. J. Dermatol.* **162**, 587–593 (2010).
57. Moore, T. L., Lunt, M., McManus, B., Anderson, M. E. & Herrick, A. L. Seventeen-point dermal ultrasound scoring system - a reliable measure of skin thickness in patients with systemic sclerosis. *Rheumatology* **42**, 1559–1563 (2003).
58. Hesselstrand, R., Scheja, A., Wildt, M. & Akesson, A. High-frequency ultrasound of skin involvement in systemic sclerosis reflects oedema, extension and severity in early disease. *Rheumatology* **47**, 84–87 (2008).
59. Liu, H. et al. A preliminary study of skin ultrasound in diffuse cutaneous systemic sclerosis: does skin echogenicity matter? *PLoS ONE* **12**, e0174481 (2017).
60. Naredo, E. et al. Performance of ultra-high-frequency ultrasound in the evaluation of skin involvement in systemic sclerosis: a preliminary report. *Rheumatology* **59**, 1671–1678 (2020).
61. Murphy, S. L. et al. Development of a musculoskeletal ultrasound protocol to examine upper extremity rehabilitation outcomes in systemic sclerosis. *J. Diagn. Med. Sonogr.* **37**, 13–23 (2021).
62. Chen, C. et al. Ultrasound assessment of skin thickness and stiffness: the correlation with histology and clinical score in systemic sclerosis. *Arthritis Res. Ther.* **22**, 197 (2020).
63. Santiago, T. et al. Ultrasonography for the assessment of skin in systemic sclerosis: a systematic review. *Arthritis Care Res.* **71**, 563–574 (2019).
64. Fercher, A. F., Drexler, W., Hitzenberger, C. & Lasser, L. Optical coherence tomography — principles and applications. *Rep. Prog. Phys.* **66**, 239–303 (2003).
65. Abignano, G. et al. Virtual skin biopsy by optical coherence tomography: the first quantitative imaging biomarker for scleroderma. *Ann. Rheum. Dis.* **72**, 1845–1851 (2013).
66. Liu, C.-H. et al. Translational optical coherence elastography for assessment of systemic sclerosis. *J. Biophotonics* **12**, e201900236 (2019).
67. Adams, D. C. et al. Assessing the progression of systemic sclerosis by monitoring the tissue optic axis using PS-OCT. *Sci. Rep.* **10**, 2561 (2020).

68. Marjanovic, E. J. et al. Polarisation-sensitive optical coherence tomography measurement of retardance in fibrosis, a non-invasive biomarker in patients with systemic sclerosis. *Sci. Rep.* **12**, 2893 (2022).
69. Merkel, P. et al. Validity, reliability, and feasibility of durometer measurements of scleroderma skin disease in a multicenter treatment trial. *Arthritis Rheum.* **59**, 699–705 (2008).
70. De Oliveira, M. F. C. et al. Durometry as an alternative tool to the modified Rodnan's skin score in the assessment of diffuse systemic sclerosis patients: a cross-sectional study. *Adv. Rheumatol.* **60**, 48 (2020).
71. Khanna, D. et al. The American College of Rheumatology provisional composite response index for clinical trials in early diffuse cutaneous systemic sclerosis. *Arthritis Rheumatol.* **68**, 299–311 (2016).
72. Khanna, D., Huang, S., Lin, C. J. F. & Spino, C. New composite endpoint in early diffuse cutaneous systemic sclerosis: revisiting the provisional American College of Rheumatology Composite Response Index in Systemic Sclerosis. *Ann. Rheum. Dis.* **80**, 641–650 (2021).
73. Rice, L. M. et al. A longitudinal biomarker for the extent of skin disease in patients with diffuse cutaneous systemic sclerosis. *Arthritis Rheumatol.* **67**, 3004–3015 (2015).
74. Rice, L. M. et al. A proteome-derived longitudinal pharmacodynamic biomarker for diffuse systemic sclerosis skin. *J. Invest. Dermatol.* **137**, 62–70 (2017).
75. Distler, O. et al. Nintedanib for systemic sclerosis–associated interstitial lung disease. *N. Engl. J. Med.* **380**, 2518–2528 (2019).
76. Kafaja, S. & Clements, P. Management of widespread skin thickening in diffuse systemic sclerosis. *Curr. Treat. Options Rheumatol.* **2**, 49–60 (2016).
77. Distler, O. et al. Factors influencing early referral, early diagnosis and management in patients with diffuse cutaneous systemic sclerosis. *Rheumatology* **57**, 813–817 (2018).
78. Matucci-Cerinic, M. et al. The challenge of early systemic sclerosis for the EULAR Scleroderma Trial and Research group (EUSTAR) community. It is time to cut the Gordian knot and develop a prevention or rescue strategy. *Ann. Rheum. Dis.* **68**, 1377–1380 (2009).
79. Sousa-Neves, J., Cerqueira, M., Santos-Faria, D., Afonso, C. & Teixeira, F. Neuropathic pain in systemic sclerosis patients: a cross-sectional study. *Reumatol. Clin.* **15**, e99–e101 (2019).
80. Gokcen, N., Badak, S. O., Sarpel, T., Sertdemir, Y. & Erken, E. The efficacy of a home-based, self-administered hand exercise program for patients with systemic sclerosis: a randomized controlled, evaluator-blind, clinical trial. *J. Clin. Rheumatol.* https://doi.org/10.1097/RHU.0000000000001752 (2021).
81. Murphy, S. L. et al. Intensive and app-delivered occupational therapy to improve upper extremity function in early diffuse cutaneous systemic sclerosis: a pilot two-arm trial. *Rheumatology* **60**, 5002–5011 (2021).
82. Denton, C. P. et al. BSR and BHPR guideline for the treatment of systemic sclerosis. *Rheumatology* **55**, 1906–1910 (2016).
83. Kowal-Bielecka, O. et al. Update of EULAR recommendations for the treatment of systemic sclerosis. *Ann. Rheum. Dis.* **76**, 1327–1339 (2017).
84. Van den Hoogen, F. H. et al. Comparison of methotrexate with placebo in the treatment of systemic sclerosis: a 24 week randomized double-blind trial, followed by a 24 week observational trial. *Br. J. Rheumatol.* **35**, 364–372 (1996).
85. Pope, J. E. et al. A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma. *Arthritis Rheum.* **44**, 1351–1358 (2001).
86. Derk, C. T. et al. A prospective open-label study of mycophenolate mofetil for the treatment of diffuse systemic sclerosis. *Rheumatology* **48**, 1595–1599 (2009).
87. Le, E. N., Wigley, F. M., Shah, A. A., Boin, F. & Hummers, L. K. Long-term experience of mycophenolate mofetil for treatment of diffuse cutaneous systemic sclerosis. *Ann. Rheum. Dis.* **70**, 1104–1107 (2011).
88. Mendoza, F. A., Nagle, S. J., Lee, J. B. & Jimenez, S. A. A prospective observational study of mycophenolate mofetil treatment in progressive diffuse cutaneous systemic sclerosis of recent onset. *J. Rheumatol.* **39**, 1241–1247 (2012).
89. Namas, R. et al. Efficacy of mycophenolate mofetil and oral cyclophosphamide on skin thickness: post hoc analyses from two randomized placebo-controlled trials. *Arthritis Care Res.* **70**, 439–444 (2018).
90. Boulos, D. et al. Long-term efficacy and tolerability of mycophenolate mofetil therapy in diffuse scleroderma skin disease. *Int. J. Rheum. Dis.* **20**, 481–488 (2017).
91. Mendoza, F. A., Lee-Ching, C. & Jimenez, S. A. Recurrence of progressive skin involvement following discontinuation or dose reduction of mycophenolate mofetil treatment in patients with diffuse systemic sclerosis. *Semin. Arthritis Rheum.* **50**, 135–139 (2020).
92. Herrick, A. L. Controversies on the use of steroids in systemic sclerosis. *J. Scleroderma Relat. Disord.* **2**, 84–91 (2017).
93. Steen, V. D. & Medsger, T. A. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. *Arthritis Rheum.* **41**, 1613–1619 (1998).
94. DeMarco, P. J. et al. Predictors and outcomes of scleroderma renal crisis: the high-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis trial. *Arthritis Rheum.* **46**, 2983–2989 (2002).
95. Guillevin, L. et al. Scleroderma renal crisis: a retrospective multicentre study on 91 patients and 427 controls. *Rheumatology* **51**, 460–467 (2012).
96. Nguyen, B. et al. HLA-DRB1*0407 and *1304 are risk factors for scleroderma renal crisis. *Arthritis Rheum.* **63**, 530–534 (2011).
97. Hamaguchi, Y. et al. Clinical and immunologic predictors of scleroderma renal crisis in Japanese systemic sclerosis patients with anti-RNA polymerase III antibodies. *Arthritis Rheumatol.* **67**, 1045–1052 (2015).
98. Stratton, R. et al. Iloprost suppresses connective tissue growth factor production in fibroblasts and in the skin of scleroderma patients. *J. Clin. Invest.* **108**, 241–250 (2001).
99. Burt, R. K. et al. Autologous non-myeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised phase 2 trial. *Lancet* **378**, 498–506 (2011).
100. Van Laar, J. M. et al. Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. *JAMA* **311**, 2490–2498 (2014).
101. Sullivan, K. M. et al. Myeloablative autologous stem-cell transplantation for severe scleroderma. *N. Engl. J. Med.* **378**, 35–47 (2018).
102. Henes, J. et al. Autologous stem cell transplantation for progressive systemic sclerosis: a prospective non-interventional study from the European Society for Blood and Marrow Transplantation Autoimmune Disease Working Party. *Haematologica* **106**, 375–383 (2021).
103. Binks, M. et al. Phase I/II trial of autologous stem cell transplantation in systemic sclerosis: procedure related mortality and impact on skin disease. *Ann. Rheum. Dis.* **60**, 577–584 (2001).
104. Spierings, J. et al. A randomised, open-label trial to assess the optimal treatment strategy in early diffuse cutaneous systemic sclerosis: the UPSIDE study protocol. *BMJ Open* **11**, e044483 (2021).
105. Spierings, J. et al. Treatment decision-making in diffuse cutaneous systemic sclerosis: a patient's perspective. *Rheumatology* **59**, 2052–2061 (2020).
106. Khanna, D. et al. Minimal clinically important differences for the modified Rodnan skin score: results from the Scleroderma Lung Studies (SLS-I and SLS-II). *Arthritis Res. Ther.* **21**, 23 (2019).
107. Clements, P. et al. Inter- and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. *J. Rheumatol.* **22**, 1281–1285 (1995).
108. Gordon, J. K. et al. Reliability and validity of the tender and swollen joint counts and the modified Rodnan skin score in early diffuse cutaneous systemic sclerosis: analysis from the prospective registry of early systemic sclerosis cohort. *J. Rheumatol.* **44**, 791–794 (2017).
109. Showalter, K., Merkel, P. A., Khanna, D. & Gordon, J. K. for the Scleroderma Clinical Trials Consortium. Assessment of skin disease in scleroderma: practices and opinions of investigators studying scleroderma. *J. Scleroderma Relat. Disord.* **5**, 167–171 (2020).
110. Park, J. W. et al. Impact of EUSTAR standardized training on accuracy of modified Rodnan skin score in patients with systemic sclerosis. *Int. J. Rheum. Dis.* **22**, 96–102 (2019).
111. Low, A. H. L. et al. Evaluation of Scleroderma Clinical Trials Consortium training recommendations on modified Rodnan skin score assessment in scleroderma. *Int. J. Rheum. Dis.* **22**, 1036–1040 (2019).
112. Czirjak, L., Foeldvari, I. & Muller-Ladner, U. Skin involvement in systemic sclerosis. *Rheumatology* **47** (Suppl. 5), v44–v45 (2008).
113. Ross, L. et al. Can patient-reported symptoms be used to measure disease activity in systemic sclerosis? *Arthritis Care Res.* **72**, 1459–1465 (2020).
114. Allanore, Y. et al. Health assessment questionnaire-disability index (HAQ-DI) use in modelling disease progression in diffuse cutaneous systemic sclerosis: an analysis from the EUSTAR database. *Arthritis Res. Ther.* **22**, 257 (2020).

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Endothelial function and endothelial progenitor cells in systemic lupus erythematosus

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Abstract | The observations that traditional cardiovascular disease (CVD) risk factors fail to fully account for the excessive cardiovascular mortality in patients with systemic lupus erythematosus (SLE) compared with the general population have prompted in-depth investigations of non-traditional, SLE-related risk factors that contribute to cardiovascular complications in patients with SLE. Of the various perturbations of vascular physiology, endothelial dysfunction, which is believed to occur in the earliest step of atherosclerosis, has been extensively investigated for its contribution to CVD risk in SLE. Endothelial progenitor cells (EPCs), which play a crucial part in vascular repair, neovascularization and maintenance of endothelial function, are quantitatively and functionally reduced in patients with SLE. Yet, the lack of a unified definition of EPCs, standardization of the quantity and functional assessment of EPCs as well as endothelial function measurement pose challenges to the translation of endothelial function measurements and EPC levels into prognostic markers for CVD in patients with SLE. This Review discusses factors that contribute to CVD in SLE, with particular focus on how endothelial function and EPCs are evaluated currently, and how EPCs are quantitatively and functionally altered in patients with SLE. Potential strategies for the use of endothelial function measurements and EPC quantification as prognostic markers of CVD in patients with SLE, and the limitations of their prognostication potential, are also discussed.

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The incidence of cardiovascular disease (CVD) is higher in patients with systemic lupus erythematosus (SLE) than in an age-matched and sex-matched healthy population[1,2]. Approximately 7% of patients with SLE will experience a cardiovascular event, and those who are between 35 and 44 years of age are ~50 times more likely to have a myocardial infarction than the general population of the same age group[3]. Although treatment-related and disease-related complications, including renal disease and infection, are common causes of death in patients with SLE, CVD remains the leading cause of death[4]. A meta-analysis of observational studies showed that patients with SLE had a standardized mortality ratio of 2.72 for cardiovascular-related death compared with the general population[5]. Although traditional CVD risk factors (as described in the Framingham study) are more prevalent in patients with SLE than in their age-matched and sex-matched healthy counterparts[6,7], they are unable to completely account for the excessive cardiovascular mortality observed in patients with SLE[8]. Over the past four decades, observational and mechanistic studies have been conducted in an attempt to understand how non-traditional and disease-specific risk factors, such as genetic susceptibility[9–14], pro-inflammatory mediators[15], SLE disease activity[16], lupus nephritis[17] and the presence of anti-phospholipid antibodies[18], adversely affect the cardiovascular system in the context of SLE by enhancing and worsening atherosclerosis. The relevance of inflammatory processes in atherosclerosis that are triggered by and related to the SLE disease process has drawn substantial attention in the past three decades, as inflammation was noted to be involved in driving the initiation, formation and eventual rupture of atherosclerotic plaques[19,20], indicating that dampening inflammation is one of the relevant strategies for reducing the burden of CVD in patients with SLE[21].

In the past two decades, endothelial dysfunction, which has increasingly been recognized to occur in the very initial step of the process of atherosclerosis[22,23], has been extensively studied in murine SLE models and human SLE. Whereas in vivo measurement of endothelial function can be readily conducted in animals[24], it used to be impractical for studying humans, and even impossible in clinical settings. Nowadays, equipment validated

Key points

- Traditional cardiovascular disease (CVD) risk factors are prevalent in patients with systemic lupus erythematosus (SLE), but this observation cannot fully explain the excess of cardiovascular mortality and morbidity in these patients.
- Endothelium-dependent flow-mediated dilation of the brachial artery, a common biophysical measure of endothelial function, is impaired in patients with SLE, even if they do not present with CVD.
- Impaired endothelial function is associated with increased diastolic blood pressure, inflammation and vertebral bone loss in patients with SLE.
- Endothelial progenitor cells (EPCs) are reduced quantitatively and functionally in patients with SLE, compared with healthy individuals.
- Antimalarial drug use might be associated with elevation of levels of circulating angiogenic cells in patients with SLE.
- Standardization of the definition and functional characterization of EPCs is paramount before EPCs can be used as prognostic biomarkers for CVD in patients with SLE.

for non-invasive measurement of biophysical endothelial function is increasingly available and decreasingly operator dependent. One of the classical non-invasive assessment tools of biophysical endothelial function in patients with SLE that has been most frequently reported is the measurement of endothelium-dependent (ED) flow-mediated vasodilatation (FMD) of the brachial artery (baED-FMD). To date, most of the observational studies revealed that baED-FMD was impaired in patients with SLE, even when they did not present with clinical CVD[25–28], supporting the hypothesis that subclinical CVD in the form of endothelial dysfunction is already active in patients with SLE prior to the occurrence of cardiovascular events.

Seeking a deeper understanding of the upstream mechanisms leading to endothelial dysfunction, various investigators have intensively studied the cellular mechanisms that lead to endothelial function impairment in patients with SLE. In particular, the study of endothelial progenitor cells (EPCs) has attracted much interest in the past 15 years. EPCs are postulated to be capable of replacing damaged endothelial cells, restoring endothelial integrity and hence endothelial function[29,30]. Observational studies and a meta-analysis revealed that the quantity of circulating EPCs was significantly reduced in patients with SLE compared with healthy people, even in patients who have not experienced a cardiovascular event[31–37]. Nevertheless, the paucity of prospective data on the effect of endothelial dysfunction on CVD risk in SLE, coupled with the lack of a clear definition and validated method of identification of EPCs, has generated substantial controversy regarding the pathological, diagnostic and prognostic roles of these early CVD biomarkers, as well as the therapeutic potential of EPCs in patients with SLE. This Review provides an overview of the epidemiology of CVD in SLE, with a special focus on the current state of research in endothelial function and circulating EPCs, and their relationships with the immune, metabolic and musculoskeletal systems. Potential utilization of endothelial function assessment and EPCs as diagnostic and prognostic biomarkers of SLE and the foreseeable challenges of bringing these biomarkers to routine clinical use will be critically discussed (FIG. 1). This Review is not intended to elaborate substantially on the molecular perturbations leading to endothelial dysfunction and perturbation of EPC quantity and function, as these topics have been thoroughly described in our and other investigators' previous work[38–40].

CVD risk and SLE

Although traditional CVD risk factors are prevalent in patients with SLE, non-traditional, disease-specific CVD risk factors have been increasingly recognized in patients with SLE. In the past two to three decades, many factors that are directly or indirectly related to SLE have been demonstrated to be detrimental to the cardiovascular health of patients with SLE (BOX 1). Of these factors, alterations of lipoprotein fractions and renal involvement of SLE deserve further elaboration, as they are relevant to routine clinical management of SLE.

HDL cholesterol. HDL cholesterol (HDL-c) classically manifests its anti-atherogenic effects through increasing cholesterol efflux capacity and scavenging oxidizing substances; however, the properties of HDL-c in patients with SLE are altered in two major ways, rendering it pro-atherogenic. First, cholesterol efflux capacity was found to be significantly reduced in patients with SLE compared with healthy individuals[41] and patients with rheumatoid arthritis (RA), despite individuals with RA having worse lipid profiles[42]. Further, reduced cholesterol efflux capacity was associated with the presence of carotid atherosclerotic plaques (as detected with ultrasonography) in patients with SLE[42]. Second, a proportion of HDL-c in patients with SLE shifted from an anti-inflammatory to a pro-inflammatory property, promoting the production of pro-inflammatory IL-6 and tumour necrosis factor (TNF) and suppressing the blockage of Toll-like receptor (TLR)-induced inflammation in macrophages, a pivotal leukocyte population that can develop into foam cells and trigger atherosclerosis[43]. Although longitudinal data have demonstrated the association between pro-inflammatory HDL-c and the development of subclinical atherosclerosis over time[44,45], whether pro-inflammatory HDL-c predicts cardiovascular events in patients with SLE remains to be studied.

Lupus glomerulonephritis. Lupus glomerulonephritis is common in patients with SLE and is one of the factors that reduce their survival[46,47]. Patients with SLE with a history of renal involvement have higher cardiovascular morbidity and mortality than those without it[48,49]. Renal impairment is a traditional CVD risk factor owing to its association with hypertension and accelerated atherosclerosis[50,51], and lupus glomerulonephritis per se is sufficient to elevate CVD risk. In a retrospective study of >3,700 patients with SLE, the odds of the presence of CVD in patients with SLE with lupus glomerulonephritis were significantly higher than in those without lupus glomerulonephritis[52], both the proliferative and the membranous types.

Pathological alterations of glomerular endothelial cells in lupus nephritis are evident[53,54]. The SLE-related inflammatory milieu that contributes to the production of TNF, IL-1β, IL-13 and IFNγ leads to the activation of nuclear factor-κB (NF-κB) and signal transducer and

activator of transcription 1 (STAT1) in resident glomerular endothelial cells, which perpetuate renal inflammation[53]. Although the evidence is limited, the mechanistic relationships between endothelial cells, T cells and lupus nephritis have been demonstrated in animal models and human lupus nephritis. Increased levels of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) in patients with active lupus glomerulonephritis were found to be significantly associated with reduced circulating levels of $CD4^+$ and $CD8^+$ regulatory T cells (T_{reg}), suggesting that low levels of T_{reg} in patients with SLE might be related to endothelial cell activation[55]. Adoptive transfer of peripherally induced $CD8^+$–$CD103^+$ T_{reg} in MRL/lpr lupus-prone mice not only reduced the circulating levels and tissue expression of various pro-inflammatory cytokines and circulating autoantibodies, but also alleviated proteinuria, the expressions of ICAM1 and VCAM1 on glomerular endothelial cells and the pathological lesions of glomerulonephritis[55]. The inhibitory action of $CD8^+$–$CD103^+$ T_{reg} on glomerular endothelial cells increased angiogenesis-related processes, including glomerular endothelial cell proliferation, migration and tube formation, leading to mitigation of lupus nephritis in the animal model[55].

In summary, the increased prevalence of traditional CVD risk factors, the presence of risk factors associated with SLE and its treatment and renal lupus increase the occurrence of cardiovascular morbidity and mortality in patients with SLE. With meticulous monitoring and control of CVD and SLE-related risk factors (such as maintenance of low disease activity state, attainment of renal remission, aggressive control of blood pressure and proteinuria in patients with lupus nephritis, and minimization of the use of glucocorticoids in all patients[56]) reduction of CVD burden in patients with SLE is not an unrealistic goal. In fact, a prospective observational study involving 33 cohorts of patients with SLE globally between 1999 and 2017 demonstrated that the prevalence and the rate of accrual of atherosclerotic vascular events have been declining over time[57]. Improved control of traditional CVD risk factors and SLE disease activity, particularly with the use of antimalarial drugs, are probably the reasons for the improving cardiovascular outcome in patients with SLE[58].

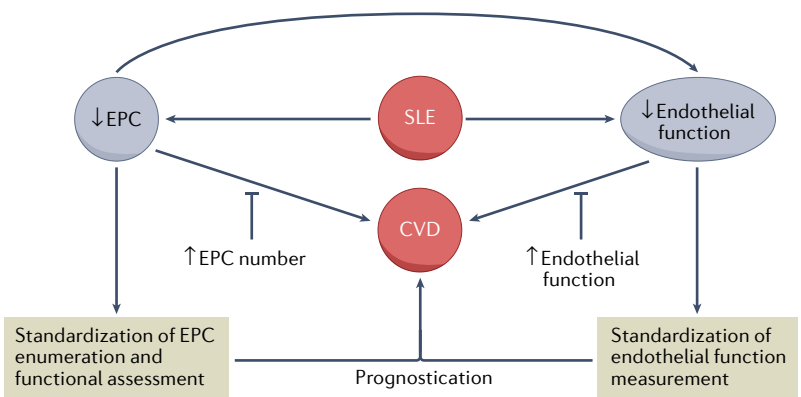

Fig. 1 | **Relationships between endothelial function, EPCs and CVD in SLE.** Reduced quantity and function of endothelial progenitor cells (EPCs) and impaired endothelial function, which are observed in patients with systemic lupus erythematosus (SLE), increase the risk of cardiovascular disease (CVD). Standardization of EPC quality and functional assessment and of endothelial function evaluation aid prognostication of CVD in patients with SLE.

Early atherosclerosis markers. Secondary prevention is inevitable once clinical atherosclerosis, which manifests as cardiovascular and/or cerebrovascular events, has developed. In the past two decades, investigators began to search for reliable surrogate markers of preclinical atherosclerosis aiming to diagnose, evaluate and prognosticate CVD in patients with SLE. In 2003, it was first reported that coronary artery calcification detected by electron-beam CT was significantly more prevalent in patients with SLE than in age-matched and sex-matched healthy individuals[59]. Subsequent cross-sectional studies confirmed these findings[60–62]. Although progression of coronary calcification was detected in short-term prospective studies[63,64], longer term longitudinal data (>8 years) that address whether this surrogate imaging marker of coronary artery disease can predict cardiovascular events are lacking.

In the past 15 years, much interest has shifted to the exploration of the earliest steps involved in the atherogenic process in patients with SLE, resulting in new understanding of how endothelial function is impaired and why the quantity and function of EPCs in patients with SLE are compromised. Nevertheless, controversy on how EPCs should be identified and functionally characterized has not been resolved.

Endothelial function in SLE

Endothelial integrity is essential for preserving endothelial function and thereby vascular health and is maintained by the homeostasis of endothelial damage and repair[65]. When endothelial damage overwhelms the repair process, endothelial dysfunction, which is believed to be the initial step of the process of atherosclerosis, ensues[22,23]. Numerous studies have demonstrated endothelial dysfunction in patients with hypertension, diabetes mellitus, metabolic syndrome and history of cardiovascular events[66–69]. Furthermore, many observational studies found that endothelial dysfunction was predictive of the occurrence of cardiovascular events[70–73]. Several observational studies have demonstrated that endothelial dysfunction is more prevalent in patients with SLE than in demographically matched healthy individuals[31–35,37].

Despite the pathological and therapeutic implications of the presence of endothelial dysfunction in patients with SLE early during their disease course, few studies thus far were specifically designed to study endothelial dysfunction in this subset of patients. In a case–control study that involved 149 patients who had paediatric-onset SLE, with a median age of 17.2 years and disease duration of 3.2 years (interquartile range 1.8–4.9 years), baED-FMD was significantly inferior in the patients with SLE compared with healthy individuals. In addition, longer disease duration was independently associated with reduced FMD[74]. In another study that assessed 20 adult patients who had new-onset SLE with a mean disease duration of 14 months

Box 1 | **Traditional and non-traditional (SLE-related) CVD risk factors**

Traditional
- Age[3,56,181,189,190]
- Elevated body mass index[191]
- Hypertension[192,193]
- Hyperlipidaemia[194,195]
- Diabetes mellitus[196,197]
- Impaired glucose tolerance[198,199]
- Hyperhomocysteinaemia[200,201]
- Elevated very-low density lipoprotein cholesterol[202]
- Hyperhomocysteine[202]
- High triglyceride levels[202]
- Obesity[202,203]
- Cigarette smoking[204]
- Post-menopausal status[3,189]
- Reduced physical exercise[205]
- Sedentary lifestyle[206]

Non-traditional, SLE-related
- Genetic predisposition[9–14]
- Increase in SLE disease activity[16]
- Lupus nephritis[17,207]
- Use of glucocorticoids[52,208]
- The presence of anti-phospholipid antibodies and lupus anticoagulants[18,81]
- Pro-inflammatory cytokines and chemokines[15,209–211]
- Neutrophil extracellular traps[212,213]
- Co-stimulatory molecule upregulation[214]
- Adipokines, such as adipocyte fatty acid binding protein[215,216]
- Increased oxidative stress[217]
- Increased levels of oxidized LDL cholesterol and pro-inflammatory HDL cholesterol[218,219]
- Reduced serum paraoxonase[220] (an enzyme associated with HDL cholesterol that protects against oxidation of LDL particles)

CVD, cardiovascular disease; SLE, systemic lupus erythematosus.

(range 1–58 months), endothelial function was significantly inferior in the SLE group compared with 20 healthy individuals matched for age, sex, BMI and blood pressure[75]. However, patients with endothelial dysfunction did not demonstrate myocardial dysfunction on transthoracic echocardiography[75]. Although the cross-sectional nature of these studies could not address the temporal relationship between the occurrence of endothelial dysfunction and that of subclinical as well as clinical impairment of cardiac function, whether the impairment of endothelial function can serve to predict CVD events in patients with SLE is worth exploring by prospective studies.

Endothelial function assessment. There are several established methods for assessing endothelial function in a non-invasive way; of these, the biophysical measurement methods, including baED-FMD and the endothelium-independent (EID) FMD of the brachial artery (baEID-FMD), are most commonly reported in patients with SLE. Briefly, baED-FMD involves temporary occlusion of the brachial artery, which results in ischaemia-induced vasodilation secondary to the release of endothelium-derived nitric oxide (eNO)[76]; eNO produced in the endothelium diffuses into the vascular smooth muscle cells and induces cGMP-mediated vasodilation[77]. By contrast, baEID-FMD requires sublingual administration of nitroglycerin as a source of nitric oxide, followed by FMD detection that is procedurally similar to that of baED-FMD measurement[78,79] (TABLE 1). In general, individuals are required to rest in supine position for at least 10 min before measurement, which takes place with the patient in the same position. The room where these tests are conducted should be quiet, dimly lit, with excellent control of temperature to minimize vascular tone fluctuations. Individuals are asked to abstain from food and exercise for 12 h, caffeine-containing food and beverages for 24 h and alcohol for 48 h prior to the procedure. In addition, individuals assuming a vasoactive drug, such as calcium channel blockers, beta-adrenergic receptor blockers and angiotensin converting enzyme inhibitors, are conventionally advised to suspend them for 36 h ahead of the test. Female individuals should be assessed at least 7 days after cessation of their last menstrual period to mitigate the effect of progesterone on endothelial reactivity.

In a meta-analysis of 13 studies that compared baED-FMD and baEID-FMD between 580 patients with SLE and 381 healthy individuals, baED-FMD was significantly reduced in patients with SLE compared with their age-matched and sex-matched healthy counterparts[80]. Increasing age and longer duration of SLE correlated significantly with lower baED-FMD in meta-regression analyses[80]. By contrast, baEID-FMD did not differ between patients with SLE and healthy individuals[80]. These findings led to the conclusion that detection of eNO release from endothelium using baED-FMD is a more reliable method of assessing endothelial function than measurement of baEID-FMD in patients with SLE[80]. Subsequent studies were designed to address whether baED-FMD is a reliable biophysical marker of CVD risk and SLE-related co-morbidities. A study of 71 patients with SLE and 71 age-matched and sex-matched healthy individuals showed that although increased diastolic blood pressure and the presence of diabetes mellitus were associated with reduced baED-FMD in patients with SLE, history of lupus glomerulonephritis and increased erythrocyte sedimentation rate were significantly related to reduced baED-FMD in patients with SLE, signifying that endothelial function is worse in patients with SLE who had a history of hypertension, diabetes mellitus and renal lupus, as well as those who were in a pro-inflammatory state, than in patients with SLE without these factors[81,82]. Furthermore, the influence of and interactions among premature menopause, glucocorticoid use and inflammation in patients with SLE prompted exploration of whether CVD and bone loss are related[83–86] (BOX 2).

Specialist equipment that assesses FMD by measuring the peripheral artery tonometry (PAT) of digital arteries with the use of the EndoPAT 2000 technology (Itamar Medical, Israel) has become available[79,80,83]. Similar to the rationale of baED-FMD, PAT measures the reactive hyperaemia index (RHI) secondary to eNO release after a brief occlusion of the brachial artery[87–89] (TABLE 1). Endothelial dysfunction measured by RHI with the EndoPAT technology was consistently shown to be more prevalent in patients with SLE than in healthy individuals[87–90]. In addition, a strong association between type 1 interferon activity and reduced RHI was evident in patients with SLE[91], implying a substantial contribution of type 1 interferon to endothelial dysfunction in patients with SLE.

Mediators of endothelial dysfunction in SLE

Inflammation is central to the pathophysiology of SLE and has long been believed to have a key role in breaching endothelial integrity, resulting in endothelial dysfunction, in patients with SLE[38,92–95]. The presence and persistence of anti-dsDNA antibodies, a pathophysiological and classification hallmark of SLE, were shown to

increase CVD risk in patients with SLE via an increase in the pro-inflammatory cytokine profile, oxidative stress and neutrophil extracellular traps (NETs) formation, which were associated with endothelial activation and biophysical endothelial dysfunction[96]. Type 1 interferon is primarily produced by plasmacytoid dendritic cells and low-density granulocytes and is a pivotal chemokine that drives the pathophysiology of SLE[38,92–95]. Type 1 interferon, particularly interferon-α (IFNα), is highly toxic to the endothelium[38,92–95] (FIG. 2).

Classically known to be atherogenic, low-density lipoprotein cholesterol (LDL-c) negatively affects endothelial physiology in patients with SLE. In addition to LDL-c levels being elevated in patients with SLE[97], LDL-c from patients with active SLE was shown to increase expression of VCAM1, monocyte chemoattractant protein 1 (MCP1, also known as CC-chemokine 2) and matrix metalloproteinase 2 (MMP2, also known as 72-kDa type IV collagenase) by up to twofold in human aortic endothelial cells compared with LDL-c from patients with SLE in remission, despite comparable circulating LDL-c levels in both groups[98], suggesting that high SLE disease activity elevates the pro-atherogenic property of LDL-c through increased expression of adhesion molecules on the endothelial cells. Pro-inflammatory cytokines, chemokines[99] and antibodies such as anti-endothelial antibodies[100] and anti-phospholipid antibodies[101] have been well described to be detrimental to the endothelium; in addition, the levels of specific soluble mediators such as annexin A5, platelet endothelial cell adhesion molecule (PECAM1, also known as CD31) and activated leukocyte cell adhesion molecule (ALCAM, also known as CD166 antigen) have been shown to be increased and could impair endothelial function in patients with SLE[102]. Additionally, serum annexin A5 levels were found to be independently associated with endothelial dysfunction and increased carotid intima-media thickness in patients with SLE[103].

Microparticles and NETs. Two important cellular events that have been increasingly described in the context of endothelial dysfunction in SLE deserve to be highlighted, namely, the formation of circulating plasma microparticles (MPs) and NETs (FIG. 2). Circulating plasma MP can directly lead to endothelial cell death in patients with SLE[104]. MPs are vesicles derived and released from apoptotic cells by blebbing, in which membrane remodelling and exposure of phosphatidylserine on the outer plasma membrane lead to the formation of vesicles containing proteins and other molecules from the apoptotic parental

Table 1 | **Comparison of non-invasive methods to assess endothelial function**

Technique	Mechanism	Procedure	Advantages	Disadvantages
baED-FMD	A duplex ultrasonographic technique to determine post-occlusion release of eNO and its effect on brachial artery vasodilation	An ultrasonography probe is steadied by a stereotactic clamp that enables fine adjustment of the probe position. The probe images the brachial artery and positions electronic tracking gates at the media–adventitia interface of opposing arterial walls. The equipment utilizes radiofrequency signals to measure vessel dimension in real time with 0.01-mm accuracy[102]. Reactive hyperaemia is induced by rapid inflation of a pneumatic cuff secured around the proximal forearm to a pressure of 50 mmHg above the SBP for 5 min, followed by deflation of the cuff. Proprietary baED-FMD software provides a real-time graphical display of minute vasodilation from baseline, cuff occlusion, vasodilation and recovery, and calculates the FMD values	Non-invasive; direct measurement of eNO, which reflects endothelial function and integrity	Operator dependent; costly equipment; narrow dynamic range of the vascular response; low signal-to-noise ratio
baEID-FMD	A duplex ultrasonographic technique to determine the vasodilation of the brachial artery by exogenous (sublingual) administration of NO	The procedure is similar to baED-FMD, except that individuals are required to take a sublingual administration of nitroglycerin as a source of NO, followed by measurement of FMD of the brachial artery as described for baED-FMD[103]. If performed alone, measuring baEID-FMD does not require brachial artery occlusion. Many centres perform baEID-FMD after baED-FMD, when patients have rested for 10 min to enable normalization of vascular tone	Non-invasive	Operator dependent; costly equipment; no direct eNO measurement; narrow dynamic range of the vascular response; low signal-to-noise ratio
PAT	A technique that measures changes of digital pulse waveforms, mediated by the release of eNO following occlusion of the brachial artery, from peripheral arterial tone signals of the digits.	Finger probes are placed on symmetric fingers, with a sphygmomanometer cuff placed on one arm and the other arm serving as a control. PAT is continuously measured for 15 to 20 min. Around 5 min into the measurement period, the sphygmomanometer cuff is inflated for 5 min to occlude the brachial artery until the SBP is raised above baseline in the test arm. At the end of the occlusion period, the cuff is released and RHI is captured as an increase in PAT signal amplitude by calculating the post-occlusion to pre-occlusion ratio[112] The index finger is recommended for measurement of RHI. If the index finger is not suitable, other fingers can be used except for the thumb. The same finger should be used for measurement in the control arm	Totally operator independent; less-costly equipment; rapid measurement of endothelial function	Investigative tool, although it seems promising for its ability to predict cardiovascular outcome

baED-FMD, endothelium-dependent flow-mediated dilatation of brachial artery; baEID-FMD, endothelium-independent flow-mediated dilatation of brachial artery; eNO, endothelium-derived nitric oxide; NO, nitric oxide; PAT, peripheral artery tonometry; RHI, reactive hyperaemic index; SBP, systolic blood pressure.

Box 2 | **CVD and bone loss in SLE**

The first study in 2001 that assessed carotid plaque index, carotid intima-media thickness and bone mineral density (BMD) of the hip and spine with dual X-ray absorptiometry (DEXA) of the lumbar spine and hip in 65 young women with systemic lupus erythematosus (SLE) found a significant relationship between high carotid plaque index and low lumbar BMD. In 13 of these 65 patients, there was a significant association between high coronary calcium score (as measured by electron beam CT) and low lumbar BMD[83]. The association between cardiovascular disease (CVD) risk and vertebral bone loss was further supported by the examination of endothelial function and BMD. In a study of 55 patients with SLE and 55 age-matched and sex-matched healthy volunteers, reduced endothelium-dependent flow-mediated dilatation of brachial artery (baED-FMD) was associated with reduced lumbar BMD in patients with SLE, after adjustment for confounding factors including age, duration of SLE, glucocorticoid use, high-sensitivity C-reactive protein level and menopausal status[84]. In addition to confirming the association between inflammation and impaired endothelial function, these novel findings imply a link between endothelial dysfunction and vertebral bone loss that was independent of the effect of glucocorticoids and inflammation in patients with SLE[84]. Stemming from these results, the postulation that endothelial nitric oxide synthase (eNOS) might have a role in promoting the homeostasis of the endothelium and osteoblasts remains to be explored. Functional variants of the exons and introns of *NOS3* (encoding eNOS) have been shown to reduce eNOS expression[85,86]. Interestingly, several *NOS3* polymorphisms have been demonstrated to be significantly more prevalent in patients with SLE than in healthy individuals and are, therefore, considered to be risk factors for SLE[85,86].

cell; the contents and composition of MPs enable their cell of origin to be identified[105]. The extruded proteins that MPs often carry are biologically active substances, such as inflammatory cytokines and nucleic acids, and even organelles, such as mitochondria, that can serve as a platform conducive to the formation of MP-immune complexes (MP-ICs), complement activation, inflammation, pro-coagulant activity and vascular thrombosis[106–110]. As a result of the increased release and reduced clearance of apoptotic cells, which are central to the pathogenesis of SLE[111], the levels of circulating MPs are elevated in patients with SLE[104]. A longitudinal study demonstrated that although an increase in circulating endothelial cell-derived MPs (which are positive for CD31 and annexin V and negative for CD42b (also known as Platelet glycoprotein Ib alpha chain), as identified by flow cytometry) was significantly associated with reduced baED-FMD in patients with SLE, subsequent reduction of SLE disease activity was related to the reduction of the level of circulating endothelial cell-derived MPs and the increase in baED-FMD[112], signifying a negative effect of SLE disease activity on endothelial cell-derived MPs and endothelial function. Mechanistically, upon internalization by endothelial cells of MPs and MP-ICs derived from patients with SLE, the expression of adhesion molecules such as CD54 (also known as ICAM1), CD102 (also known as ICAM2) and CC-chemokine ligand 2 (CCL2) is increased on endothelial cells, along with increased production of pro-inflammatory mediators including IL-6 and CCL5, leading to increased adherence of classical monocytes on the endothelium, which triggers endothelial injury[113]. Furthermore, SLE-derived MPs and MP-ICs increased disruption of endothelial microstructure, such as depolymerization of actin filaments and formation of intracellular spaces that promote endothelial cell permeability and death[113]. Of note, the origin of MPs is not restricted to endothelial cells: MPs derived from platelets, monocytes, granulocytes and lymphocytes were found to be increased in patients with SLE[114], although their relevance to CVD risk in patients with SLE has not been adequately established.

NETs are an antimicrobial mechanism characterized by the formation of traps that comprise microbicidal proteins such as LL37 (also known as cathelicidin antimicrobial peptide), human neutrophils peptides and DNA[115]. The presence of antibodies against ribonucleoproteins triggers NETs formation in patients with SLE[116]. NETs directly lead to endothelial cell apoptosis[117]; endothelial cell death through endothelial MMP2 activation[118]; activation of platelets, coagulation cascade and thrombosis via the release of serine proteases that degrade tissue factor pathway inhibitor and activate factor XII[119,120]; and vascular leakage[121] (FIG. 3). Indirectly, NETs induce massive IFNα production by plasmacytoid dendritic cells and low-density granulocytes[122] that perpetuates IFNα-induced endothelial toxicity[123], mediates oxidation of HDL-c and impairs cholesterol outflow capacity[124] (FIG. 2).

Endothelial progenitor cells in SLE

EPCs originate from the bone marrow, can participate in vasculogenesis through migration, replication and terminal differentiation into mature endothelial cells, and were initially thought to be restricted to the embryonic state[125]. EPCs were subsequently described in the postnatal state through the isolation of either haematopoietic progenitor cell antigen $CD34^+$ or fetal liver kinase 1 (also known as vascular endothelial growth factor receptor 2 or CD309)-positive ($FLK1^+$) circulating cells[126]. These cells can expand in culture, express the endothelial markers CD31 and E-selectin, produce endothelial nitric oxide and incorporate acetylated LDL[126]. This discovery was followed by a massive expansion of the use of EPCs in both regenerative medicine and as a marker of disease activity in many conditions, including cardiovascular and autoimmune diseases[127]. However, the precise methodology and criteria by which these rare populations of circulating EPCs are identified and characterized have not been formally established, leading to conflicting reports on their ability to incorporate into vasculature[128], their role and nature in disease states[129,130] and their different circulating levels in patients with SLE compared with healthy donors[31–35,37].

EPC measurement and identification techniques. Several methods have been described to identify EPCs (TABLE 2). One method involves in-bulk culturing of circulating peripheral blood mononuclear cells (PBMCs) on fibronectin and endothelial cell growth medium; however, this method results in high contamination rates of myeloid monocytic cell types that can adopt an endothelial phenotype by the uptake of platelet-associated CD31, or even by expressing von Willebrand factor (vWF), incorporating acetylated LDL and upregulating endothelial nitric oxide expression, when circulating cells are cultured in the presence of vascular endothelial growth factor (VEGF)[131–133]. These adherent contaminating monocytic cells are now recognized as myeloid angiogenic cells (MACs), which have a role in potentiating vasculogenesis in a paracrine fashion[134].

Another method of identifying putative EPCs is a flow-cytometry based approach, which enables circulating angiogenic cells (CACs) to be identified among the PBMCs by their co-expression of CD34, CD133 (also known as prominin 1), CD309 and other CAC markers[135]. Although the flow-cytometry approach is frequently adopted in EPC research pertaining to SLE and other autoimmune conditions, two major issues exist. First, none of the described cell-surface markers is specific for EPCs[135]. Second, CACs, the $CD34^+$–$CD133^+$–$CD309^+$ population, are exceedingly rare, with mean levels ranging from 0 to ~500 cells per millilitre of peripheral blood[36]. These limitations hamper the efforts to identify and quantify the true EPCs within the PBMC population, despite the high volume of blood required for high-quality flow-cytometry analysis.

Last, a more robust method identifies EPCs through their ability to form colonies, self-renew (through re-plating), express EPC markers, form tubing in vitro and participate in vessel regeneration in vitro[136–138]. These cells have been termed endothelial colony-forming cells (ECFCs)[136–139]. The terminologies MAC, CAC and ECFC have now been adopted in an international consensus on the nomenclature of EPCs[139]. Despite the heterogeneity of methods, the majority of studies using either CACs or ECFCs has demonstrated reduced EPC function in patients with SLE compared with healthy individuals[33,34,140–143], although a minority did not[144,145].

To date, there is no standardized method of identifying and quantifying circulating EPCs in patients with SLE. In 2009, the European Alliance of Associations for Rheumatology Scleroderma Trials and Research group (EUSTAR) published a group statement and recommendations to unify the methodology of EPCs quantification, with the aim of standardizing EPC research primarily conducted for systemic sclerosis[146]. In brief, the EUSTAR recommendation for EPC identification and quantification via flow cytometry involves the determination of cells that co-express CD34, CD133 and CD309 with a multicolour fluorescent staining approach, and the necessity to combine with a viability marker, collect a large number of events (cells detected), use Fc blockers prior to immunostaining to minimize non-specific binding, exclude lineage-positive cells (that is, lineage-committed cells expressing $CD3^+$ (also known as T cell surface glycoprotein CD3), $CD19^+$ (B lymphocyte antigen CD19),

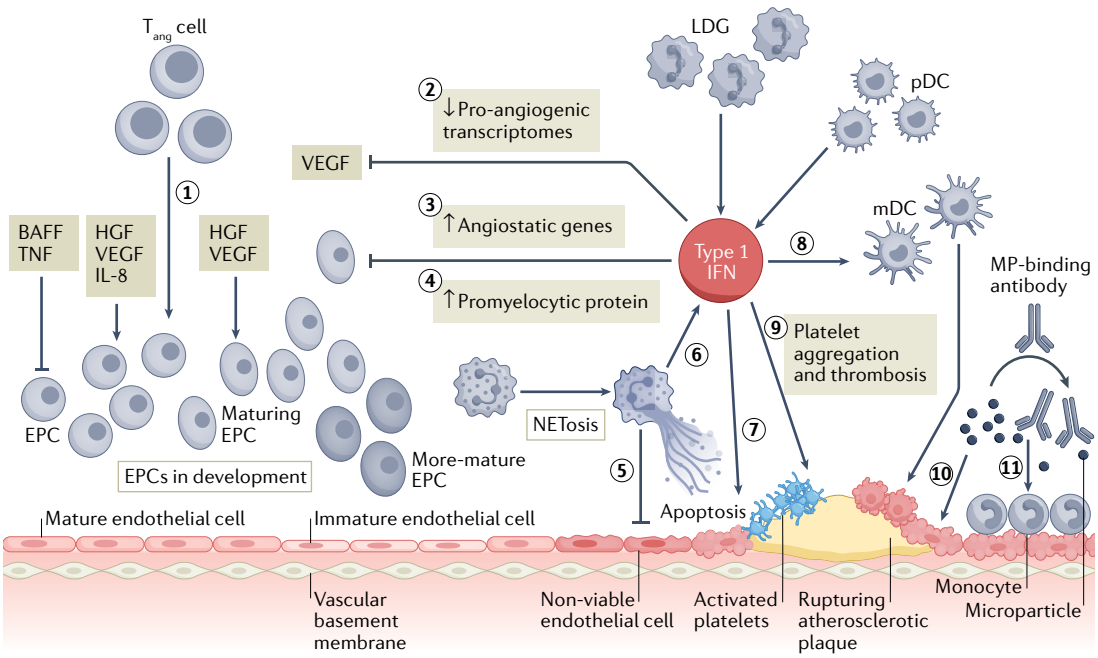

Fig. 2 | **Type 1 interferon and other key mediators detrimental to endothelial cells and EPCs.** Angiogenic T (T_{ang}) cells, working in concert with IL-8 and vascular endothelial growth factor (VEGF), promote differentiation and function of endothelial progenitor cells (EPCs) (**1**). Interferon-α (IFNα) inhibits pro-angiogenic transcriptomes in circulating mononuclear cells induced by IL-1β, resulting in reduction of expression of VEGF (**2**). IFNα directly inhibits endothelial cell proliferation and migration by upregulating the transcription of angiostatic genes including *TRAIL* (also known as *TNFSF10*), *CXCL10* and *CXCL11* in circulating mononuclear cells (**3**)[92]. IFNα inhibits endothelial cell growth and tube formation by inducing the promyelocytic leukaemia protein, which stimulates STAT1 and STAT2 and feeds back negatively with STAT-3 (**4**)[93]. Neutrophil extracellular traps (NETs) cause endothelial cell apoptosis through endothelial MMP2 activation (**5**). NETs induce massive production of IFNα by low-density granulocytes (LDGs) and plasmacytoid dendritic cells (pDCs), which perpetuates IFNα-induced endothelial toxicity (**6**). IFNα causes direct apoptosis of endothelial cells (**7**)[38]. IFNα induces myeloid dendritic cells (mDCs) in diseased endothelium and atherosclerotic plaque to produce various pro-inflammatory cytokines and matrix metalloproteinases (MMPs) that destabilize plaque (**8**)[94]. (**9**) IFNα induces platelet aggregation and thrombosis via a P-selectin-mediated mechanism on diseased endothelium (**9**)[95]. Microparticles cause apoptosis of endothelial cells (**10**). Microparticles bind with antibodies to form immune complexes and induce expressions of adhesion molecules and increase production of pro-inflammatory cytokines and chemokines in endothelial cells, leading to increased adherence of classical monocytes on the endothelium and endothelial injury (**11**). BAFF, B cell activating factor; HGF, hepatocyte growth factor.

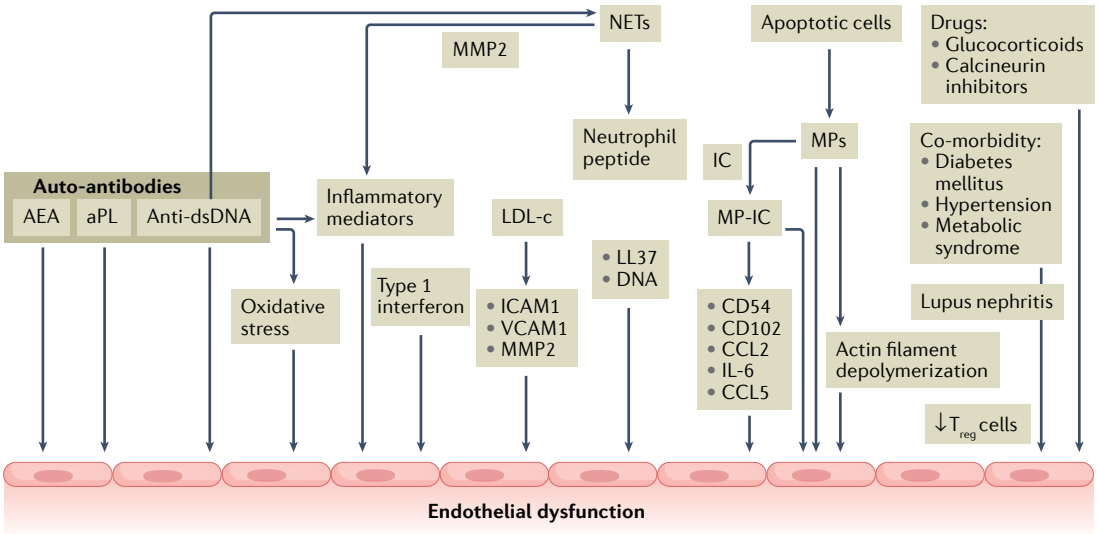

Fig. 3 | **Factors that influence endothelial function in SLE.** The presence of autoantibodies, increase in neutrophil extracellular traps (NETs) formation and apoptosis, the presence of associated co-morbidities and systemic lupus erythematosus (SLE)-related drug use, among other factors, trigger various downstream events that increase endothelial dysfunction in patients with SLE. AEA, anti-endothelial cell antibodies; Anti-dsDNA, anti-double-stranded DNA antibodies; aPL, anti-phospholipid antibodies; IC, immune complexes; ICAM1, intercellular adhesion molecule 1; LDL-c, low-density lipoprotein cholesterol; MMP2, matrix metalloproteinase 2; MPs, microparticles; T_{reg}, regulatory T cells; VCAM1, vascular cell adhesion molecule 1.

$CD14^+$, or $CD56^+$ (neural cell adhesion molecule 1)) and involve an investigator experienced in flow cytometry[146]. In 2012, the EUSTAR recommendation was validated, with an additional recommendation of calibration with fluorospheres to obtain the absolute number of circulating EPCs per millilitre of peripheral blood[147]. Although not all studies addressing EPCs in patients with SLE strictly followed the EUSTAR recommendations, studies that involved identification of CACs co-expressing CD34 and either CD309 or CD133 or both revealed relatively consistent reductions of CAC level in patients with SLE compared with healthy individuals[31–37]. On the basis of our experience (A.M. and colleagues), the scarcity of CACs and the fact that CD133 and CD309 are weakly expressed (leading to missed events) often compromise the accuracy and validity of CAC quantification, even when the EUSTAR recommendations were followed. To circumvent this limitation, the florescent-minus-one (FMO) technique involved in the cell staining and gating step was adopted. This technique involves the analysis of samples stained for all fluorophores in the panel except for one, to determine where the gates for each signal should be set and improve the accuracy of signal detection[36]. With this modified approach, the levels of CACs, defined as Lin^- (lineage-negative) $DAPI^-$ (that is, viable cells) $CD34^+CD133^+CD309^+$ and identified using the FMO method, were found to be significantly lower in patients with SLE than in age-matched and sex-matched healthy counterparts[36]. Interestingly, when applying meta-regression analysis in the case–control study and published data[36], the use of antimalarial drugs was found to be associated with a higher level of CACs in patients with SLE who took these drugs than in those who did not[36]. Although the long-observed benefits of antimalarial drugs have been well established in patients with SLE, the potential mechanistic effect of these agents on EPCs that leads to cardiovascular benefits is worth further exploration.

EPC number and function reduction in SLE

A few observations explain the general reduction in circulating numbers of EPCs in patients with SLE. Altered morphology[35] and reduced expression of VEGF and hence reduced VEGF-driven mobilization of EPCs[33,148] have been demonstrated in patients with SLE. In addition to reduced quantity, the crucial functions of EPCs, including migration and tube- and colony-forming abilities, were found to be reduced in patients with SLE compared with healthy individuals[33,149].

Type 1 interferon exerts a similar effect on EPCs to the effect it has on endothelial cells, as exemplified in murine lupus models and patients with SLE. When EPCs isolated from C57BL/6 mice were co-cultured with plasmacytoid dendritic cells that had been treated with double-stranded DNA that triggered IFNα production, apoptosis and senescence of EPCs were increased, and EPCs were arrested at the G0/G1 phase[150]. Treatment of pristine-induced murine lupus mice with IFNα-neutralizing antibodies rescued all these in vivo changes of EPCs induced by IFNα and activated plasmacytoid dendritic cells[150]. In an IFNα receptor knockout murine model, the numbers and functions of EPCs (in terms of differentiation and neoangiogenesis) were increased compared with New Zealand mixed 2328 lupus-prone mice with intact interferon receptors, along with improved ED vasorelaxation[151]. Exposure to IFNα impaired ED vasorelaxation and EPC function and accelerated vascular thrombosis and platelet activation in New Zealand mixed 2328 lupus-prone mice[151].

Studies in human SLE confirmed the deleterious effect of type 1 interferon on EPCs demonstrated in animal models[34,152]. Upregulation of the genes encoding

Table 2 | **Identification and properties of putative EPCs**

Cell population	Phenotype	Identification procedure	Advantages	Disadvantages
CACs	$CD34^+$, $CD133^+$, $CD309^+$	Flow cytometry with multicolour immunostaining for $CD34^+CD133^+CD309^+$ cells from PBMCs	Direct quantification of EPCs in circulating blood; EPC quantification can be done within a day; EUSTAR recommendations have been established for comparison between studies, although they are primarily meant for research of patients with systemic sclerosis	CACs are rare in the population of circulating PBMCs; require expertise in flow cytometry; identifying CACs as EPCs is still being debated; conflicting data on CAC ability to differentiate into endothelial cells, as some remain haematopoietic
MACs	$CD45^+$, $CD14^+$, $CD31^+$, $CD146^-$, $CD34^-$	Culture of PBMCs on fibronectin-coated plates in EGM with 2% fetal calf serum. Colonies emerge within a week as early outgrowth cells	MACs are generally believed to be circulating early EPCs. MACs can promote angiogenesis through a paracrine mechanism	MACs are haematopoietic, not endothelial in origin; MACs per se do not have the capacity to differentiate into endothelial cells
ECFCs	$CD31^+$, $CD105^+$, $CD146^+$, $^aCD34^+$, $CD309^+$, $CD144^+$, vWF^+, $CD14^-$, $CD45^-$	Culture of PBMCs on collagen-coated plates in EGM with 10% fetal calf serum. Colonies emerge in 2–4 weeks	ECFCs possess potent intrinsic angiogenic capacity, which can give rise to genuine endothelial cells; these cells are 'bona fide EPCs' owing to their capability to form vascular networks in vitro and in vivo	Long and tedious culture process from plating and re-plating until ECFC emergence, with identification taking up to 4 weeks

CAC, circulating angiogenic cell; ECFC, endothelial colony-forming cell; EGM, endothelial-cell growth medium; EPC, endothelial progenitor cells; EUSTAR, European Scleroderma Trials and Research; MAC, myeloid angiogenic cell; PBMC, peripheral blood mononuclear cell; vWF, von Willebrand factor. ^{a}CD34 expression on ECFCs is reduced during in vitro expansion.

IFNα was observed in EPCs in patients with SLE, which suppressed VEGF and hepatocyte growth factor expression and subsequent angiogenic activity[33]. IFNα induces EPC apoptosis, reduces proliferation of EPCs[33,34] and inhibits pro-angiogenic transcriptomes induced by IL-1β, leading to reduction of VEGF expression and increase of IL-18-induced inflammasome activation[142,143], a crucial downstream pathway that promotes aberrant vasculogenesis. Inhibition of inflammasome activation by caspase 1 blockage restores EPC differentiation[142]. B cell activating factor (BAFF, also known as tumour necrosis factor ligand superfamily member 13B), which is overexpressed in patients with SLE[153], plays a part in reducing EPC quantity and function. BAFF receptors are expressed on EPCs and mediate EPC apoptosis when engaged with BAFF[140]. EPC apoptosis and impairment of colony structure were shown to be reversible by treating EPCs from patients with SLE with belimumab, a fully humanized monoclonal antibody against BAFF, suggesting that BAFF has a pro-apoptotic effect on EPCs[140]. Last, TNF has been shown to suppress proliferation, migration and tube formation and to increase apoptosis of EPCs[154]. As serum TNF levels are increased in patients with SLE[155], whether TNF exerts a negative effect on EPCs in patients with SLE requires further study.

Of note, circulating angiogenic T (T_{ang}) cells, which are $CD3^+CD31^+CXCR4$ (CXC-chemokine receptor 4)$^+$, are implicated in repair of damaged endothelium by orchestrating the functions of EPCs[156]. Working in concert with pro-angiogenic factors including IL-8 and VEGF, T_{ang} cells increase differentiation and function of EPCs[156] (FIG. 2). In patients with SLE, although the overall T_{ang} cell levels were increased compared with those in healthy individuals[157,158], expansion of T_{ang} cells was observed in the subset of T_{ang} cells that exhibited immunosenescent features, as indicated by the loss of T cell-specific surface glycoprotein CD28. T_{ang} cells were shown to exert an anti-inflammatory effect on endothelial cells and might mediate EPC function through the action of IL-8 (REF.[153]). High SLE disease activity was related to reduced circulating T_{ang} proportion, and in young patients with SLE who had not experienced any cardiovascular events, contraction of the T_{ang} compartment occurred early in the course of SLE and preceded overt cardiovascular events[159].

Potential therapies to restore EPCs

To date, there have been to our knowledge no clinical trials specifically designed to evaluate the long-term cardiovascular outcome of pharmacological EPC manipulation in patients with SLE. Yet, a limited number of short-term observational studies that attempted to address the effects of immunomodulatory and immunosuppressive therapies on the number and function of EPCs in patients with various autoimmune conditions provide preliminary concepts on the potential of therapeutic manipulation of EPCs in autoimmunity[160–164]. For example, the use of intravenous immunoglobulins and aspirin was shown to be associated with an increase in EPC proliferation and migration in children with Kawasaki disease after 7 days of treatment, along with the significantly correlated reduction of serum inflammatory markers including TNF and high sensitivity C-reactive protein[160].

The potential benefits of B cell depletion therapy in endothelial activity and function have been demonstrated[161]. In a mechanistic study, in vitro treatment of human umbilical vein endothelial cells with sera from patients with SLE and RA before and after therapy with rituximab (a chimeric antibody against the B cell surface marker CD20) revealed a significant reduction in the expression of endothelial cell activation markers, including VEGF, IL-8, ICAM1 and endothelial nitric oxide synthase (eNOS), in umbilical vein endothelial cells that had been exposed to rituximab-treated sera[161]. In a clinical study, which was limited to assessment of patients with RA, treatment with rituximab (1 g, 2 weeks apart) improved endothelial function[162] and

reduced clinical RA disease activity and serum levels of inflammatory markers[162].

The effects of anti-IFNα and anti-BAFF therapies on the quantity and function of EPCs in SLE have been addressed. In addition to reaching the primary end points of reducing SLE disease activities in their respective trials[163,164], both anti-IFNα and anti-BAFF therapies were shown to normalize the quantity and function of SLE EPCs; the potential benefits of the anifrolumab (a human monoclonal antibody against type I interferon receptor subunit 1) and belimumab for cardiovascular protection in SLE are worth further exploration.

Tofacitinib, one of the Janus kinase (JAK) inhibitors, has been evaluated for its efficacy in ameliorating murine lupus[165] and safety in patients with SLE[87]. The inhibition of the JAK–STAT pathway is therapeutically relevant because many pro-inflammatory cytokines, chemokines and growth factors involved in the pathophysiology of SLE, including type 1 interferon, are implicated in SLE-related inflammation and cardiovascular pathology[166]. In addition to a significant reduction in SLE disease activity, including lupus-related nephritis and dermatitis as well as autoantibody and pro-inflammatory cytokine production, treatment with tofacitinib in MRL/lpr lupus-prone mice led to a reduction in NETs formation, an increase in endothelial cell differentiation and an improvement of ED vasorelaxation[165]. In a phase I double-blind controlled trial that evaluated the safety of tofacitinib in patients with SLE, tofacitinib (5 mg twice daily) led to the improvement of endothelial-dependent vasorelaxation (assessed by PAT) and other markers of CVD risk, including HDL-c, cholesterol efflux capacity and arterial stiffness, compared with the placebo group[87]. Intriguingly, improvement of some of the CVD risk surrogates, such as RHI and arterial stiffness, was significantly more apparent in patients with SLE bearing the *STAT4* risk allele (rs7574865)[87] — an allele that was shown to be associated with a significantly increased risk of CVD in patients with SLE[167].

In addition to immunomodulatory therapies, health supplements and therapeutic agents for CVD and related comorbidities were shown to exert beneficial quantitative and qualitative effects on EPCs. For instance, vitamin D increased the proliferative and migratory activities of EPCs that were cultured from lupus PBMCs from mice and patients[24,168,169]. Furthermore, vitamin D upregulated NO production in EPCs, which was correlated with increased FMD in patients with SLE[168]. Indeed, supplementation of vitamin D to SLE EPCs with calcitriol restored angiogenicity with CXC-chemokine ligand 10 (CXCL10) reduction[169]. The definite beneficial effects in bones and the potential favourable effect on EPCs render vitamin D supplementation in patients with SLE an attractive strategy (FIG. 4).

Last, there are functional benefits to endothelial reactivity and EPCs related to the use of statins in myocardial infarction and stroke[170,171], angiotensin II receptor antagonists in patients with type II diabetes mellitus and myocardial infarction[172,173], antiplatelet agents in CVD[174] and a variety of anti-glycaemic agents, including gliclazide, pioglitazone and sitagliptin, in type II diabetes mellitus[175–177].

Of note, calcineurin inhibitors that were shown to be efficacious and safe in the treatment of lupus nephritis[178,179] potentially worsen cardiovascular outcome via their negative effects on EPCs. For example, cyclosporine A was shown to suppress the proliferation and increase the apoptosis of EPCs in vitro, possibility through reduced NO production in EPCs[180]. EPCs isolated from umbilical cord blood mononuclear cells revealed reduced proliferation, migration and tube formation upon in vitro treatment with cyclosporine A and tacrolimus[181]. Furthermore, these calcineurin inhibitors increased NF-κB p65 phosphorylation and nuclear translocation and upregulated expression of the mRNAs for TNF, IL-6, ICAM and VCAM mRNA expression[181]. These observations collectively suggest that calcineurin inhibitors mitigate the proliferation and function of EPCs and augment pro-inflammatory signalling and apoptosis of EPCs, potentially engendering adverse cardiovascular outcomes in addition to their off-target lipidogenic and diabetogenic effects.

FMD and EPCs as prognostic biomarkers

Although using FMD and EPCs as markers to diagnose endothelial dysfunction and prognosticate for CVD is practically feasible and scientifically sound, there are some foreseeable challenges to the application of these approaches in patients with SLE. As for FMD, one of the major drawbacks that confound the interpretation of baED-FMD is that its evaluation is highly operator dependent, in addition to the technical difficulty

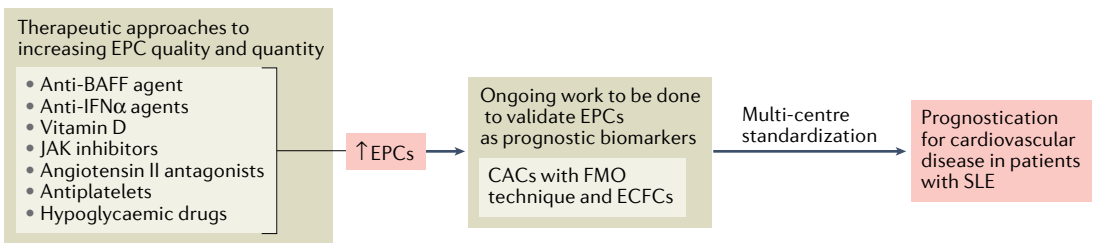

Fig. 4 | **Regulation of EPCs by different therapeutic approaches and their prognostic potential for CVD in SLE.** Improvements in the function and quantity of endothelial progenitor cell (EPCs) are observed with systemic lupus erythematosus (SLE)-related and non-SLE-related therapeutic approaches in patients with SLE. Further multi-centre validation work that involves flow cytometry (with the florescent-minus-one (FMO) technique) for the enumeration of circulating angiogenic cells (CACs) and assessment of endothelial colony-forming cells (ECFCs) is fundamental to prognosticating cardiovascular disease in patients with SLE. BAFF, B cell activating factor; CVD, cardiovascular disease; IFNα, interferon-α; JAK Janus kinase.

in tracking and recording the brachial artery waveforms generated from an unsteady forearm during the assessment procedure. To mitigate the issue of operator dependency, measurement of RHI with PAT is a newer alternative[87–89]. Similar to FMD, whereas endothelial dysfunction measured by RHI was consistently shown to be more prevalent in patients with SLE than in healthy individuals[87–89], further validation is required to advocate RHI use as a prognostic biophysical marker of CVD in patients with SLE, despite the absence of operator dependency, shorter assessment time and lower operative cost than baED-FMD (TABLE 1).

As elaborated in previous sections, no standardized method exists to identify EPCs that are consistently reliable for prognostication for CVD in patients with SLE. The robustness of ECFCs as putative EPCs is compromised by the tedious culture methods, whereas the extreme rarity of CACs augments the technical difficulty for their identification. Although the FMO technique minimizes inaccurate enumeration of CACs as a result of their scarcity in the peripheral blood and weak expressions of CD133 and CD309, the necessary expertise of the operator in designing fluorochrome-conjugated antibody panels, cellular event gating and subsequent flow cytometric analyses implies that methodological validation across different centres is fundamental before a scientifically robust and practical consensus can be reached. Taking into account all the potential technical difficulties and applications discussed, it is likely that the enumeration of CACs identified by multi-colour flow cytometry following the EUSTAR recommendations and application of the FMO staining and gating technique, coupled with the isolation and characterization of ECFCs via meticulous culture techniques and assays, will be the foundations for CVD prognostication in patients with SLE[33,34,140–143] (FIG. 4). The standardization and implementation of these techniques for studying EPCs in larger cohorts of patients with SLE and the collection of long-term prospective data are possibly the pre-requisites for advocating the potential of EPCs as a prognostic biomarker of CVD in SLE (FIG. 4). Nevertheless, a tremendous ongoing effort is required to align centres with interest in EPC research to reach a consensus of the standardized methodologies in flow cytometry and ECFC culture, because these methodologies require sophisticated laboratory conditions and expertise, which might not be readily accessible in some centres.

Although the bone marrow is likely to be the main source of circulating ECFCs, there is emerging evidence of small foci of ECFCs in different tissues that contribute towards vascular repair in conjunction with MACs, with several lines of evidence pointing towards their collaborative role in tissue repair[182,183]. These tissue-resident ECFCs are activated where there is tissue injury to repair the vasculature, with their function and quantity varying through different tissues and disease states[184,185]. With the development of sensitive methods of assessing both number and function of circulating ECFCs, such as with a microfluidic capture system[186], a more complete picture of the ECFC population can be determined through a much lower blood volume. Correspondingly, the development of single-cell genomics technologies should enable the heterogeneity and rarity of these ECFCs to be investigated with regard to their differentiation trajectories and hierarchies, along with probable cell–cell interactions, in the near future[187,188]. These new developments may usher in a new understanding of the role of ECFCs in SLE and other disease states.

Conclusion

Although traditional CVD risk factors (which have increased prevalence in individuals with SLE) lead to excess cardiovascular morbidity and mortality in patients with SLE, the mechanisms leading to the heightened prevalence and incidence of CVD beyond the effect of traditional risk factors remain incompletely understood. Among these mechanisms, the reduced circulating level and impaired function of EPCs in vitro and impaired biophysical endothelial function are postulated to have a negative effect on the cardiovascular outcome of patients with SLE. With properly conducted prospective observational studies and clinical trials based on a consensus with a clear definition of EPCs and standardized methodologies for endothelial function evaluation, quantitative and functional parameters of EPCs and endothelial function measures with baED-FMD can be potential prognostication biomarkers of CVD in patients with SLE (FIG. 4). Taking together the promising in vitro findings and murine model studies related to the reversal of reduction of EPC number and function with anti-type 1 IFNα and anti-BAFF therapies, it is worth studying the potential cardiovascular benefits of the use of anti-IFNα and anti-BAFF agents and JAK inhibitors in patients with SLE. In concert, cost-effective and safe therapeutic strategies such as vitamin D supplementation and antimalarial drugs are highly recommended for their potential cardiovascular benefits in patients with SLE for whom these treatments are not contraindicated.

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1. Barbhaiya, M. et al. Comparative risks of cardiovascular disease in patients with systemic lupus erythematosus, diabetes mellitus, and in general Medicaid recipients. *Arthritis Care Res.* **72**, 1431–1439 (2020).
2. Jonsson, H., Nived, O. & Sturfelt, G. Outcome in systemic lupus erythematosus: a prospective study of patients from a defined population. *Medicine* **68**, 141–150 (1989).
3. Manzi, S. et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am. J. Epidemiol.* **145**, 408–415 (1997).
4. Bernatsky, S. et al. Mortality in systemic lupus erythematosus. *Arthritis Rheum.* **54**, 2550–2557 (2006).
5. Yurkovich, M., Vostretsova, K., Chen, W. & Aviña-Zubieta, J. A. Overall and cause-specific mortality in patients with systemic lupus erythematosus: a meta-analysis of observational studies. *Arthritis Care Res.* **66**, 608–616 (2014).
6. Goldberg, R. J., Urowitz, M. B., Ibañez, D., Nikpour, M. & Gladman, D. D. Risk factors for development of coronary artery disease in women with systemic lupus erythematosus. *J. Rheumatol.* **36**, 2454–2461 (2009).
7. Colombo, B. M. et al. Traditional and non traditional risk factors in accelerated atherosclerosis in systemic lupus erythematosus: role of vascular endothelial growth factor (VEGATS Study). *Autoimmun. Rev.* **8**, 309–315 (2009).
8. Moghaddam, B., Marozoff, S., Li, L., Sayre, E. C. & Zubieta, J. A. A. All-cause and cause-specific mortality in systemic lupus erythematosus: a population-based study. *Rheumatology* https://doi.org/10.1093/rheumatology/keab362 (2021).
9. Leonard, D. et al. Coronary heart disease in systemic lupus erythematosus is associated with interferon regulatory factor-8 gene variants. *Circ. Cardiovasc. Genet.* **6**, 255–263 (2013).
10. Bahrehmand, F. et al. Matrix metalloproteinase-2 functional promoter polymorphism G1575A is associated with elevated circulatory MMP-2 levels and increased risk of cardiovascular disease in systemic lupus erythematosus patients. *Lupus* **21**, 616–624 (2012).

11. Bicakcigil, M., Tasan, D., Tasdelen, N., Mutlu, N. & Yavuz, S. Role of fibrinolytic parameters and plasminogen activator inhibitor 1 (PAI-1) promoter polymorphism on premature atherosclerosis in SLE patients. *Lupus* **20**, 1063–1071 (2011).
12. Troelsen, L. N., Garred, P., Christiansen, B., Torp-Pedersen, C. & Jacobsen, S. Genetically determined serum levels of mannose-binding lectin correlate negatively with common carotid intima-media thickness in systemic lupus erythematosus. *J. Rheumatol.* **37**, 1815–1821 (2010).
13. Marasini, B. et al. Genetic contribution to carotid vascular disease in patients with systemic lupus erythematosus. *J. Clin. Immunol.* **28**, 131–133 (2008).
14. Szalai, A. J. et al. Systemic lupus erythematosus in a multiethnic US Cohort (LUMINA). XXX: association between C-reactive protein (CRP) gene polymorphisms and vascular events. *Rheumatology* **44**, 864–868 (2005).
15. Frieri, M. Accelerated atherosclerosis in systemic lupus erythematosus: role of proinflammatory cytokines and therapeutic approaches. *Curr. Allergy Asthma Rep.* **12**, 25–32 (2012).
16. Medeiros, P. B. S. et al. Disease activity index is associated with subclinical atherosclerosis in childhood-onset systemic lupus erythematosus. *Pediatr. Rheumatol. Online J.* **19**, 35 (2021).
17. Faurschou, M. et al. High risk of ischemic heart disease in patients with lupus nephritis. *J. Rheumatol.* **38**, 2400–2405 (2011).
18. Ajeganova, S., Hafström, I. & Frostegård, J. Patients with SLE have higher risk of cardiovascular events and mortality in comparison with controls with the same levels of traditional risk factors and intima-media measures, which is related to accumulated disease damage and antiphospholipid syndrome: a case-control study over 10 years. *Lupus Sci. Med.* **8**, e000454 (2021).
19. Montarello, N. J., Nguyen, M. T., Wong, D. T. L., Nicholls, S. J. & Psaltis, P. J. Inflammation in coronary atherosclerosis and its therapeutic implications. *Cardiovasc Drugs Ther.* https://doi.org/10.1007/s10557-020-07106-6 (2020).
20. Oikonomou, E. et al. A link between inflammation and thrombosis in atherosclerotic cardiovascular diseases: clinical and therapeutic implications. *Atherosclerosis* **309**, 16–26 (2020).
21. Miller, A. M. & McInnes, I. B. Cytokines as therapeutic targets to reduce cardiovascular risk in chronic inflammation. *Curr. Pharm. Des.* **17**, 1–8 (2011).
22. Steyers, C. M. 3rd & Miller, F. J. Jr Endothelial dysfunction in chronic inflammatory diseases. *Int. J. Mol. Sci.* **15**, 11324–11349 (2014).
23. Sitia, S. et al. From endothelial dysfunction to atherosclerosis. *Autoimmun. Rev.* **9**, 830–834 (2010).
24. Reynolds, J. A. et al. Brief report: vitamin D deficiency is associated with endothelial dysfunction and increases type I interferon gene expression in a murine model of systemic lupus erythematosus. *Arthritis Rheumatol.* **68**, 2929–2935 (2016).
25. Stalc, M., Tomsic, M., Jezovnik, M. K. & Poredos, P. Endothelium-dependent and independent dilation capability of peripheral arteries in patients with systemic lupus erythematosus and antiphospholipid syndrome. *Clin. Exp. Rheumatol.* **29**, 616–623 (2011).
26. Johnson, S. R. et al. Impaired brachial artery endothelium dependent flow mediated dilation in systemic lupus erythematosus: preliminary observations. *Lupus* **13**, 590–593 (2004).
27. Kiss, E. et al. Reduced flow-mediated vasodilation as a marker for cardiovascular complications in lupus patients. *J. Autoimmun.* **27**, 211–217 (2006).
28. Ghosh, P. et al. Subclinical atherosclerosis and endothelial dysfunction in young South-Asian patients with systemic lupus erythematosus. *Clin. Rheumatol.* **28**, 1259–1265 (2009).
29. Go, E. & Yoder, M. C. Identification of endothelial cells and their progenitors. *Methods Mol. Biol.* **2206**, 27–37 (2021).
30. Mao, S. Z., Ye, X., Liu, G., Song, D. & Liu, S. F. Resident endothelial cells and endothelial progenitor cells restore endothelial barrier function after inflammatory lung injury. *Arterioscler. Thromb. Vasc. Biol.* **35**, 1635–1644 (2015).
31. Baker, J. F. et al. Circulating endothelial progenitor cells are reduced in SLE in the absence of coronary artery calcification. *Rheumatol. Int.* **32**, 997–1002 (2012).
32. Westerweel, P. E. et al. Haematopoietic and endothelial progenitor cells are deficient in quiescent systemic lupus erythematosus. *Ann. Rheum. Dis.* **66**, 865–870 (2007).
33. Denny, M. F. et al. Interferon-alpha promotes abnormal vasculogenesis in lupus: a potential pathway for premature atherosclerosis. *Blood* **110**, 2907–2915 (2007).
34. Lee, P. Y. et al. Type I interferon as a novel risk factor for endothelial progenitor cell depletion and endothelial dysfunction in systemic lupus erythematosus. *Arthritis Rheum.* **56**, 3759–3769 (2007).
35. Moonen, J. R. et al. Reduced number and impaired function of circulating progenitor cells in patients with systemic lupus erythematosus. *Arthritis Res. Ther.* **9**, R84 (2007).
36. Huang, J., Kow, N. Y., Lee, H. Y., Fairhurst, A. M. & Mak, A. $CD34^+CD133^+CD309^+$ circulating angiogenic cell level is reduced but positively related to hydroxychloroquine use in SLE patients — a case-control study and meta-regression analysis. *Rheumatology* **60**, 3936–3944 (2021).
37. Kim, J. Y. et al. Osteoprotegerin causes apoptosis of endothelial progenitor cells by induction of oxidative stress. *Arthritis Rheum.* **65**, 2172–2182 (2013).
38. Mak, A. & Kow, N. Y. Imbalance between endothelial damage and repair: a gateway to cardiovascular disease in systemic lupus erythematosus. *Biomed. Res. Int.* **2014**, 178721 (2014).
39. Ding, X., Xiang, W. & He, X. IFN-I mediates dysfunction of endothelial progenitor cells in atherosclerosis of systemic lupus erythematosus. *Front. Immunol.* **11**, 581385 (2020).
40. Komici, K. et al. Systemic lupus erythematosus, endothelial progenitor cells and intracellular Ca^{2+} signaling: a novel approach for an old disease. *J. Autoimmun.* **112**, 102486 (2020).
41. Sánchez-Pérez, H. et al. Impaired HDL cholesterol efflux capacity in systemic lupus erythematosus patients is related to subclinical carotid atherosclerosis. *Rheumatology* **59**, 2847–2856 (2020).
42. Quevedo-Abeledo, J. C. et al. Differences in HDL-cholesterol efflux capacity between patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Care Res.* https://doi.org/10.1002/acr.24407 (2020).
43. Li, J. et al. Novel insights: dynamic foam cells derived from the macrophage in atherosclerosis. *J. Cell Physiol.* **236**, 6154–6167 (2021).
44. Smith, C. K. et al. Lupus high-density lipoprotein induces proinflammatory responses in macrophages by binding lectin-like oxidised low-density lipoprotein receptor 1 and failing to promote activating transcription factor 3 activity. *Ann. Rheum. Dis.* **76**, 602–611 (2017).
45. McMahon, M. et al. A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheumatol.* **66**, 130–139 (2014).
46. Parikh, S. V., Almaani, S., Brodsky, S. & Rovin, B. H. Update on lupus nephritis: core curriculum 2020. *Am. J. Kidney Dis.* **76**, 265–281 (2020).
47. Mak, A., Cheung, M. W., Chiew, H. J., Liu, Y. & Ho, R. C. Global trend of survival and damage of systemic lupus erythematosus: meta-analysis and meta-regression of observational studies from the 1950s to 2000s. *Semin. Arthritis Rheum.* **41**, 830–839 (2012).
48. Hermansen, M. L., Lindhardsen, J., Torp-Pedersen, C., Faurschou, M. & Jacobsen, S. The risk of cardiovascular morbidity and cardiovascular mortality in systemic lupus erythematosus and lupus nephritis: a Danish nationwide population-based cohort study. *Rheumatology* **56**, 709–715 (2017).
49. Liu, Y. & Kaplan, M. J. Cardiovascular disease in systemic lupus erythematosus: an update. *Curr. Opin. Rheumatol.* **30**, 441–448 (2018).
50. Sethna, C. B., Merchant, K. & Reyes, A. Cardiovascular disease risk in children with kidney disease. *Semin. Nephrol.* **38**, 298–313 (2018).
51. Kahn, M. R., Robbins, M. J., Kim, M. C. & Fuster, V. Management of cardiovascular disease in patients with kidney disease. *Nat. Rev. Cardiol.* **10**, 261–273 (2013).
52. Sun, E. Y., Alvarez, C. & Sheikh, S. Z. Association of lupus nephritis with coronary artery disease by ISN/RPS classification: results from a large real-world lupus population. *ACR Open Rheumatol.* **1**, 244–250 (2019).
53. Frangou, E., Georgakis, S. & Bertsias, G. Update on the cellular and molecular aspects of lupus nephritis. *Clin. Immunol.* **216**, 108445 (2020).
54. Dimou, P. et al. The human glomerular endothelial cells are potent pro-inflammatory contributors in an in vitro model of lupus nephritis. *Sci. Rep.* **9**, 8348 (2019).
55. Deng, W. et al. $CD8^+CD103^+$ iTregs inhibit the progression of lupus nephritis by attenuating glomerular endothelial cell injury. *Rheumatology* **58**, 2039–2050 (2019).
56. Choe, J. Y., Lee, S. S., Kwak, S. G. & Kim, S. K. Anti-Sm antibody, damage index, and corticosteroid use are associated with cardiac involvement in systemic lupus erythematosus: data from a prospective registry study. *J. Korean Med. Sci.* **35**, e139 (2020).
57. Urowitz, M. B. et al. Accrual of atherosclerotic vascular events in a multicenter inception systemic lupus erythematosus cohort. *Arthritis Rheumatol.* **72**, 1734–1740 (2020).
58. Fasano, S. et al. Prolonged remission is associated with a reduced risk of cardiovascular disease in patients with systemic lupus erythematosus: a GIRRCS (Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale) study. *Clin. Rheumatol.* **38**, 457–463 (2019).
59. Asanuma, Y. et al. Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N. Engl. J. Med.* **349**, 2407–2415 (2003).
60. Rho, Y. H. et al. Novel cardiovascular risk factors in premature coronary atherosclerosis associated with systemic lupus erythematosus. *J. Rheumatol.* **35**, 1789–1794 (2008).
61. Yiu, K. H. et al. Pattern of arterial calcification in patients with systemic lupus erythematosus. *J. Rheumatol.* **36**, 2212–2217 (2019).
62. Kiani, A. N. et al. Coronary calcification in SLE: comparison with the multi-ethnic study of atherosclerosis. *Rheumatology* **54**, 1976–1981 (2015).
63. Khan, A., Arbab-Zadeh, A., Kiani, A. N., Magder, L. S. & Petri, M. Progression of noncalcified and calcified coronary plaque by CT angiography in SLE. *Rheumatol. Int.* **37**, 59–65 (2017).
64. Kiani, A. N., Magder, L. S. & Petri, M. Mycophenolate mofetil (MMF) does not slow the progression of subclinical atherosclerosis in SLE over 2 years. *Rheumatol. Int.* **32**, 2701–2705 (2012).
65. Evans, C. E., Iruela-Arispe, M. L. & Zhao, Y. Y. Mechanisms of endothelial regeneration and vascular repair and their application to regenerative medicine. *Am. J. Pathol.* **191**, 52–65 (2021).
66. Konukoglu, D. & Uzun, H. Endothelial dysfunction and hypertension. *Adv. Exp. Med. Biol.* **956**, 511–540 (2017).
67. Bai, B. et al. NLRP3 inflammasome in endothelial dysfunction. *Cell Death Dis.* **11**, 776 (2020).
68. Tabrizi, R. et al. The effects of vitamin D supplementation on markers related to endothelial function among patients with metabolic syndrome and related disorders: a systematic review and meta-analysis of clinical trials. *Horm. Metab. Res.* **50**, 587–596 (2018).
69. Simsek, E. C. et al. Endothelial dysfunction in patients with myocardial ischemia or infarction and nonobstructive coronary arteries. *J. Clin. Ultrasound* **49**, 334–340 (2021).
70. Koo, B. K., Chung, W. Y. & Moon, M. K. Peripheral arterial endothelial dysfunction predicts future cardiovascular events in diabetic patients with albuminuria: a prospective cohort study. *Cardiovasc. Diabetol.* **19**, 82 (2020).
71. Migliacci, R. et al. Walking-induced endothelial dysfunction predicts ischemic cardiovascular events in patients with intermittent claudication. *Vasc. Med.* **26**, 394–400 (2021).
72. Schächinger, V., Britten, M. B. & Zeiher, A. M. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* **101**, 1899–1906 (2000).
73. Halcox, J. P. et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* **106**, 653–658 (2002).
74. Barsalou, J. et al. Impact of disease duration on vascular surrogates of early atherosclerosis in childhood-onset systemic lupus erythematosus. *Arthritis Rheumatol.* **68**, 237–246 (2016).
75. Taraborelli, M. et al. Endothelial dysfunction in early systemic lupus erythematosus patients and controls without previous cardiovascular events. *Arthritis Care Res.* **70**, 1277–1283 (2018).
76. Wennmalm, A. Endothelial nitric oxide and cardiovascular disease. *J. Intern. Med.* **235**, 317–327 (1994).

77. Furchgott, R. F. & Zawadzki, J. V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373–376 (1980).
78. Soga, J. et al. Relationship between augmentation index and flow-mediated vasodilation in the brachial artery. *Hypertens. Res.* **31**, 1293–1298 (2008).
79. Gokce, N. et al. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension* **38**, 1349–1354 (2001).
80. Mak, A., Liu, Y. & Ho, R. C. Endothelium-dependent but not endothelium-independent flow-mediated dilation is significantly reduced in patients with systemic lupus erythematosus without vascular events: a metaanalysis and metaregression. *J. Rheumatol.* **38**, 1296–1303 (2011).
81. Mak, A. et al. Endothelial dysfunction in systemic lupus erythematosus — a case-control study and an updated meta-analysis and meta-regression. *Sci. Rep.* **7**, 7320 (2017).
82. Riancho-Zarrabeitia, L. et al. Antiphospholipid syndrome (APS) in patients with systemic lupus erythematosus (SLE) implies a more severe disease with more damage accrual and higher mortality. *Lupus* **29**, 1556–1565 (2020).
83. Ramsey-Goldman, R. & Manzi, S. Association of osteoporosis and cardiovascular disease in women with systemic lupus erythematosus. *Arthritis Rheum.* **44**, 2338–2341 (2001).
84. Mak, A. et al. Lumbar spine bone mineral density predicts endothelial reactivity in patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **29**, 261–268 (2011).
85. Zhu, J. et al. The association of endothelial nitric oxide synthase gene single nucleotide polymorphisms with paediatric systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **36**, 508–512 (2018).
86. Katkam, S. K., Indumathi, B., Tasneem, F. S. D., Rajasekhar, L. & Kutala, V. K. Impact of eNOS 27-bp VNTR (4b/a) gene polymorphism with the risk of Systemic Lupus Erythematosus in south Indian subjects. *Gene* **658**, 105–112 (2018).
87. Hasni, S. A. et al. Phase 1 double-blind randomized safety trial of the Janus kinase inhibitor tofacitinib in systemic lupus erythematosus. *Nat. Commun.* **12**, 3391 (2021).
88. Santos, M. J. et al. Early vascular alterations in SLE and RA patients — a step towards understanding the associated cardiovascular risk. *PLoS One* **7**, e44668 (2012).
89. Tydén, H. et al. Low plasma concentrations of apolipoprotein M are associated with disease activity and endothelial dysfunction in systemic lupus erythematosus. *Arthritis Res. Ther.* **21**, 110 (2019).
90. Fusco, E. et al. Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique. *Nutr. Metab. Cardiovasc. Dis.* **30**, 306–312 (2020).
91. Tydén, H. et al. Endothelial dysfunction is associated with activation of the type I interferon system and platelets in patients with systemic lupus erythematosus. *RMD Open* **3**, e000508 (2017).
92. Indraccolo, S. et al. Identification of genes selectively regulated by IFNs in endothelial cells. *J. Immunol.* **178**, 1122–1135 (2007).
93. Hsu, K. S. et al. Dual regulation of Stat1 and Stat3 by the tumor suppressor protein PML contributes to interferon α-mediated inhibition of angiogenesis. *J. Biol. Chem.* **292**, 10048–10060 (2017).
94. Niessner, A. & Weyand, C. M. Dendritic cells in atherosclerotic disease. *Clin. Immunol.* **134**, 25–32 (2010).
95. Higashiyama, M. et al. Interferon-α increases monocyte migration via platelet-monocyte interaction in murine intestinal microvessels. *Clin. Exp. Immunol.* **162**, 156–162 (2010).
96. Patiño-Trives, A. M. et al. Anti-dsDNA antibodies increase the cardiovascular risk in systemic lupus erythematosus promoting a distinctive immune and vascular activation. *Arterioscler. Thromb. Vasc. Biol.* **41**, 2417–2430 (2021).
97. Campos-López, B. et al. Association of cardiometabolic risk status with clinical activity and damage in systemic lupus erythematosus patients: a cross-sectional study. *Clin. Immunol.* **222**, 108637 (2021).
98. Rodríguez-Calvo, R. et al. Low-density lipoprotein from active SLE patients is more atherogenic to endothelial cells than low-density lipoprotein from the same patients during remission. *Rheumatology* **60**, 866–871 (2021).
99. Theofilis, P. et al. Inflammatory mechanisms contributing to endothelial dysfunction. *Biomedicines* **9**, 781 (2021).
100. Hernandez, N. M., Casselbrant, A., Joshi, M., Johansson, B. R. & Sumitran-Holgersson, S. Antibodies to kidney endothelial cells contribute to a "leaky" glomerular barrier in patients with chronic kidney diseases. *Am. J. Physiol. Renal Physiol.* **302**, F884–F894 (2012).
101. Velásquez, M., Rojas, M., Abrahams, V. M., Escudero, C. & Cadavid, Á. P. Mechanisms of endothelial dysfunction in antiphospholipid syndrome: association with clinical manifestations. *Front. Physiol.* **9**, 1840 (2018).
102. Mancardi, D. et al. Endothelial dysfunction and cardiovascular risk in lupus nephritis: new roles for old players? *Eur. J. Clin. Invest.* **51**, e13441 (2021).
103. Valer, P., Paul, B., Eugenia, B. & Camelia, B. Annexin A5 as independent predictive biomarker for subclinical atherosclerosis and endothelial dysfunction in systemic lupus erythematosus patients. *Clin. Lab.* **59**, 359–367 (2013).
104. Mobarrez, F. et al. Microparticles in the blood of patients with systemic lupus erythematosus (SLE): phenotypic characterization and clinical associations. *Sci. Rep.* **6**, 36025 (2016).
105. Martínez, M. C., Tesse, A., Zobairi, F. & Andriantsitohaina, R. Shed membrane microparticles from circulating and vascular cells in regulating vascular function. *Am. J. Physiol. Heart Circ. Physiol.* **288**, H1004–H1009 (2005).
106. Shao, W. H. The role of microparticles in rheumatic diseases and their potentials as therapeutic tools. *J. Mol. Immunol.* **1**, 101 (2016).
107. Puddu, P., Puddu, G. M., Cravero, E., Muscari, S. & Muscari, A. The involvement of circulating microparticles in inflammation, coagulation and cardiovascular diseases. *Can. J. Cardiol.* **26**, 140–145 (2010).
108. Markiewicz, M., Richard, E., Marks, N. & Ludwicka-Bradley, A. Impact of endothelial microparticles on coagulation, inflammation, and angiogenesis in age-related vascular diseases. *J. Aging Res.* **2013**, 734509 (2013).
109. Mobarrez, F. et al. Microparticles in the blood of patients with SLE: size, content of mitochondria and role in circulating immune complexes. *J. Autoimmun.* **102**, 142–149 (2019).
110. Combes, V. et al. In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *J. Clin. Invest.* **104**, 93–102 (1999).
111. Tsokos, G. C., Lo, M. S., Costa Reis, P. & Sullivan, K. E. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* **12**, 716–730 (2016).
112. Parker, B. et al. Suppression of inflammation reduces endothelial microparticles in active systemic lupus erythematosus. *Ann. Rheum. Dis.* **73**, 1144–1150 (2014).
113. Atehortúa, L. et al. Endothelial activation and injury by microparticles in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Res. Ther.* **21**, 34 (2019).
114. López, P., Rodríguez-Carrio, J., Martínez-Zapico, A., Caminal-Montero, L. & Suárez, A. Circulating microparticle subpopulations in systemic lupus erythematosus are affected by disease activity. *Int. J. Cardiol.* **236**, 138–144 (2017).
115. Brinkmann, V. et al. Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
116. Garcia-Romo, G. S. et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* **3**, 73ra20 (2011).
117. Gupta, A. K. et al. Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death. *FEBS Lett.* **584**, 3193–3197 (2010).
118. Carmona-Rivera, C., Zhao, W., Yalavarthi, S. & Kaplan, M. J. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. *Ann. Rheum. Dis.* **74**, 1417–1424 (2015).
119. von Brühl, M. L. et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J. Exp. Med.* **209**, 819–835 (2012).
120. Massberg, S. et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat. Med.* **16**, 887–896 (2010).
121. Pieterse, E. et al. Neutrophil extracellular traps drive endothelial-to-mesenchymal transition. *Arterioscler. Thromb. Vasc. Biol.* **37**, 1371–1379 (2017).
122. Lande, R. et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci. Transl. Med.* **3**, 73ra19 (2011).
123. Döring, Y. et al. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. *Circulation* **125**, 1673–1683 (2012).
124. Knight, J. S. et al. Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. *Ann. Rheum. Dis.* **74**, 2199–2206 (2015).
125. Pardanaud, L., Altmann, C., Kitos, P., Dieterlen-Lievre, F. & Buck, C. A. Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. *Development* **100**, 339–349 (1987).
126. Asahara, T. et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275**, 964–967 (1997).
127. Chong, M. S., Ng, W. K. & Chan, J. K. Concise review: endothelial progenitor cells in regenerative medicine: applications and challenges. *Stem Cell Transl. Med.* **5**, 530–538 (2016).
128. Purhonen, S. et al. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. *Proc. Natl Acad. Sci. USA* **105**, 6620–6625 (2008).
129. Barber, C. L. & Iruela-Arispe, M. L. The ever-elusive endothelial progenitor cell: identities, functions and clinical implications. *Pediatr. Res.* **59**, 26R–32R (2006).
130. Basile, D. P. & Yoder, M. C. Circulating and tissue resident endothelial progenitor cells. *J. Cell Physiol.* **229**, 10–16 (2014).
131. Schmeisser, A. & Strasser, R. H. Phenotypic overlap between hematopoietic cells with suggested angioblastic potential and vascular endothelial cells. *J. Hematother. Stem Cell Res.* **11**, 69–79 (2002).
132. Schmeisser, A., Graffy, C., Daniel, W. G. & Strasser, R. H. Phenotypic overlap between monocytes and vascular endothelial cells. *Adv. Exp. Med. Biol.* **522**, 59–74 (2003).
133. Desai, A. et al. Microarray-based characterization of a colony assay used to investigate endothelial progenitor cells and relevance to endothelial function in humans. *Arterioscler. Thromb. Vasc. Biol.* **29**, 121–127 (2009).
134. Medina, R. J. et al. Myeloid angiogenic cells act as alternative M2 macrophages and modulate angiogenesis through interleukin-8. *Mol. Med.* **17**, 1045–1055 (2011).
135. Zhao, H. & He, Y. The inhibitory effect of lysophosphatidylcholine on proangiogenesis of human $CD34^+$ cells derived endothelial progenitor cells. *Front. Mol. Biosci.* **8**, 682367 (2021).
136. Liu, Y. et al. Contrasting effects of vasculogenic induction upon biaxial bioreactor stimulation of mesenchymal stem cells and endothelial progenitor cells cocultures in three-dimensional scaffolds under in vitro and in vivo paradigms for vascularized bone tissue engineering. *Tissue Eng. A* **19**, 893–904 (2013).
137. Ingram, D. A. et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* **104**, 2752–2760 (2004).
138. Yoder, M. C. et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* **109**, 1801–1809 (2007).
139. Medina, R. J. et al. Endothelial progenitors: a consensus statement on nomenclature. *Stem Cell Transl. Med.* **6**, 1316–1320 (2017).
140. Spinelli, F. R. et al. B lymphocyte stimulator modulates number and function of endothelial progenitor cells in systemic lupus erythematosus. *Arthritis Res. Ther.* **21**, 245 (2019).
141. Deng, X. L., Li, X. X., Liu, X. Y., Sun, L. & Liu, R. Comparative study on circulating endothelial progenitor cells in systemic lupus erythematosus patients at active stage. *Rheumatol. Int.* **30**, 1429–1436 (2010).
142. Kahlenberg, J. M. et al. Inflammasome activation of IL-18 results in endothelial progenitor cell dysfunction in systemic lupus erythematosus. *J. Immunol.* **187**, 6143–6156 (2011).

143. Thacker, S. G. et al. The detrimental effects of IFN-α on vasculogenesis in lupus are mediated by repression of IL-1 pathways: potential role in atherogenesis and renal vascular rarefaction. *J. Immunol.* **185**, 4457–4469 (2010).
144. Ablin, J. N. et al. Enhanced adhesive properties of endothelial progenitor cells (EPCs) in patients with SLE. *Rheumatol. Int.* **31**, 773–778 (2011).
145. Grisar, J. et al. Systemic lupus erythematosus patients exhibit functional deficiencies of endothelial progenitor cells. *Rheumatology* **47**, 1476–1483 (2008).
146. Distler, J. H. et al. EULAR Scleroderma Trials and Research group statement and recommendations on endothelial precursor cells. *Ann. Rheum. Dis.* **68**, 163–168 (2009).
147. Kuwana, M. & Okazaki, Y. Quantification of circulating endothelial progenitor cells in systemic sclerosis: a direct comparison of protocols. *Ann. Rheum. Dis.* **71**, 617–620 (2012).
148. Williamson, K. A. et al. Age-related impairment of endothelial progenitor cell migration correlates with structural alterations of heparan sulfate proteoglycans. *Aging Cell* **12**, 139–147 (2013).
149. Ebner, P. et al. Accumulation of VEGFR-2$^+$/CD133$^+$ cells and decreased number and impaired functionality of CD34$^+$/VEGFR-2$^+$ cells in patients with SLE. *Rheumatology* **49**, 63–72 (2010).
150. Ding, X. et al. Neutralizing interferon-α blocks inflammation-mediated vascular injury via PI3K and AMPK in systemic lupus erythematosus. *Immunology* https://doi.org/10.1111/imm.13379 (2021).
151. Thacker, S. G. et al. Type I interferons modulate vascular function, repair, thrombosis, and plaque progression in murine models of lupus and atherosclerosis. *Arthritis Rheum.* **64**, 2975–2985 (2012).
152. Denny, M. F. et al. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. *J. Immunol.* **184**, 3284–3297 (2010).
153. Möckel, T., Basta, F., Weinmann-Menke, J. & Schwarting, A. B cell activating factor (BAFF): structure, functions, autoimmunity and clinical implications in Systemic Lupus Erythematosus (SLE). *Autoimmun. Rev.* **20**, 102736 (2021).
154. Naserian, S. et al. The TNF/TNFR2 signaling pathway is a key regulatory factor in endothelial progenitor cell immunosuppressive effect. *Cell Commun. Signal.* **18**, 94 (2020).
155. Mak, A., Tang, C. S. & Ho, R. C. Serum tumour necrosis factor-alpha is associated with poor health-related quality of life and depressive symptoms in patients with systemic lupus erythematosus. *Lupus* **22**, 254–261 (2013).
156. Hur, J. et al. Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. *Circulation* **116**, 1671–1682 (2007).
157. Miao, J. et al. Circulating angiogenic T cells and their subpopulations in patients with systemic lupus erythematosus. *Mediators Inflamm.* **2016**, 2842143 (2016).
158. Zhao, P. et al. Circulating angiogenic T cells are increased in lupus nephritis patients. *Med. Sci. Monit.* **24**, 5384–5390 (2018).
159. López, P., Rodríguez-Carrio, J., Martínez-Zapico, A., Caminal-Montero, L. & Suarez, A. Senescent profile of angiogenic T cells from systemic lupus erythematosus patients. *J. Leukoc. Biol.* **99**, 405–412 (2016).
160. Xu, M. G. et al. The functions of endothelial progenitor cells were significantly improved after treatment with intravenous immunoglobulin and aspirin in children with Kawasaki disease. *Pediatr. Cardiol.* **32**, 455–460 (2011).
161. Pérez-Sánchez, C. et al. Early restoration of immune and vascular phenotypes in systemic lupus erythematosus and rheumatoid arthritis patients after B cell depletion. *J. Cell Mol. Med.* **23**, 6308–6318 (2019).
162. Hsue, P. Y. et al. Depletion of B-cells with rituximab improves endothelial function and reduces inflammation among individuals with rheumatoid arthritis. *J. Am. Heart Assoc.* **3**, e001267 (2014).
163. Morand, E. F. et al. Trial of anifrolumab in active systemic lupus erythematosus. *N. Engl. J. Med.* **382**, 211–221 (2020).
164. Navarra, S. V. et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* **377**, 721–731 (2011).
165. Furumoto, Y. et al. Tofacitinib ameliorates murine lupus and its associated vascular dysfunction. *Arthritis Rheumatol.* **69**, 148–160 (2017).
166. Kaul, A. et al. Systemic lupus erythematosus. *Nat. Rev. Dis. Prim.* **2**, 16039 (2016).
167. Svenungsson, E. et al. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. *Ann. Rheum. Dis.* **69**, 834–840 (2010).
168. Huang, Z. et al. Vitamin D (1,25-(OH)$_2$D$_3$) improves endothelial progenitor cells function via enhanced no secretion in systemic lupus erythematosus. *Cardiol. Res. Pract.* **2020**, 6802562 (2020).
169. Reynolds, J. A. et al. Vitamin D improves endothelial dysfunction and restores myeloid angiogenic cell function via reduced CXCL-10 expression in systemic lupus erythematosus. *Sci. Rep.* **6**, 22341 (2016).
170. Sobrino, T., Blanco, M., Pérez-Mato, M., Rodríguez-Yáñez, M. & Castillo, J. Increased levels of circulating endothelial progenitor cells in patients with ischaemic stroke treated with statins during acute phase. *Eur. J. Neurol.* **19**, 1539–1546 (2012).
171. Baran, Ç. et al. Effects of preoperative short term use of atorvastatin on endothelial progenitor cells after coronary surgery: a randomized, controlled trial. *Stem Cell Rev. Rep.* **8**, 963–971 (2012).
172. Bahlmann, F. H. et al. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* **45**, 526–529 (2005).
173. Cangiano, E. et al. ACE inhibition modulates endothelial apoptosis and renewal via endothelial progenitor cells in patients with acute coronary syndromes. *Am. J. Cardiovasc. Drugs* **11**, 189–198 (2011).
174. França, C. N. et al. Endothelial progenitor cell mobilization and platelet microparticle release are influenced by clopidogrel plasma levels in stable coronary artery disease. *Circ. J.* **76**, 729–736 (2012).
175. Chen, L. L. et al. Effects of gliclazide on endothelial function in patients with newly diagnosed type 2 diabetes. *Eur. J. Pharmacol.* **659**, 296–301 (2011).
176. Fadini, G. P. et al. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. *Diabetes Care* **33**, 1607–1609 (2010).
177. Esposito, K. et al. Effects of pioglitazone versus metformin on circulating endothelial microparticles and progenitor cells in patients with newly diagnosed type 2 diabetes — a randomized controlled trial. *Diabetes Obes. Metab.* **13**, 439–445 (2011).
178. Mok, C. C. et al. Long-term outcome of a randomised controlled trial comparing tacrolimus with mycophenolate mofetil as induction therapy for active lupus nephritis. *Ann. Rheum. Dis.* **79**, 1070–1076 (2020).
179. Mok, C. C. et al. Tacrolimus versus mycophenolate mofetil for induction therapy of lupus nephritis: a randomised controlled trial and long-term follow-up. *Ann. Rheum. Dis.* **75**, 30–36 (2016).
180. Yang, L. et al. Cyclosporin A suppresses proliferation of endothelial progenitor cells: involvement of nitric oxide synthase inhibition. *Intern. Med.* **47**, 1457–1464 (2008).
181. Meyer, N., Brodowski, L., von Kaisenberg, C., Schröder-Heurich, B. & von Versen-Höynck, F. Cyclosporine A and tacrolimus induce functional impairment and inflammatory reactions in endothelial progenitor cells. *Int. J. Mol. Sci.* **22**, 9696 (2021).
182. Ingram, D. A. et al. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood* **105**, 2783–2786 (2005).
183. Yoon, C. H. et al. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation* **112**, 1618–1627 (2005).
184. Schwartz, S. M. & Benditt, E. P. Clustering of replicating cells in aortic endothelium. *Proc. Natl Acad. Sci. USA* **73**, 651–653 (1976).
185. Yoder, M. C. Is endothelium the origin of endothelial progenitor cells? *Arterioscler. Thromb. Vasc. Biol.* **30**, 1094–1103 (2010).
186. Lin, R. Z., Hatch, A., Antontsev, V. G., Murthy, S. K. & Melero-Martin, J. M. Microfluidic capture of endothelial colony-forming cells from human adult peripheral blood: phenotypic and functional validation in vivo. *Tissue Eng. C. Methods* **21**, 274–283 (2015).
187. Karlsson, G., Sommarin, M. N. E. & Böiers, C. Defining the emerging blood system during development at single-cell resolution. *Front. Cell Dev. Biol.* **9**, 660350 (2021).
188. Bian, Z. et al. Deciphering human macrophage development at single-cell resolution. *Nature* **582**, 571–576 (2020).
189. Tselios, K., Sheane, B. J., Gladman, D. D. & Urowitz, M. B. Optimal monitoring for coronary heart disease risk in patients with systemic lupus erythematosus: a systematic review. *J. Rheumatol.* **43**, 54–65 (2016).
190. Sella, E. M., Sato, E. I., Leite, W. A., Oliveira Filho, J. A. & Barbieri, A. Myocardial perfusion scintigraphy and coronary disease risk factors in systemic lupus erythematosus. *Ann. Rheum. Dis.* **62**, 1066–1070 (2003).
191. Schanberg, L. E. et al. Premature atherosclerosis in pediatric systemic lupus erythematosus: risk factors for increased carotid intima-media thickness in the atherosclerosis prevention in pediatric lupus erythematosus cohort. *Arthritis Rheum.* **60**, 1496–1507 (2009).
192. Lu, X. et al. Patients with systemic lupus erythematosus face a high risk of cardiovascular disease: a systematic review and meta-analysis. *Int. Immunopharmacol.* **94**, 107466 (2021).
193. Munguia-Realpozo, P. et al. Systemic lupus erythematosus and hypertension. *Autoimmun. Rev.* **18**, 102371 (2019).
194. Molina, M. J. et al. Prevalence of systemic lupus erythematosus and associated comorbidities in Puerto Rico. *J. Clin. Rheumatol.* **13**, 202–204 (2007).
195. Font, J. et al. Cardiovascular risk factors and the long-term outcome of lupus nephritis. *QJM* **94**, 19–26 (2001).
196. Quinones, A., Lobach, I., Maduro, G. A. Jr., Smilowitz, N. R. & Reynolds, H. R. Diabetes and ischemic heart disease death in people age 25–54: a multiple-cause-of-death analysis based on over 400 000 deaths from 1990 to 2008 in New York City. *Clin. Cardiol.* **38**, 114–120 (2015).
197. Yang, L. et al. Prevalence and correlation of conventional and lupus-specific risk factors for cardiovascular disease in Chinese systemic lupus erythematosus patients. *J. Eur. Acad. Dermatol. Venereol.* **26**, 95–101 (2012).
198. Paolisso, G. et al. Evidence for peripheral impaired glucose handling in patients with connective tissue diseases. *Metabolism* **40**, 902–907 (1991).
199. Zeng, Y. J. et al. Characteristics and risk factors for hyperglycemia in Chinese female patients with systemic lupus erythematosus. *Lupus* **19**, 1344–1350 (2010).
200. Moroni, G. et al. Oxidative stress and homocysteine metabolism in patients with lupus nephritis. *Lupus* **19**, 65–72 (2010).
201. Lazzerini, P. E. et al. Hyperhomocysteinemia: a cardiovascular risk factor in autoimmune diseases? *Lupus* **16**, 852–862 (2007).
202. Bruce, I. N., Urowitz, M. B., Gladman, D. D., Ibañez, D. & Steiner, G. Risk factors for coronary heart disease in women with systemic lupus erythematosus: the Toronto Risk Factor Study. *Arthritis Rheum.* **48**, 3159–3167 (2003).
203. Teh, P., Zakhary, B. & Sandhu, V. K. The impact of obesity on SLE disease activity: findings from the Southern California Lupus Registry (SCOLR). *Clin. Rheumatol.* **38**, 597–600 (2019).
204. Tedeschi, S. K. et al. Obesity and the risk of systemic lupus erythematosus among women in the Nurses' Health Studies. *Semin. Arthritis Rheum.* **47**, 376–383 (2017).
205. Ghaussy, N. O., Sibbitt, W. L. Jr & Qualls, C. R. Cigarette smoking, alcohol consumption, and the risk of systemic lupus erythematosus: a case-control study. *J. Rheumatol.* **28**, 2449–2453 (2001).
206. Legge, A., Blanchard, C. & Hanly, J. G. Physical activity, sedentary behaviour and their associations with cardiovascular risk in systemic lupus erythematosus. *Rheumatology* **59**, 1128–1136 (2020).
207. Mak, A. Physical exercise and systemic lupus erythematosus. *Rheumatology* **59**, 921–922 (2020).
208. Manzi, S. et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 51–60 (1999).
209. López-Pedrera, C. et al. Oxidative stress in the pathogenesis of atherothrombosis associated with

anti-phospholipid syndrome and systemic lupus erythematosus: new therapeutic approaches. *Rheumatology* **55**, 2096–2108 (2016).
210. Chorin, E. et al. Soluble ST2 and CXCL-10 may serve as biomarkers of subclinical diastolic dysfunction in SLE and correlate with disease activity and damage. *Lupus* **29**, 1430–1437 (2020).
211. Sagar, D. et al. LOX-1: A potential driver of cardiovascular risk in SLE patients. *PLoS One* **15**, e0229184 (2020).
212. Tripi, L. M. et al. Relationship of serum paraoxonase 1 activity and paraoxonase 1 genotype to risk of systemic lupus erythematosus. *Arthritis Rheum.* **54**, 1928–1939 (2006).
213. Giannelou, M. & Mavragani, C. P. Cardiovascular disease in systemic lupus erythematosus: a comprehensive update. *J. Autoimmun.* **82**, 1–12 (2017).
214. Apel, F., Zychlinsky, A. & Kenny, E. F. The role of neutrophil extracellular traps in rheumatic diseases. *Nat. Rev. Rheumatol.* **14**, 467–475 (2018).
215. Al Gadban, M. M., Alwan, M. M., Smith, K. J. & Hammad, S. M. Accelerated vascular disease in systemic lupus erythematosus: role of macrophage. *Clin. Immunol.* **157**, 133–144 (2015).
216. Parra, S. et al. Circulating FABP4 is a marker of metabolic and cardiovascular risk in SLE patients. *Lupus* **23**, 245–254 (2014).
217. Nived, O. et al. Disease duration, age at diagnosis and organ damage are important factors for cardiovascular disease in SLE. *Lupus Sci. Med.* **7**, e000398 (2020).
218. Kow, N. Y. & Mak, A. Costimulatory pathways: physiology and potential therapeutic manipulation in systemic lupus erythematosus. *Clin. Dev. Immunol.* **2013**, 245928 (2013).
219. Ganjali, S., Shirmohammadi, L., Read, M. I. & Sahebkar, A. High-density lipoprotein functionality in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **50**, 769–775 (2020).
220. Frostegård, J. Autoimmunity, oxidized LDL and cardiovascular disease. *Autoimmun. Rev.* **1**, 233–237 (2002).

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Author contributions
A.M. conceptualized the framework and content of the article; A.M. and J.K.Y.C. researched data for the article, made substantial contributions to discussions of the content, co-wrote the article, reviewed and edited the manuscript before submission.

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Oral surveillance and JAK inhibitor safety: the theory of relativity

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Abstract | The published results of the post-marketing ORAL Surveillance study, which compared the Janus kinase (JAK) inhibitor tofacitinib with anti-TNF therapy in older patients with rheumatoid arthritis who have cardiovascular risk factors, have led to changes in the recommendations for the use of JAK inhibitors. Although new safety signals have emerged for tofacitinib, namely malignancy and cardiovascular disease, it should be noted that these signals are relative to those seen with TNF blockers. The new data further raise our intrigue that venous thromboembolism might be a true risk related to JAK inhibition. Reassuringly, the totality of the findings from this newly published study and the other data collected to date suggest that JAK inhibitors can be used safely at approved doses by many patients with rheumatoid arthritis.

Tofacitinib was the first Janus kinase (JAK) inhibitor to be approved for the treatment of rheumatoid arthritis (RA) nearly 10 years ago. This oral medication, which diminishes the activity of JAK1, JAK2 and JAK3, was proven in phase III trials to offer an alternative of similar efficacy to injectable biologic DMARDs (bDMARDs). Since that time, other, more selective JAK inhibitor compounds have been approved, each with the idea that greater selectivity for one or more kinases might offer improvements in efficacy or safety (FIG. 1). So, where are we today in understanding the relative safety of these compounds? In the 'old days' we compared the safety of TNF inhibitors with non-biologic DMARDs. Over time, as more bDMARDs were approved, we began to shift our frame of reference and compare the safety of TNF inhibitors with other bDMARDs, and now compare JAK inhibitors and their relative safety with the bDMARDs. With so many choices for patients starting therapy, these relative comparisons are important, and very recently, the large post-marketing ORAL Surveillance (ORALSURV) trial has reported its outcomes in the peer-reviewed literature[1]. Although ORALSURV studied only patients with RA aged >50 years old with cardiovascular risk factors who started treatment with either tofacitinib or anti-TNF therapy (etanercept or adalimumab, depending on the region), the FDA extrapolated the study's findings beyond tofacitinib to all JAK inhibitors currently in use for immune-mediated inflammatory diseases, and restricted use of this class of drugs to patients with RA only after TNF inhibitor failure[2]. While the practicing rheumatologist attempts to digest this information, which will no doubt change clinical practice recommendations, it is useful to put the ORALSURV findings in the context of the JAK inhibitor studies to date.

The ORALSURV study

In 2012, when tofacitinib received FDA approval for use in RA, the agency mandated the drug's manufacturer, Pfizer, to conduct an additional post-marketing clinical trial owing to concerns regarding a potential increased risk of cancer, cardiovascular events and serious infections (SIEs) observed in the developmental programme in patients who received the higher, unapproved dose of 10 mg twice daily. Dose-dependent safety signals were noted in relation to a number of adverse events of special interest, leading to the conclusion that the benefit:risk ratio of tofacitinib was optimal with 5 mg twice daily, leading to the approval of only this dosage for use in RA[3]. ORALSURV was this FDA-mandated post-marketing phase IIIb–IV study, which enrolled 4,362 patients with RA aged >50 years who had at least one cardiovascular risk factor. Patients on background methotrexate therapy were randomly allocated to receive treatment either with tofacitinib at a dose of 5 mg or 10 mg twice daily or with a TNF inhibitor (etanercept or adalimumab, depending on the region). The trial's primary end points were major adverse cardiovascular events (MACEs) and malignancy, and the trial was designed as an event-driven, non-inferiority study with regard to these two outcomes. The trial could only be concluded when at least 1,500 patients had been followed for 3 years, and 103 MACEs (including cardiovascular death, non-fatal myocardial infarction and non-fatal stroke) and 138 malignancies (excluding non-melanoma skin cancers) had occurred. Non-inferiority of the tofacitinib regimens to the TNF inhibitor control regimen was to be concluded if the upper confidence limits for the hazard ratios for malignancy (total time analysis) or MACEs (on-treatment time analysis) were less than 1.8 (REF.[4]).

Interestingly, in 2019, during the ORALSURV trial, a statistically significant elevation in the risk for pulmonary embolism was noted with the 10-mg dose of tofacitinib relative to TNF inhibitor treatment, and all patients on this dose were moved to the 5-mg dosage, although their data continued to be analysed as part of the 10-mg cohort[1]. This switch perhaps complicates interpretation of dose effect, and potentially brings the risk estimates for the two doses closer together, given the blending of these groups. Although this study was conceived primarily with cardiovascular and malignancy outcomes in mind, the study focused on the usual adverse events of interest for JAK inhibitor therapies: SIEs, herpes zoster infection, venous thromboembolism (VTE), MACEs, malignancy and mortality. For each of these adverse events, we lend context below, and in the end, conclude that the data are not dissimilar to those from the original developmental programme, which suggested additional safety concerns at the 10-mg dosage and that resulted in the 5-mg twice daily dosage as the approved dose for RA.

Infection

ORALSURV's findings are consistent with clinical-trial and real-world studies of JAK inhibitors to date, and highlight that JAK

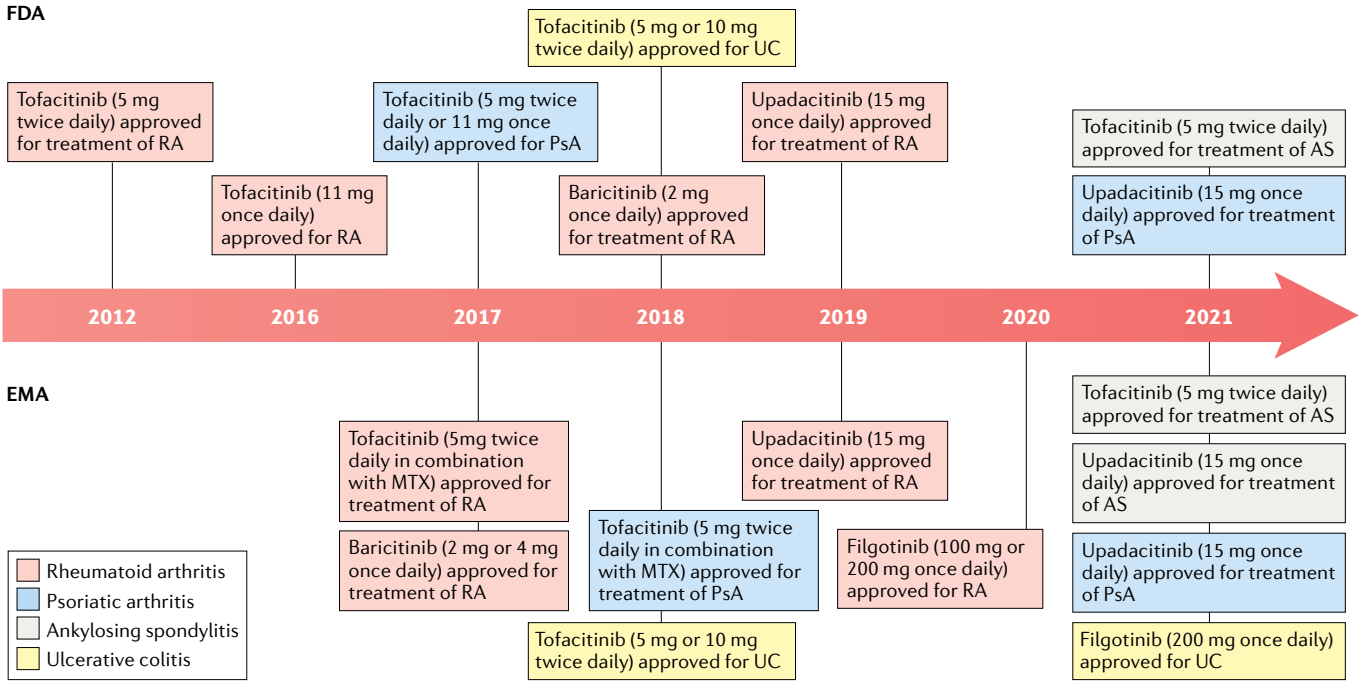

Fig. 1 | **Timeline of approved indications for Janus kinase inhibitors for rheumatic diseases.** In 2012 tofacitinib became the first Janus kinase (JAK) inhibitor to be indicated for a rheumatic disease, when the FDA approved its use in the treatment of rheumatoid arthritis (RA); EMA approval came in 2017. In addition to tofacitinib, other JAK inhibitors (baricitinib, upadacitinib and filgotinib) have also been approved for use in the treatment of RA, and the indications for JAK inhibitors have expanded to include psoriatic arthritis (PsA), ankylosing spondylitis (AS) and ulcerative colitis (UC). MTX, methotrexate.

inhibitors confer similar risk of infection to TNF inhibitors, with the exception of their propensity to reactivate latent viruses (such as varicella zoster virus, herpes simplex virus and cytomegalovirus). Unsurprisingly, ORALSURV reported rates of varicella zoster virus reactivation (that is, herpes zoster) several fold higher than for tofacitinib. To date, all JAK inhibitors approved in the USA seem to confer similar herpes zoster risk based on experience from phase II–III trials[5], although direct comparator data between the different JAK inhibitor compounds are lacking. Filgotinib, a compound with selectivity for JAK1 and approved for use in Europe, had a lower herpes zoster incidence in more recently conducted phase II–III trials, although a dose-dependent elevation was observed[6]. With regard to SIEs, importantly, ORALSURV reported a similar risk for the tofacitinib 5-mg dose and TNF inhibitors, even in patients >65 years old. Although incidence rates were slightly higher in those >65 years old than in those 50–65 years old, there was no effect modification due to age, and the hazard ratios comparing tofacitinib with TNF inhibitors were similar in older and younger individuals. The ORALSURV SIE data were reassuring, and consistent with data from the RA development programmes of currently approved JAK inhibitors and bDMARDs, in which SIE rates are similar[7] and generally in the range of 3–4 events per 100 patient-years, with elevated rates as expected in more elderly individuals.

Venous thromboembolism

ORALSURV's finding of an increased risk of VTE with the tofacitinib 10-mg dose relative to TNF inhibition supports the idea, first raised in RA clinical trials of baricitinib's 4-mg dose[5], that VTE might be a true JAK inhibitor-related adverse event. Although a biological explanation is currently lacking, it is tempting to speculate that greater modulation of JAK2, which would be observed with higher doses of tofacitinib and baricitinib, could offer an eventual explanation[8]. Reassuringly, however, JAK inhibitors used in RA at their currently approved doses do not yet seem to carry excess risk. ORALSURV reported the tofacitinib 5-mg dose and TNF inhibitors to be associated with a similar risk of VTE, and this is consistent with real-world data (from the CORRONA registry) indicating that patients starting treatment with tofacitinib and those starting treatment with TNF inhibitors had a similar risk[9]. Furthermore, the incidence rates of VTE observed in pivotal trials of tofacitinib and upadacitinib were similar (and were even lower in filgotinib trials), and the rates within the active comparator groups of those programmes (that is, methotrexate and adalimumab) were similar or in some cases even higher[6,10,11]. Even for baricitinib, for which the initial imbalance in VTE risk between the 2-mg and 4-mg doses in the first 12 weeks of phase III trials raised eyebrows, similar long-term incidence rates were reported for both doses, of 0.5 events per 100 patient-years, a rate in line with RA population-based studies[5,12]. Lastly, baricitinib 4 mg given for 2 weeks did not increase the risk of VTE in the treatment of COVID-19, a condition with heightened VTE risk at baseline[13]. As the findings of ORALSURV suggest that there is a dose-dependent risk with tofacitinib relative to TNF inhibitors, until more basic and population-based research has been conducted with each of these compounds it seems at least prudent to steer JAK inhibitors away from those with a strong risk of VTE, particularly those with a history of VTE who are not presently anti-coagulated.

Major adverse cardiovascular events

Within ORALSURV, tofacitinib at both the 5-mg and the 10-mg twice daily doses failed to demonstrate non-inferiority for MACEs in comparison with TNF inhibitors, as the 95% CI exceeded the pre-specified

upper boundary of 1.8. The incidence rate for the tofacitinib 5-mg dose was 0.91 per 100 patient-years and for the TNF inhibitors it was 0.73 per 100 patient-years (HR 1.24; 95% CI 0.81–1.91). Interestingly, the MACE incidence rate for TNF inhibitors in ORALSURV was markedly lower than that seen for etanercept (1.70 per 100 patient-years) in a similar trial evaluating patients with RA and cardiovascular risk factors[14]. Real-world data have established that TNF inhibitors are protective with regard to MACEs compared with non-biologic DMARDs, and some studies suggest that tocilizumab (an IL-6 receptor inhibitor) or even abatacept (a selective co-stimulation modulator) might be more protective than TNF inhibitors[15]. The 2022 STAR-RA population-based study, evaluating commercial and Medicare data on patients with RA initiating treatment with tofacitinib or TNF inhibitors, found no difference in the incidence rates of myocardial infarction and stroke between these treatment groups (HR 1.01; 95% CI 0.83–1.23)[16]. Of interest, however, when the analysis was restricted to patients with similar cardiovascular risk factors to those of patients enrolled in the ORALSURV study, again, no statistical difference in cardiovascular outcomes was found, but the HR of 1.24 (95% CI 0.90–1.69) for tofacitinib compared with TNF inhibitors was the same as that reported in the ORALSURV for tofacitinib 5-mg twice daily, noted above. As for JAK inhibitor data from studies prior to ORALSURV, data from the RA developmental programme for all approved JAK inhibitors suggest incidence rates similar to those observed for the bDMARD comparators in those phase III trials[10,13,17].

Malignancy

The overall rate of malignancy for JAK inhibitors in RA randomized clinical trials and long-term extension studies has been reported to be similar to that seen with bDMARDs, and lower than that observed for tofacitinib in this study. In the ORALSURV study, the incidence rate for malignancy was 1.13 (95% CI 0.87–1.14) for patients treated with tofacitinib 5 mg twice daily and 1.13 (95% CI 0.86–1.14) for those treated with tofacitinib 10 mg twice daily, compared with 0.77 (95% CI 0.55–1.04) for the TNF inhibitor-treated patients (HR 1.48; 95% CI 1.04–2.09). This signal was driven by differential rates of several cancers (particularly lung cancer and lymphoma) primarily seen in the North American strata of the study (compared with the rest of the world), and among older individuals and in those with a history of tobacco smoking. An increased risk of non-melanoma skin cancer was also noted, which has been noted previously with use of the tofacitinib 10-mg dose in ulcerative colitis[18]. Conversely, numerically higher rates of melanoma (per 100 patient-years) were observed for patients using TNF inhibitors (0.09 (95% CI 0.03–0.21) versus 0.02 (95% CI 0.0–0.10) for either tofacitinib dosage. The mechanism by which JAK inhibitors could be associated with some types of cancer is unknown, but we would speculate that some JAK inhibitors, depending on their selectivity and effect on natural killer cells, could potentially diminish the host's immunosurveillance for cancer, making an existing or de novo cancer more likely to progress[19]. In general, long-term data from large numbers of individuals is required to evaluate these long-latency events, and the real world RA data evaluated to date suggest no difference in cancer risk between patients treated with tofacitinib 5 mg or bDMARDs[9]. The extent to which tofacitinib or other JAK inhibitors might increase the risk of malignancy within specific high-risk groups (for example, elderly smokers) deserves further study.

Mortality

For all the approved JAK inhibitors, mortality rates have generally been reported to be similar to those associated with bDMARDs including TNF inhibitors, with standard incidence ratios in the Surveillance, Epidemiology, and End Results (SEER) database of around 1 with no statistical difference[5,6,10,17]. In the ORALSURV study, there was a statistically significant increase in overall mortality for the 10-mg dose (HR 2.37; 95% CI 1.34–4.18) and non-statistically significant trend for the 5-mg dose (HR 1.49; 95% CI 0.81–2.74) compared with TNF inhibitor-treated patients. These data were reflective of the differential rates of MACEs and malignancy observed in the trial.

How should we use JAK inhibitors?

In our minds, ORALSURV raises more questions than it answers, but it does help to inform treatment decision-making for physicians and patients, particularly if patients are at a high risk of certain outcomes. The issues raised by this study are reminiscent of the concerns raised about the risk of tuberculosis and other opportunistic infections with the use of TNF inhibitors, which was recognized in post-marketing surveillance, or of the association between cyclooxygenase 2 (COX2) inhibitors and increased cardiovascular risk, which resulted in rofecoxib and valdecoxib being withdrawn from the market[20,21]. As with ORALSURV, when signals of concern arose with TNF inhibitors and COX2 inhibitors there was substantial controversy and conflicting opinions. Over time, research confirmed these signals, and we look forward to additional mechanistic and clinical research to confirm or refute the observations from ORALSURV. What we do have to acknowledge is that we clearly have a signal of concern in a high-risk population and need to grapple with how this signal should influence our treatment decision-making. We must also acknowledge that ORALSURV reflects only a comparison of TNF inhibitors relative to JAK inhibitors in a specific population of patients with RA, and that both treatments might be protective against many of the outcomes under study if compared with no therapy, non-biologic DMARDs, or even other bDMARDs. It all boils down to the population under study

Box 1 | FDA and EMA responses to ORAL Surveillance

The results of the ORAL Surveillance study[1] have led regulatory authorities such as the FDA and EMA to recommend different changes to the utilization of Janus kinase inhibitors.

EMA[22]

- For patients ≥65 years old, those with a history of smoking and those with risk factors for cardiovascular disease or malignancy, tofacitinib should be used only if no suitable alternatives exist

FDA[2]

- Use of tofacitinib, baricitinib and upadacitinib is recommended for use only in patients who have had an inadequate response to, or intolerance of, one or more TNF inhibitors
- Boxed warnings for tofacitinib, baricitinib and upadacitinib updated to include information about the risks of serious heart-related events, cancer, blood clots and death
- Health-care professionals should consider the benefits and risks for an individual patient prior to initiating or continuing treatment with tofacitinib, baricitinib or upadacitinib, particularly for patients with a history of smoking, those with risk factors for cardiovascular disease and those with a malignancy

and the referent group, and the fact that RA disease control is protective against all of the outcomes under study.

What does the clinician do now? Regulatory authorities such as the FDA and EMA have arrived at different conclusions with different modifications of JAK inhibitor utilization (BOX 1). The EMA recommended that for patients ≥65 years old with a history of smoking or risk factors for cardiovascular disease or malignancy, tofacitinib should be used only if no suitable alternatives exist[22]; the FDA extrapolated the ORALSURV data beyond tofacitinib to include baricitinib and upadacitinib, with use of these agents recommended in such patients only in the case of prior TNF inhibition failure[2]. We believe that the ORALSURV data highlight the already described narrow safety window of JAK inhibitors, which was noted in the clinical trials, where the higher doses of the JAK inhibitors were not approved owing to increased toxicity. In general, these medicines, like all medicines, should be steered towards patients for whom the benefit:risk ratio is maximal, and it underscores the importance of screening patients for various risk factors prior to therapy selection. Fortunately, a number of highly effective alternatives are available for the treatment of rheumatic disease that we can utilize for patients at an increased risk of certain outcomes. For now, however, it seems that JAK inhibitors can be used at approved doses with safety similar to that of TNF blockade in many patients with RA, particularly in younger individuals and in older individuals who lack certain risk factors (for example, smoking).

Conclusions

Although the FDA's 'better to be safe than sorry' approach might ultimately prove correct, there is certainly a need for studies comparing JAK inhibitor compounds, and comparing JAK inhibitors with other DMARDs, as well as further mechanistic studies to explain the safety signals observed in comparison with TNF inhibitors to date. How JAK inhibitor compounds compare with one another, and how they compare with other bDMARDs beyond TNF inhibitors, in regard to these outcomes is unknown. Although we clinicians navigate now perhaps murkier waters in the wake of this one study, we remind ourselves that we must always remember our frame of reference.

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1. Ytterberg, S. R. et al. Cardiovascular and cancer risk with tofacitinib in rheumatoid arthritis. *N. Engl. J. Med.* **386**, 316–326 (2022).
2. U.S. Food and Drug Administration. FDA approves Boxed Warning about increased risk of blood clots and death with higher dose of arthritis and ulcerative colitis medicine tofacitinib (Xeljanz, Xeljanz XR). https://www.fda.gov/drugs/drug-safety-and-availability/fda-approves-boxed-warning-about-increased-risk-blood-clots-and-death-higher-dose-arthritis-and (2021).
3. U.S. Food and Drug Administration. Center for Drug Evaluation and Research: NDA 203,214 Tofacitinib for Rheumatoid Arthritis, Addendum to Primary Clinical Review. Edited by Department of Health and Human Services, 26 September 2012 edn. Silver Spring, MD. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203214Orig1s000Approv.pdf (2012).
4. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/results/NCT02092467 (2021).
5. Genovese, M. C. et al. Safety profile of baricitinib for the treatment of rheumatoid arthritis over a median of 3 years of treatment: an updated integrated safety analysis. *Lancet Rheumatol.* **2**, e347–e357 (2020).
6. Winthrop, K. L. et al. Integrated safety analysis update for filgotinib in patients with moderately to severely active rheumatoid arthritis receiving treatment over a median of 2.2 years [abstract]. *Arthritis Rheumatol.* **73**, 1698 (2021).
7. Strand, V. et al. Systematic review and meta-analysis of serious infections with tofacitinib and biologic disease-modifying antirheumatic drug treatment in rheumatoid arthritis clinical trials. *Arthritis Res. Ther.* **17**, 362 (2015).
8. Parra-Izquierdo, I. et al. Janus kinase inhibitors ruxolitinib and baricitinib impair glycoprotein-VI mediated platelet function. *Platelets* https://doi.org/10.1080/09537104.2021.1934665 (2021).
9. Kremer, J. M. et al. Postapproval comparative safety study of tofacitinib and biological disease-modifying antirheumatic drugs: 5-year results from a United States-based rheumatoid arthritis registry. *ACR Open Rheumatol.* **3**, 173–184 (2021).
10. Cohen, S. B. et al. Long-term safety of tofacitinib up to 9.5 years: a comprehensive integrated analysis of the rheumatoid arthritis clinical development programme. *RMD Open* **6**, e001395 (2020).
11. Winthrop, K. L. et al. Integrated safety analysis of filgotinib in patients with moderately to severely active rheumatoid arthritis receiving treatment over a median of 1.6 years. *Ann. Rheum. Dis.* **81**, 184–192 (2022).
12. Desai, R. J., Pawar, A., Khosrow-Khavar, F., Weinblatt, M. E. & Kim, S. C. Risk of venous thromboembolism associated with tofacitinib in patients with rheumatoid arthritis: a population-based cohort study. *Rheumatology* **61**, 121–130 (2021).
13. Marconi, V. C. et al. Efficacy and safety of baricitinib for the treatment of hospitalised adults with COVID-19 (COV-BARRIER): a randomised, double-blind, parallel-group, placebo-controlled phase 3 trial. *Lancet Respir. Med.* **9**, 1407–1418 (2021).
14. Giles, J. T. et al. Cardiovascular safety of tocilizumab versus etanercept in rheumatoid arthritis: a randomized controlled trial. *Arthritis Rheumatol.* **72**, 31–40 (2020).
15. Singh, S. et al. Comparative risk of cardiovascular events with biologic and synthetic disease-modifying antirheumatic drugs in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Arthritis Care Res.* **72**, 561–576 (2020).
16. Khosrow-Khavar, F., Kim, S. C., Lee, H., Lee, S. B. & Desai, R. J. Tofacitinib and risk of cardiovascular outcomes: results from the Safety of TofAcitinib in Routine care patients with Rheumatoid Arthritis (STAR-RA) study. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-221915 (2022).
17. Cohen, S. B. et al. Safety profile of upadacitinib in rheumatoid arthritis: integrated analysis from the SELECT phase III clinical programme. *Ann. Rheum. Dis.* **80**, 304–311 (2020).
18. Sands, B. E. et al. Tofacitinib for the treatment of ulcerative colitis: analysis of nonmelanoma skin cancer rates from the Ulcerative Colitis Clinical Program. *Inflamm. Bowel Dis.* **2**, 234–245 (2022).
19. Nocturne, G., Pascaud, J., Ly, B., Tahmasebi, F. & Mariette, X. JAK inhibitors alter NK cell functions and may impair immunosurveillance against lymphomagenesis. *Cell. Mol. Immunol.* **17**, 552–553 (2020).
20. Bhala, N. et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* **382**, 769–779 (2013).
21. Wallis, R. S., Broder, M. S., Wong, J. Y., Hanson, M. E. & Beenhouwer, D. O. Granulomatous infectious diseases associated with tumor necrosis factor antagonists. *Clin. Infect. Dis.* **38**, 1261–1265 (2004).
22. European Medicines Agency. Meeting highlights from the Pharmacovigilance Risk Assessment Committee (PRAC) 7–10 June 2021 https://www.ema.europa.eu/en/news/meeting-highlights-pharmacovigilance-risk-assessment-committee-prac-7-10-june-2021 (2021).

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

K.L.W. declares that he has acted as a consultant for AbbVie, Union AstraZeneca, Bristol Myers Squibb (BMS), Chimique Belge (UCB), Eli Lilly & Company, Galapagos, Gilead, GlaxoSmithKline, Novartis, Pfizer, Regeneron, Roche and Sanofi, and has received research funding from Bristol Myers Squibb and Pfizer. S.B.C. declares that he has acted as a consultant for and received research funding from AbbVie, Amgen, Gilead, Lilly and Pfizer.

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