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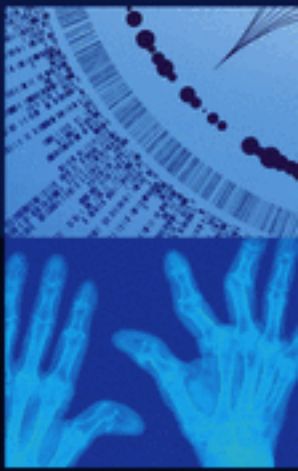

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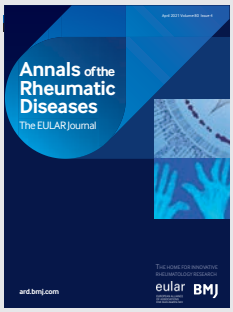

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Rheumatoid arthritis, bone and drugs: a dangerous interweave

Salvatore Minisola , Jessica Pepe, Cristiana Cipriani

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory condition that leads, per se, to bone loss by three main mechanisms. The first one is represented by bone erosions around the involved joints. Second, there is focal subchondral bone loss owing to increased osteoclast activity. Finally, systemic inflammation causes universal bone loss, mainly manifesting at the axial skeleton (ie, vertebral bodies and hip).[1] On the top of this, physical inactivity, also related to lower muscle mass and sarcopenia, further exacerbates bone loss. As a consequence, osteoporosis can be detected in 30%–50% of patients with RA, also depending on age and sex; prevalence and fracture risk both raise with disease duration and seropositivity.[2] The importance of RA in determining both local and systemic osteoporosis is emphasised by the fact that RAis an input variable, as independent risk factor, in the calculation of future fracture risk by FRAX.

It is also well known that patients with RA consume a number of drugs to mainly counteract inflammation and pain. However, some of the drugs used have detrimental effects on skeletal tissue, reducing bone strength and ultimately leading to fractures.

About 1% of adults in Britain and USA receive long-term oral glucocorticoids mainly for the treatment of joint problems but also for digestive and cutaneous diseases.[3 4] The long-term or high doses of glucocorticoids use is associated with reduced skeletal strength; indeed, glucocorticoid induced osteoporosis is the most prevalent cause of secondary osteoporosis. Glucocorticoids exert their deleterious effects on bone by acting on the three main cell skeletal lines (osteoblasts, osteoclasts end osteocytes). Among the most important negative effects are: (1) a preferential differentiation of pluripotent precursor cells to adipocytes rather than osteoblasts; (2) a stimulation of the receptor activator of nuclear factor-kB (RANK)-RANK ligand (RANKL) production which at least initially increase bone resorption and (3) an increased apoptosis of osteocytes with changes in the physical and fluid characteristics of the surrounding territory. These negative effects at the cellular levels contribute to increase the risk of fracture, together with the effects on other organs such as muscle (steroid myopathy) and eye (increased intra-articular pressure and formation of posterior subcapsular cataracts).[5]

Proton pump inhibitors (PPI) are drugs widely prescribed in the world. When Food and Drug Administration approved its use as an 'over-the-counter' drug, there has been a skyrocketing increase in their consumption. Data in Europe mirrored this trend.[6] However, PPIs are associated with a number of side effects among which an increased risk of fracture. Indeed, a number of meta-analyses showed a significantly increased risk of hip and vertebral fractures, with some of them also suggesting a dose–dependent relationship.[7 8] However, while the biological mechanisms leading to fractures in patients taking glucocorticoids are well ascertained, the same is not true for PPI. Hypothetical mechanisms include a reduced intestinal calcium absorption related to the hypochloridria induced by the negative effects on H^+/K^+ ATPase activity. The absorption of other micronutrients important for skeletal health are also adversely affected by long-term PPI use; in particular, PPIs use may be associated with hypomagnesaemia and a dose-response between the PPIs use and development of hypomagnesaemia has been reported.[9] Other putative mechanisms include an excessive histamine production driven by hypergastrinaemia activated enterochromaffin-like cells (possibly leading to enhanced osteoclastogenesis and bone resorption) together with vitamin B_{12} deficiency. The last one has been associated with an increased risk of falls and with an increased risk of fractures.[10 11]

In this context, Abtahi *et al*[12] add important information with implications in clinical practice that should change our behaviour. In summary, they showed that patients with RA taking both GC and PPI have a 1.6-fold increased risk of osteoporotic fractures (hip, clinical vertebral, pelvis and ribs) compared with non-users but also with single users or oral GC or PPI.[12] Merits of this investigation include the large database used together with a long observation period (ie, more than 9 years). However, there are also important weaknesses in addition to those listed by the authors. The most important is represented by the lack of full control for bone active drugs taken by the patients, also including calcium and vitamin D. They claim that these drugs 'were not considered in the main analysis because of the accompaniment of their prescription with those of oral glucocorticoids and we expect them to lie in the causal pathway of the intended associations of mediators'. However, they do not show any evidence of this assumption nor the use of these drugs in the past 6 months is a guarantee for continuous use during the whole observation period. In addition, and most importantly, their data are not from a randomised controlled study; therefore, confounding cannot be excluded. For example, the populations considered might differ in terms of other factors that predispose or prevent fractures, such as the frequency and type of contact with care health system, physician prescribing attitudes and so on.

Bearing in mind these limitations, the most important message coming from this paper is a call for a watchful behaviour by doctors taking care of patients with RA on concurrent GC and PPI treatment. Interestingly, this dangerous liaison has been highlighted in an almost contemporary paper by Miyano *et al*[13] in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis.

Abtahi and coworkers conclude that fracture risk assessment could be considered when a patient with RA is coprescribed oral glucocorticoids and PPIs. This approach that we would say 'must' be considered, does not preclude an integration with another approach represented by reducing or de-prescribing drugs when they are not needed or when their use is not fully supported by the evidence. This is particularly true, considering the significant number of drugs prescribed to patients with RA that have been associated with an increased risk of fractures.[14]

As far as glucocorticoids, the first step should be an attempt to minimise the use of oral glucocorticoids in terms of both dosage and duration. Then, there is clear evidence that the combination of glucocorticoids with non-steroidal anti-inflammatory drugs increase the risk of peptic ulcer disease, thus justifying the concomitant use of PPI. However, there is conflicting evidence

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about the risk of peptic ulcer disease in patients taking glucocorticoid monotherapy. In a nested case–control study of Medicaid patients, there was no increased risk of peptic ulcer disease at any dose or length of glucocorticoid treatment.[15] Looking for example at figure 1 (considering the pharmacological history in the 6 months before), only in 2954 patients the use of PPIs seems justified in those already taking steroids (2309 patients taking NSAIDs, 409 Cox-2 selective inhibitors, 198 with gastro-oesophageal reflux and 38 with peptic ulcer disease) leaving the remaining 30.5% coprescriptions not appropriate or at least debatable. On the other hand, inappropriateness is also present in those taking steroids alone (1202, 205, 94 and 15 patients respectively) and in majority of those taking PPIs alone. This last group, points once again on the long-standing debate on overcoming resistance on deprescribing PPI.[16]

In those taking PPIs, an alternative approach to circumvent low calcium absorption due to hypochloridria (with subsequent skeletal loss owing to secondary hyperparathyroidism) may be represented by the administration of calcium citrate. This is because calcium carbonate is not soluble in water thus needing adequate acid secretion for ideal absorption. It has also the advantage of an optimal absorption independent of food intake.[17] Furthermore, alternative therapies (at least in the first instance) including antacids, alginates or histamine type-2 receptor antagonists can be attempted.

In conclusion, the paper by Abtahi *et al*,[12] brings to the light an important issue focusing on multiple coprescription of drugs potentially detrimental for skeletal health in patients ith RA. This aspect is often disregarded in clinical practice not only by general practitioners but also in referral centres. Add another drug to protect bone is an option; a number of studies in general not specifically targeted to patients with RA,[18] have shown satisfactory results in terms of bone mineral density increase and fracture risk reduction in patients taking oral glucocorticoids.[19–22] However, also reconsidering drug prescription is a suitable alternative to follow. Our suggested approach to protect bone in patients with RA taking steroids in addition to multidrug therapy is summarised in box 1.

Box 1 Skeletal protection in patients with rheumatoid arthritis on steroids and multidrug therapy

1. Reduce glucocorticoids dose and consider glucocorticoids sparing treatments.
2. Improve nutrition (protein, calcium and vitamin D).
3. Adopt a healthy lifestyle (avoid tobacco, alcohol and perform physical activities and weight-bearing exercises).
4. Evaluate the panel of drugs prescribed.
5. Deprescribe, if possible, or consider alternatives drugs not harmful to bone.
6. Consider prescription of bone active drugs.
7. Monitor and reassess when indicated.

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EULAR December 2020 viewpoints on SARS-CoV-2 vaccination in patients with RMDs

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The COVID-19 pandemic has severely influenced all aspects of life in 2020. This pandemic also affected patients with rheumatic and musculoskeletal diseases (RMDs) and impacted the care given to them. With the development of vaccines, the future is becoming brighter. The possibility of vaccination however also raises a lot of questions, especially for patients with inflammatory RMDs and patients who are treated with drugs that may influence their immune system. To address these questions EULAR has formed a Task Force of representatives of its constituents, patients, health professionals and rheumatologists experienced in the field which addressed pertinent aspects.

This information is based on knowledge available at this moment in time, realising that specific data about the performance of the emerging vaccines to COVID-19 in patients with RMDs and in patients treated with drugs that influence the immune system are not yet available. In the coming months we expect that more relevant information will be collected. When you read this information, please realise that this text will need to be updated when new information becomes available.

In general, several different kinds of vaccines will be used in national vaccination programmes. Vaccines that are presently being used or under development specifically for COVID-19 are non-live vaccines, that cannot give you the viral disease, that cannot transfer infection to you, nor can they change your genetic information. These vaccines can be used safely in patients with RMDs as well as in patients receiving drugs that influence the immune system. Other non-live vaccines have been proven to work for immune-suppressed patients. To say it more strongly, there is no reason to withhold these vaccines from patients with RMDs and patients treated with drugs that influence the immune system.

The following different SARS-CoV-2 vaccines are presently in a more advanced stage of development and some have been approved in different countries. Vaccines based on mRNA (such as those from Pfizer/BioNTech and from Moderna), on proteins with adjuvant (such as from Novavax) and on non-replicable vectors (such as from AstraZeneca and from Janssen).

Vaccinations should preferably be given when the disease is in a quiet phase; it is also preferred to vaccinate before planned immunosuppression if feasible. But of course, this is not always possible. A vaccination is most effective when the amount of, or level of immune suppression is low; however, the risk of a flare of the disease is real, and therefore it is **not** advised to decrease your medication.

Of course, in specific cases your physician can make other choices, based on your personal condition and/or on the drugs you are using; if you are in doubt consult your rheumatologist.

Independent from vaccination for SARS-CoV-2, vaccination against pneumococcus and influenza is highly recommended in patients with RMDs and patients treated with drugs that influence the immune system.

FREQUENTLY ASKED QUESTIONS BY PATIENTS WITH RMDS AND PATIENTS USING DRUGS THAT INFLUENCE THE IMMUNE SYSTEM

Do I need to be vaccinated? It is wise for everybody to be vaccinated against COVID-19.

Do I need to get an urgent vaccination? Countries have completely different rules. Many countries place, at this moment, age and residents and staff in care homes at the top of their priorities list.

Is one vaccine better for me than another one? Too early to say and there are not studies comparing vaccines; with the present info vaccination by any vaccine is better than no vaccination at all.

I had COVID-19 and recovered from it. Should I be vaccinated? At present, there are no data; but vaccination after COVID-19 is considered to be safe and potentially confers additional protection.

Can I get the vaccination if I take my anti-rheumatic or immunosuppressive drugs? Yes, you can. The only exception could be rituximab; in case you use rituximab it will depend on when you last received the drug, please consult your rheumatologist.

Do vaccines interfere with my medication? No.

Who should I consult before vaccination—my GP or my rheumatologist? If you have specific questions your rheumatologist would be the preferred source of information.

What data are necessary to take the right decision? Knowledge of your disease activity, drug treatment and possible comorbidities.

What about side effects? It is rather early for a definite answer, but so far, the vaccines that are tested are remarkably safe, comparable with those we know from the influenza vaccination.

What should I do in case of a flare? Contact your rheumatologist to discuss.

In case I have worrying side effects? This is unlikely, but contact your rheumatologist.

Does the vaccine activate my illness? This is unlikely, but we don't have enough experience yet.

Will I need a vaccination annually as with other vaccinations for example, influenza? Not known yet, but quite likely.

What about long-term effects? It is rather early for a definite answer, but so far, the vaccines that are tested are remarkably safe.

Am I more at risk of getting COVID-19 disease? No there is no evidence that the risk of getting the disease is higher in patients with RMDs.

Am I more at risk of getting worse COVID-19 disease? Not by your disease itself; but—like in everybody—when there is major organ damage (such as renal dialysis for kidney failure, severe lung involvement) the risk can be higher.

Do my treatments increase the risk of worse disease? Most of the drugs used in RMD have not been associated with worse disease. To date the only treatments that have been shown to be associated with a worse COVID-19 outcome are using more than 10 mg glucocorticoids daily or being treated with rituximab.

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Microenvironment in subchondral bone: predominant regulator for the treatment of osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease in the elderly. Although OA has been considered as primarily a disease of the articular cartilage, the participation of subchondral bone in the pathogenesis of OA has attracted increasing attention. This review summarises the microstructural and histopathological changes in subchondral bone during OA progression that are due, at the cellular level, to changes in the interactions among osteocytes, osteoblasts, osteoclasts (OCs), endothelial cells and sensory neurons. Therefore, we focus on how pathological cellular interactions in the subchondral bone microenvironment promote subchondral bone destruction at different stages of OA progression. In addition, the limited amount of research on the communication between OCs in subchondral bone and chondrocytes (CCs) in articular cartilage during OA progression is reviewed. We propose the concept of 'OC–CC crosstalk' and describe the various pathways by which the two cell types might interact. Based on the 'OC–CC crosstalk', we elaborate potential therapeutic strategies for the treatment of OA, including restoring abnormal subchondral bone remodelling and blocking the bridge—subchondral type H vessels. Finally, the review summarises the current understanding of how the subchondral bone microenvironment is related to OA pain and describes potential interventions to reduce OA pain by targeting the subchondral bone microenvironment.

INTRODUCTION

Osteoarthritis (OA) is the most frequent form of arthritis with a high incidence and a prolonged course.[1] OA affects articular and periarticular tissues, such as articular cartilage, subchondral bone and synovium.[2] Over recent years, the role of subchondral bone during OA progression has gradually attracted researchers' attention.[3 4] Imaging techniques have revealed microstructural alterations in subchondral bone in OA joints, including early-stage bone loss, late-stage bone sclerosis and histopathological alterations, caused by subchondral bone cysts, bone marrow oedema-like lesions (BMOLs) and osteophyte formation.[5] These alterations are caused by biological processes involving uncoupling and coupling interactions among osteocytes, osteoblasts (OBs), osteoclasts (OCs), endothelial cells (ECs) and sensory neurons in the subchondral bone microenvironment,[6] and therefore they will help in understanding OA pathogenesis from the perspective of subchondral bone. Notably, bone remodelling rates are altered during the development of OA due to the spontaneous activation or inactivation of osteoclastic bone resorption activity. As a result, activation of bone resorption may be evident in the subchondral bone microenvironment in early-stage OA, while late-stage OA is characterised by inactivation of bone resorption activity and a bias towards activation of bone formation activity.[7] Subchondral bone and cartilage form a functional complex called the bone–cartilage unit, which is involved in the pathophysiology of OA at the biochemical and mechanical levels.[8 9] In this review, we summarise the various pathways by which OCs interact with CCs, thus providing a novel research direction for the investigation of the crosstalk between these two types of cells in OA. Furthermore, we have noted the reported and potential communication pathways between OCs and CCs, and we propose promising therapeutic strategies to restrain the progression of OA by targeting the subchondral bone microenvironment. Moreover, arthritic pain is a major complaint of patients with OA during the progression of the disease. Recent studies indicate that neuronal factors may contribute to the innervation of pain-related sensory nerves in OA subchondral bone.[10 11] Intriguingly, the evidence suggests a close relationship between OCs/OBs and sensory nerves in the microenvironment of subchondral bone.[10 11] Based on this, it may be useful to develop specific drugs for the treatment of OA-related pain by targeting the subchondral bone microenvironment.

OSTEOARTHRITIC SUBCHONDRAL BONE MICROENVIRONMENT

Normal subchondral bone architecture

Subchondral bone is divided into two anatomical entities: the subchondral bone plate and subchondral trabeculae. Subchondral bone plate is a thin cortical plate subjacent to calcified cartilage. It is a penetrable structure with interconnected porosity. Numerous vessels and nerves pass through the porosity, sending branches into calcified cartilage.[12] The subchondral trabeculae, which are subjacent to the subchondral bone plate, are porous structures with abundant vessels and nerves that play an important role in load absorption and structural support as well as nutritional supply to cartilage.[13] Subchondral bone adapts to mechanical forces exerted on the joint dynamically via coordinated bone remodelling.[14] Bone remodelling involves the coupling of osteoclastic bone resorption and osteoblastic bone formation to replace damaged bone with new bone.[15] However, subchondral bone and cartilage exhibit distinct capacities of mechanical adaptation. Although cartilage modulates the functional state in response to mechanical damage, its capacity to repair and modify the surrounding extracellular matrix is more limited than that of

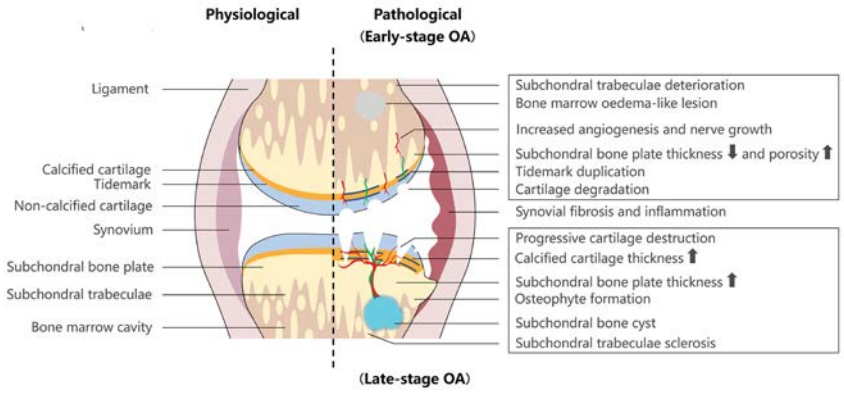

Figure 1 Microstructural and histopathological alterations in osteoarthritis (OA) subchondral bone. In early-stage OA, subchondral bone plate becomes thinner and more porous, together with deteriorated subchondral trabeculae and initial cartilage degradation. In late-stage OA, calcified cartilage and subchondral bone plate become thicker, along with sclerotic subchondral trabeculae and progressive cartilage destruction. During OA progression, growing vessels and nerves send branches from subchondral bone into cartilage. OA subchondral bone exhibits subchondral bone cysts, bone marrow oedema-like lesions and osteophyte formation.

subchondral bone.[16] Subchondral bone responds rapidly to mechanical loading by bone remodelling and then re-establishes normal physiological conditions.[17]

Microstructural and histopathological alterations in OA subchondral bone

The occurrence of cartilage degeneration and subchondral bone destruction has always been a controversial issue.[18] Not all patients with OA exhibit the progression from abnormal bone formation in subchondral bone. Moreover, a fraction of patients with OA exhibit the earliest changes at the sites of subchondral bone. OA is commonly thought to be a degenerative disease related to ageing and trauma. In ageing-induced OA, it could be confirmed that aberrant chondrocyte metabolism plays a crucial role in the occurrence of cartilage damage prior to abnormal subchondral bone formation.[19] Conversely, early microdamage at the sites of subchondral bone is detected in trauma-induced OA.[20] Notably, the alterations of subchondral bone are not exactly the same in different articulating joints in OA. There is good evidence that pathological alterations in different joints (such as the knee, spine and temporomandibular joint) exhibit several kinds of features.[21–25]

At different stages of OA, there are distinct microstructural alterations in subchondral bone. In early OA, enhanced subchondral bone turnover is observed. In addition, the subchondral bone sclerosis is observed during the advanced and late stages.[26–28] In early OA, subchondral bone plate becomes thinner and more porous during the initial cartilage degeneration. Subchondral trabeculae deteriorate, with increased trabecular separation and decreased trabecular thickness.[29] Conversely, the subchondral bone plate and trabeculae become thicker, which is accompanied by subchondral bone sclerosis and decreased bone marrow spacing in late OA. At the same time, non-calcified cartilage shows progressive damage, and becomes thicker with tidemark replication.[29] Despite the increased bone volume, high local bone turnover and a decreased calcium:collagen ratio lead to insufficient bone mineralisation and a decreased bone tissue elastic modulus. Consequently, the mechanical property is compromised, and it becomes easier to deform bone under mechanical loads (figure 1).[30 31]

Abnormal cellular interactions in the OA subchondral bone microenvironment

Subchondral bone in OA undergoes an uncoupling of remodelling process, in which enhanced osteoclast-mediated bone resorption and osteoblast-mediated bone formation could be displayed at different stages during OA progression.[32] Normally, biomechanical coupling of articular cartilage and subchondral bone has been well established. In early-stage OA, the self-repair of articular cartilage reduces excessive mechanical loads on subjacent subchondral bone. As a result, loading of subchondral bone falls below a predetermined level. In turn, this underloading increases the ratio of the expression of receptor activator of nuclear factor κB ligand (RANKL)/osteoprotegerin (OPG) in osteocytes, which leads to excessive osteoclastogenesis and enhanced bone resorption activity.[33 34] Overactivated bone remodelling is commonly found at microdamage sites in subchondral bone in patients with OA and OA animal models.[35 36] Osteocytes directly adjacent to microdamage sites undergo apoptosis, whereas osteocytes adjacent to apoptotic populations upregulate the expression of pro-osteoclastic molecules at the early stage of OA.[37 38] Conversely, osteocytes also regulate osteoblast mineralisation by activating the Wnt signalling pathway via increased production of Wnt proteins and decreased secretion of sclerostin (SOST) in response to increased mechanical loading, which is caused by progressive cartilage destruction in OA during progression to the advanced and late stages.[39 40] In addition, it was confirmed in vitro that transforming growth factor-β1 (TGF-β1) from osteocytes could enhance osteoblast-mediated bone anabolic metabolism by activating Smad2/3 in the subchondral bone in advanced-stage OA.[41] As a result, the concomitant increase in osteoblast activity leads to spatial remineralisation and osteosclerosis in the end stage of OA.

In parallel, osteoclastic bone resorption leads to a sharp increase in active TGF-β1 in OA subchondral bone, recruiting osteoprogenitors to bone remodelling sites via activation of the Smad2/3 pathway to promote the formation of osteoid islets.[42] Abnormal mechanical strain triggers dysregulated metabolism in osteoblasts, which is characterised by increased expression of interleukin (IL)−6, prostaglandin E2 (PGE2), the degradative metalloproteinases matrix metalloproteinase (MMP)–3,

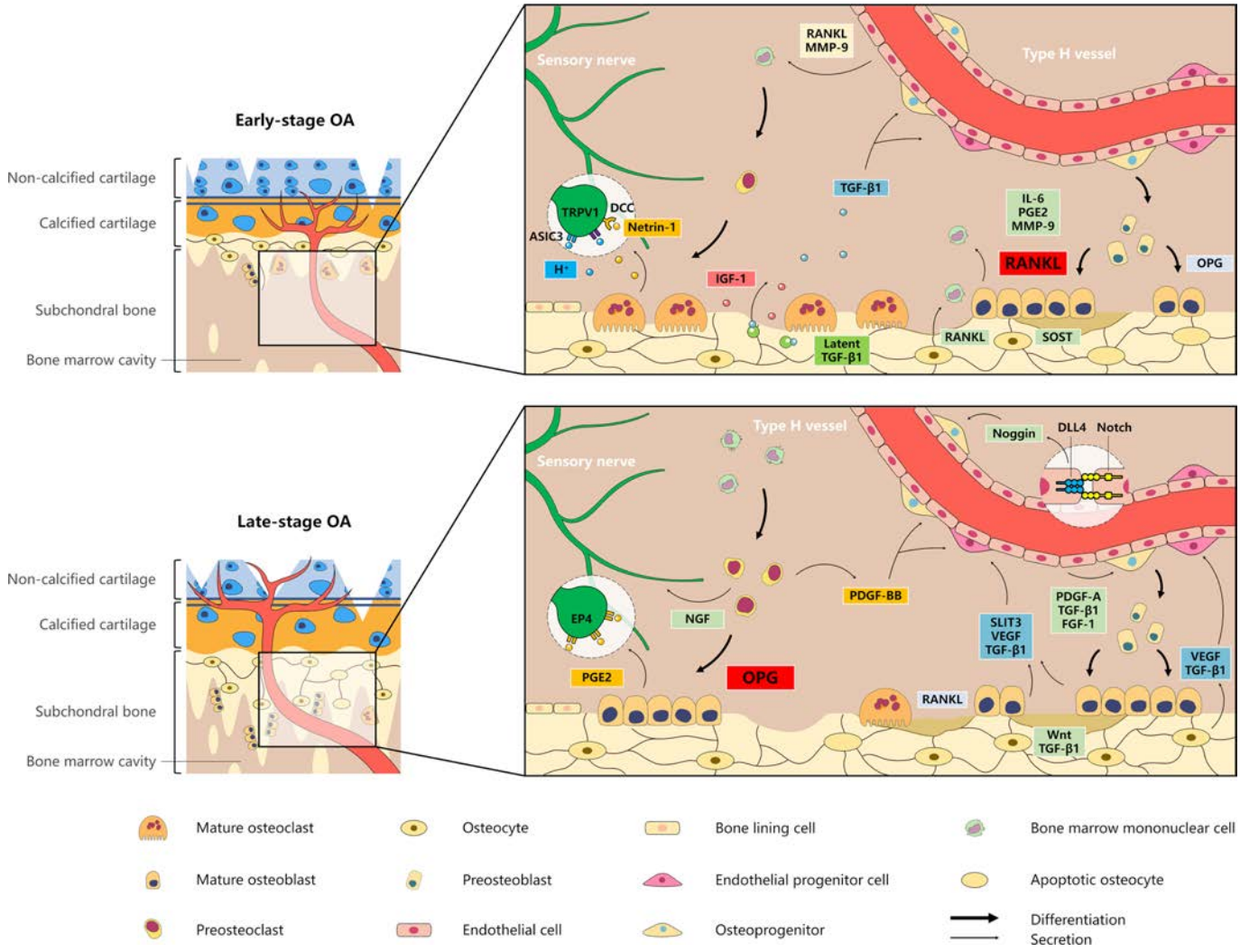

Figure 2 Pathological cellular interactions in the osteoarthritis (OA) subchondral microenvironment. (A) In early-stage OA, osteocytes upregulate the expression of RANKL:OPG ratio to enhance osteoclast differentiation. According to relative production of PGE2, IL-6 and OPG to RANKL, osteoblasts are separated into two subgroups: 'low-synthesiser cells' and 'high-synthesisers'. PGE2, IL-6, MMP-9 and VEGF from the two subgroups mediate the pro-osteoclastic effect, while the former acts as primary effectors of subchondral bone loss by high levels of RANKL. In parallel, osteoclastic bone resorption is primarily responsible for angiogenesis and osteogenesis by released TGF-β1. Moreover, sensory innervation is induced by H^+ and Netrin-1 secreted from mature osteoclasts. RANKL and MMP-9 produced by type H ECs may facilitate osteoclast chemotaxis and formation. (B) In late-stage OA, osteocytes regulate osteoblast mineralisation by increased Wnt proteins and TGF-β1 in response to increased mechanical loading. Multiple cells produce factors to support type H vessel formation, including VEGF and TGF-β1 from osteocyte, PDGF-BB from pre-osteoclasts, and VEGF, TGF-β1 and SLIT3 from osteoblasts. Sustained nerve sprouting is supported by NGF from preosteoclasts and PGE2 from osteoblasts. The latter subgroup promotes subchondral bone sclerosis, primarily regulated by angiocrine factors (PDGF-A, TGF-β1 and FGF-1). ASIC, acid-sensing ion channel; DCC, deleted in colon cancer; DLL4, delta-like protein 4; DP1R, DP1 receptor; IL-6, interleukin-6; MMP-9, matrix metalloproteinase-9; PDGF, platelet-derived growth factor; PG, prostaglandin; RANKL, receptor activator of NF-κB ligand; SLIT3, slit guidance ligand 3; SOST, sclerostin; TGF-β1, transforming growth factor-β1; TRPV1, transient receptor potential vanilloid 1; VEGF, vascular endothelial growth factor.

–9, −13 and RANKL and decreased production of OPG.[43] IL-6 and PGE2 stimulate osteoclast formation by inhibiting the secretion of OPG and stimulating the production of RANKL in osteoblasts or by upregulating the expression of RANK in osteoclasts.[44] Moreover, PGE2 promotes the secretion of IL-6, and in turn, IL-6 promotes the secretion of PGE2 by osteoblasts.[45] Hence, the positive feedback loop between PGE2 and IL-6 signalling promotes osteoclast differentiation via affecting the OPG/RANKL/RANK system. In addition, RANKL and vascular endothelial growth factor (VEGF) secreted by osteoblasts in subchondral bone in OA could trigger osteoclast chemotaxis by inducing extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation.[46–48]

Evidence has shown that the crosstalk between osteoblast or osteoclast lineage cells and type H ECs promotes subchondral angiogenesis and aggravates subchondral bone remodelling.[49–51] Type H ECs surrounded by osterix-expressing osteoprogenitors produce high levels of angiocrine factors (such as platelet-derived growth factor (PDGF)–A, TGF-β1 and fibroblast growth factor (FGF)−1), stimulating survival, proliferation and differentiation of these osteoprogenitors to promote local bone formation.[52 53] Type H ECs intercommunicate via the intercellular Notch/delta-like protein 4 (DLL4) signalling pathway to induce the production of Noggin,[54] which stimulates the differentiation of osteoprogenitors surrounding vessels.[55] Type H vessels also stimulate osteoclast migration and differentiation

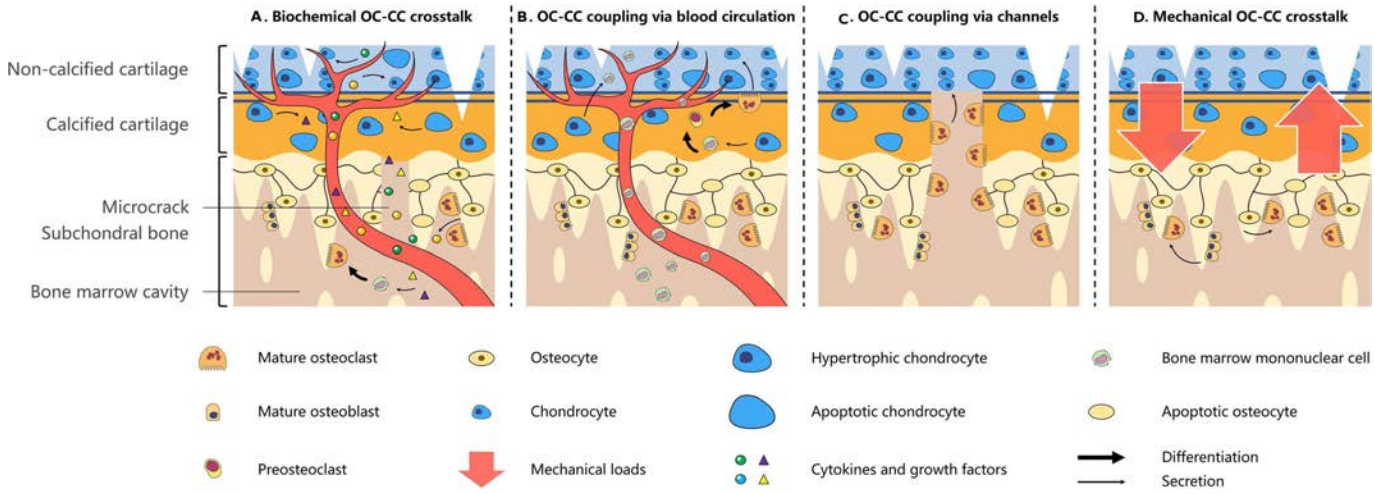

Figure 3 Various pathways for the 'osteoclast–chondrocyte crosstalk'. (A) Osteoclasts (OC) and chondrocytes (CC) interplay through secreted mediators crossing microcracks and vessels. (B) Bone marrow mononuclear cells are brought to the cartilage layer through invasive vessels. Osteoclast lineage cells directly contact with chondrocytes at different stages of differentiation. (C) Mature osteoclasts tunnel their way into subchondral bone and overlying cartilage and interact with chondrocytes in the cartilage layer. (D) Subchondral bone destruction mediated by osteoclasts transfers shear forces to the cartilage layer and consequently leads to abnormal chondrocyte metabolism. In turn, osteocytes and osteoblasts sense overloads from the damaged cartilage layer and send pro-osteoclastic signals, resulting in accelerated subchondral bone remodelling.

by producing RANKL and MMP-9, which regulate bone remodelling to promote longitudinal bone growth.[56] In addition, slit guidance ligand 3 (SLIT3) and TGF-β1 derived from osteoblasts acts as pro-angiogenic factors to increase the number of type H ECs.[57–59] Notably, TGF-β1 derived from osteoclastic resorption is primarily responsible for subchondral angiogenesis in early-stage OA,[60] while the increase in preosteoclast-derived PDGF-BB plays a relatively predominant role in angiogenic and osteogenic differentiation in late-stage OA (figure 2).[61]

REGULATION FEEDBACK LOOP OF 'OSTEOCLAST–CHONDROCYTE CROSSTALK'

Various pathways for the 'osteoclast–chondrocyte crosstalk'

A large number of vessels from subchondral bone penetrate calcified cartilage and invade non-calcified cartilage through vertical microcracks observed in OA joints.[62] Consequently, mediators originating from osteoclasts and chondrocytes may diffuse and transport across microcracks or via invasive vessels. Intriguingly, osteoclast precursors invade the hypertrophic area of cartilage during the growth of periosteal vessels and then function together with hypertrophic chondrocytes to remodel cartilage matrix and form a primary ossification centre.[63 64] Similarly, an in vivo cell tracking technique revealed that bone marrow–derived CX3CR1-positive osteoclast precursors enter the inflammatory cartilage layer via the blood circulation and differentiate into mature osteoclasts, promoting cartilage destruction in rheumatoid arthritis.[65] Collectively, these data suggest that osteoclast precursors migrate into the cartilage layer and then make direct contact with hypertrophic chondrocytes and even interact with chondrocytes with normal phenotype. In addition, recent data have identified the capability of osteoclasts to degrade the osteochondral junction and articular cartilage in an MMP-dependent and cysteine protease–dependent manner,[66] indicating the potential of mature osteoclasts to function as direct regulators of neighbour chondrocytes. During 'mechanical OC-CC crosstalk', on the one hand, the cartilage layer exhibits abnormal alterations in OA progression, which reduce its ability to absorb mechanical pressure and result in excessive loads on subchondral bone.[67] On the other hand, high turnover of subchondral bone leads to alterations in the biomechanical properties of bone tissue in early OA, transferring shear forces to the cartilage layer and causing continued cartilage damage (figure 3).[68]

Regulation of chondrocytes by osteoclasts promotes cartilage deterioration

Growth factors released from the bone matrix through osteoclastic bone resorption regulate chondrocyte metabolism and participate in cartilage deterioration. Mature osteoclasts attach to the bone surface through sealing zones and dissolve bone during bone remodelling. Consequently, various factors are released from the bone matrix, including TGF-β1, insulin-like growth factor (IGF)−1 and calcium-phosphate complexes.[69] Zhang *et al*[70] found that the expression of TGF-β1 in osteoclasts was significantly upregulated in a time-dependent and dose-dependent manner under mechanical stimulation. Meanwhile, chondrocytes showed increased apoptosis when cultured with osteoclasts. Furthermore, intraperitoneal injection of TGF-β1R inhibitors reversed chondrocyte apoptosis and reduced cartilage degradation in a rat OA model.[70] TGF-β1 is not derived from osteoclastic bone resorption in the study, no matter what, it implied that TGF-β1 in subchondral bone could be transferred to the cartilage layer by diffusion or blood transport to adversely affect chondrocytes. Intriguingly, IGF-1, another bone-released growth factor, was shown to play a protective role in chondrocyte anabolism. IGF-1 promotes the expression of Col2a1 and inhibits the expression and enzyme activity of MMP-13 by activating the phosphatidylinositol 3 kinase (PI3K)/Akt and ERK1/2 pathways in rat endplate chondrocytes.[71] In addition, IGF-1 signalling protects chondrocytes from apoptosis by reducing caspase-3 activity and DNA fragmentation.[72 73] Cartilage also obtains calcium–phosphate complexes from subchondral bone, which increases the production of MMP-13 in chondrocytes via activation of nuclear factor-kappa B (NF-κB), p38 and ERK1/2, and signal transducer and activator of transcription 3 (STAT3) signalling.[74] Lu *et al*[50] reported a nutrient-sensing mechanism in which vascular-derived nutrients (such as amino acids) induced hypertrophic differentiation by activating mechanistic target of rapamycin complex 1 (mTORC1). Osteoclasts at distinct stages

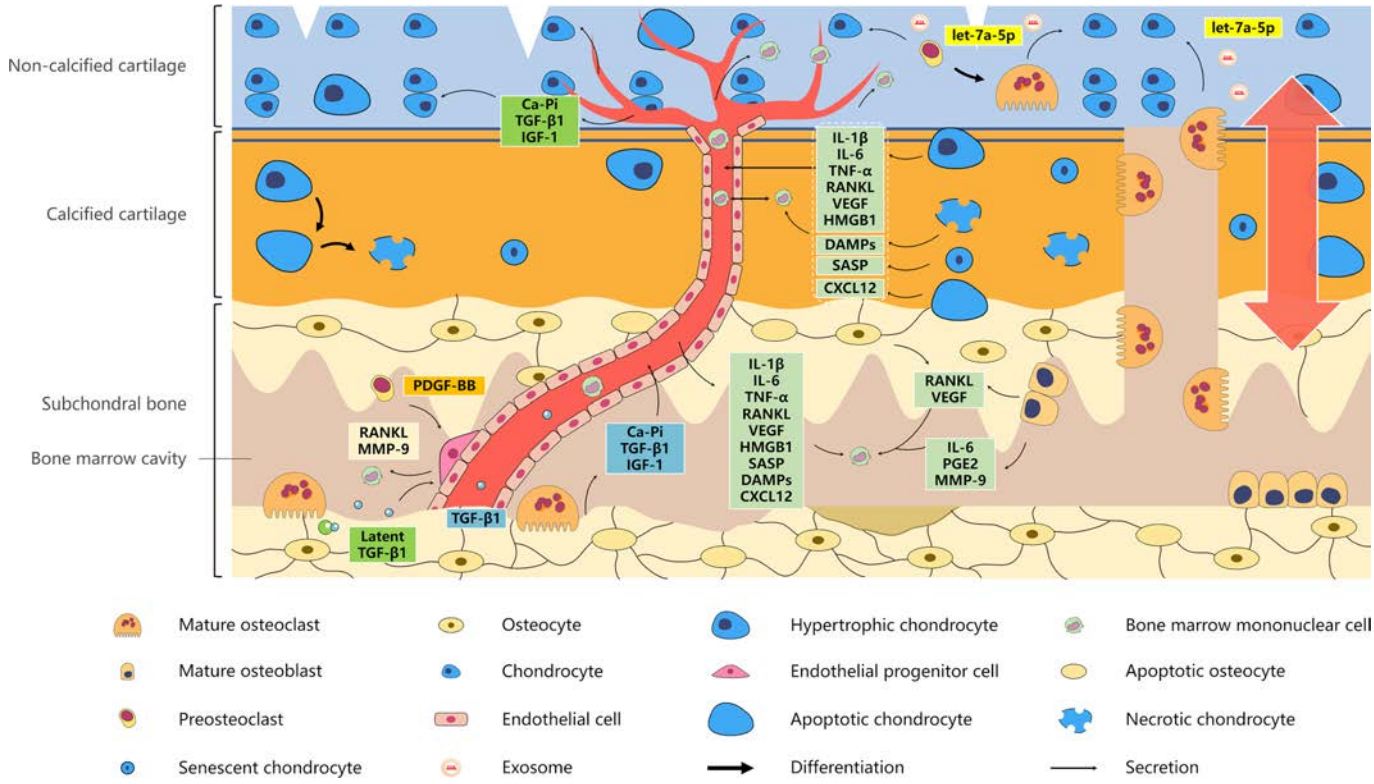

Figure 4 Role of the 'osteoclast–chondrocyte crosstalk' in the pathogenesis of OA. Multiple subchondral factors arrive at the cartilage layer through blood transport to regulate chondrocyte metabolism. For example, various factors are released from bone matrix, including TGF-β1, IGF-1 and Ca–Pi complexes. Moreover, BMMCs migrate into the cartilage layer. Mediators produced by chondrocytes are transported to the subchondral bone layer through blood transport. Hypertrophic, senescent and necrotic chondrocytes produce high levels of pro-osteoclastic molecules, which act on BMMCs in the subchondral bone or cartilage layer to promote osteoclast recruitment and formation. Preosteoclasts and mature osteoclasts in the cartilage layer induce chondrocyte hypertrophy through exosomal let-7a-5p. In addition, osteoclasts and chondrocytes influence each other by 'OC–CC coupling via channels' or 'mechanical OC–CC crosstalk'. BMMC, bone marrow mononuclear cell; CXCL12, CXC motif chemokine 12; DAMP, damage-associated molecular pattern; FGF, fibroblast growth factor; HMGB1, high mobility group box 1; IGF, insulin-like growth factor; IL, interleukin; MMP-9, matrix metalloproteinase-9; PDGF, platelet-derived growth factor; PG, prostaglandin; RANKL, receptor activator of NF-κB ligand; SASP, senescence-associated secretory phenotype; SLIT3, slit guidance ligand 3; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor.

of differentiation derived from bone marrow mononuclear cells (BMMCs) may affect the normal phenotype of chondrocytes. Our group reported that exosomal let-7a-5p from preosteoclasts and mature osteoclasts targets Smad2 to promote the hypertrophic differentiation of chondrocytes,[75] providing insights into 'OC–CC coupling' during OA progression.

Regulation of osteoclasts by chondrocytes promotes subchondral bone loss

Subchondral bone cells may be exposed to various pro-inflammatory cytokines produced by OA chondrocytes. Changes in joint biomechanical properties induce the upregulation of IL-1β in primary chondrocytes.[76] IL-1β upregulates the expression of RANKL by osteoblasts to indirectly induce osteoclast formation and directly induces osteoclast precursors to form multinucleated osteoclasts.[77] The excessive production of tumour necrosis factor (TNF)-α and IL-6 in chondrocytes in OA was detected in a surgical OA model of destabilisation of the medial meniscus.[78] TNF-α directly induces osteoclast differentiation by activating NF-κB and c-Jun NH2-terminal protein kinase (JNK) in a RANKL-independent manner[79] and indirectly induces osteoclastogenesis by stimulating osteoblasts to express RANKL.[80] IL-6 induces CD14-positive peripheral blood mononuclear cells to form tartrate-resistant acid phosphatase (TRAP) and calcitonin receptor–positive osteoclasts in a RANKL-independent manner by activating the signal transduction factor gp130.[81] In addition, VEGF-positive and RANKL-positive chondrocytes are increased in the hypertrophic layer by applying mechanical stress to the temporomandibular joint. In parallel, TRAP-positive osteoclasts increase in the mineralised layer subjacent to the hypertrophic layer.[82] Furthermore, RANKL and VEGF induced osteoclast chemotaxis through the phosphorylation of ERK1/2 in a modified model of osteoclasts cultured in a Boyden chamber.[83] High-mobility group box 1 (HMGB1) is expressed in and around OA chondrocytes in vivo.[84] Taniguchi *et al*[85] analysed the bone development of *Hmgb1*$^{-/-}$ in hypertrophic chondrocytes in the growth plate of mice and found that the endochondral bone formation was disrupted due to the delayed invasion of osteoclast precursors into the primary ossification centre. In addition, senescent chondrocytes occur alongside hypertrophic chondrocytes, which produce catabolic enzymes, pro-inflammatory mediators and chemokines (collectively known as the senescence-associated secretory phenotype (SASP)),[86 87] potentially modulating the behaviours of subchondral osteoclast lineage cells.

The presence of chondrocytes with morphological features consistent with apoptosis in OA cartilage is positively correlated with OA severity.[88–90] Tang *et al*[91] found that the conditioned

Table 1 Role of the 'osteoclast–chondrocyte crosstalk' in the pathogenesis of OA

Origins	Factors	Effects	References
Bone resorption	TGF-β1	Induce endothelial progenitor cell and osteoprogenitor migration and chondrocyte hypertrophy and apoptosis	42 60 70
	IGF-1	Induce chondrocyte anabolism and prevent chondrocyte maturation and apoptosis	71–73
	Ca-Pi	Induce chondrocyte catabolism	74
Preosteoclast	PDGF-BB	Modulate chondrocytes through abnormal angiogenesis	61
	Exosomal let-7a-5p	Promote the hypertrophic differentiation of chondrocytes by targeting Smad2	75
Mature osteoclast	Exosomal let-7a-5p	Promote the hypertrophic differentiation of chondrocytes by targeting Smad2	75
Type H endothelial cell	MMP-9, RANKL	Stimulate osteoclast migration to indirectly affect chondrocytes	56
Mature osteoblast	IL-6, PGE2	Enhance osteoclast formation to indirectly regulate chondrocytes	43–45
	VEGF	Stimulate angiogenesis and osteoclast recruitment to indirectly affect chondrocytes	46 47
	RANKL	Stimulate osteoclast recruitment and differentiation to indirectly regulate chondrocytes	46 47
	MMP-9	Promote osteoclast recruitment to indirectly affect chondrocytes	43
	SLIT3, TGF-β1	Induce subchondral angiogenesis to indirectly affect chondrocytes	57–59
Osteocyte	VEGF, TGF-β1	Stimulate angiogenesis to indirectly regulate chondrocytes	33 34 41
	RANKL	Induce osteoclast recruitment and differentiation to indirectly modulate chondrocytes	33 34
Hypertrophic chondrocyte	IL-1β, IL-6, TNF-α	Induce osteoclast differentiation directly or indirectly	76–81
	RANKL, VEGF	Induce osteoclast chemotaxis and differentiation	82 83
	HMGB1	Promote osteoclast recruitment to indirectly affect chondrocytes	84 85
Senescent chondrocyte	SASP	Promote osteoclast chemotaxis and differentiation	86 87
Apoptotic chondrocyte	CXCL12	Enhance osteoclast recruitment and differentiation	91–93
Necrotic chondrocyte	DAMPs	Promote osteoclast formation	94 95

medium of apoptotic chondrocytes following dexamethasone treatment enhanced the recruitment of RAW264.7 osteoclast precursor cells and increased their differentiation potential. Further explorations confirmed that CXC motif chemokine 12 (CXCL12) released from apoptotic chondrocytes had the strongest pro-osteoclastic effect by activating the ERK1/2 and p38 pathways in BMMCs.[91] AMD3100 (an inhibitor of CXCR4) effectively prevented subchondral trabecular destruction and cartilage loss in the tibia of mice after anterior cruciate ligament transection (ACLT).[92 93] The cartilage matrix is the main obstacle for phagocytic cells, resulting in late apoptotic chondrocytes undergoing the transition to necrosis, which is called secondary necrosis.[94] Necrosis causes plasma membrane rupture and the release of damage-associated molecular patterns (DAMPs), such as nucleotides, HMGB1 and pro-inflammatory cytokines.[95] DAMPs act on nearby cartilage and synovium to trigger inflammation, and may regulate the behaviours of subchondral osteoclast lineage cells (figure 4, table 1).

TARGETING THE SUBCHONDROL BONE MICROENVIRONMENT FOR THE TREATMENT OF OA

Restoring abnormal subchondral bone remodelling

In fact, the efficacy of antiresorptive agents in OA treatment has been evaluated in clinical trials by restoring abnormal subchondral bone remodelling. Regrettably, there are currently few or no data on the beneficial effect of strategies targeting abnormal bone remodelling in patients with OA. Bisphosphonates approved for osteoporosis management belong to classical antiresorptive agents. Risedronate reduced biochemical markers of cartilage degradation but did not improve signs or symptoms or slow radiographic progression in a prospective 2-year trial involving 2483 patients with medial compartment knee OA at dosages of 5 mg/day, 15 mg/day, 35 mg/week or 50 mg/week.[96] Alendronate treatment improved the Western Ontario and McMaster University Osteoarthritis Index pain score, decreased biochemical markers and increased the BMD in a prospective 2-year trial

Table 2 Targeting the subchondral bone microenvironment for the treatment of osteoarthritis (OA)

Therapeutic strategy	Agents	Effects	References
Restoring abnormal subchondral bone remodelling	Bisphosphonate, osteoprotegerin, cathepsin K inhibitor, strontium ranelate	Relieve pain, improve joint structure, and reduce bone and cartilage degradation markers	96–101
	Calcitonin	Prevent bone pathology development and promote chondrocyte anabolism	102 103
	TGF-β1 inhibitor	Reform subchondral bone remodelling and inhibit subchondral angiogenesis	60
Blocking the bridge—subchondral type H vessels	Bevacizumab	Attenuate subchondral angiogenesis	50
	Halofuginone	Restore coupled bone remodelling and alleviate type H vessel formation by inhibiting TGF-β1 signalling	105
Ameliorating OA-related pain by modulating the subchondral bone microenvironment	Tanezumab	Reduce pain and improve joint function by binding NGF specifically	111 112
	SB366791, APETx2	Improve acidic subchondral bone microenvironment and acid-induced pain by inhibiting TRPV1 and ASIC3, respectively	110 115
	COX2 inhibitor, $Na_v1.8$ inhibitor, EP4 receptor inhibitor	Blunt nociceptive signals in subchondral sensory neurons	11 113 114

involving 50 patients with symptomatic hip OA.[97] Moreover, compared with those receiving placebo, patients with symptomatic knee OA who received intravenous zoledronic acid yearly did not show a significant reduction in cartilage volume loss, the size of BMOLs or the pain score over 24 months.[98] There are other antiresorptive agents (such as OPG, cathepsin K (CTSK) inhibitors and strontium ranelate) that may exert protective effects on subchondral bone and cartilage in animal models and serve as disease-modifying OA drugs for clinical treatment of OA.[99–101] Intriguingly, calcitonin, which is known for targeting subchondral bone remodelling, also leads to intracellular cAMP accumulation and then promotes chondrocyte anabolism by binding to its receptors on human OA chondrocytes.[102] Two phase III trials have reported a beneficial effect of bioactive oral calcitonin on joint pain and biochemical indicators of bone and cartilage degradation in patients with OA.[103] Enhanced osteoclast activity leads to the overactivation of TGF-β1 signalling in subchondral bone, and therefore subchondral TGF-β1 is a pharmacological target for OA. Implantation of alginic acid microbeads with TGF-β1 antibody into subchondral bone or deletion of *Tgfbr2* prevented the phosphorylation of Smad2/3 in osteoblastic precursor cells, thus reducing their subchondral localisation and improving bone parameters and cartilage structure in a mouse ACLT model.[60] Accumulating evidence suggests that restoring subchondral bone remodelling could improve OA symptoms and the structure of bone and cartilage, but these agents require large clinical trials with plenty of subjects to verify their effects.

Blocking the bridge—subchondral type H vessels

Invasive subchondral type H vessels serve as a bridge between subchondral bone and articular cartilage. Current treatments for OA focus on the inhibition of inflammation and subchondral bone remodelling, while therapeutic strategies targeting subchondral angiogenesis are limited. In fact, blocking type H vessel formation in animal models of OA has been shown to reduce cartilage destruction and subchondral bone loss.[104] For example, bevacizumab (a VEGF blocking antibody) attenuated the formation of subchondral type H vessels in an OA model, thereby inhibiting chondrocyte hypertrophy and delaying OA progression.[50] In addition to pharmacological VEGF inhibition, secretory factors derived from osteoclast or osteoblast lineage cells in the OA subchondral bone microenvironment, such as TGF-β1, PDGF-BB and SLIT3, promote subchondral angiogenesis. Therefore, antagonists of those molecules might be developed as potential agents for OA. For example, the small molecule compound halofuginone inhibits Smad2/3-dependent TGF-β1 signalling to restore the coupling of subchondral bone remodelling, alleviate type H vessel formation and attenuate cartilage degradation in the rodent ACLT joint.[105]

Ameliorating OA-related pain by modulating subchondral bone microenvironment

The detailed mechanisms of OA contributing to pain remained unclear for decades until recent studies found that particular neuronal factors related to aberrant bone remodelling cause the innervation of sensory nerves in the subchondral bone of patients with OA.[106 107] Bone-resorbing osteoclasts create an acidic microenvironment by secreting H^+ to cause bone pain in animal models of bone metastasis. Mechanistically, acidosis induces the expression and activation of acid-sensing receptor transient receptor potential vanilloid 1 (TRPV1) in dorsal root ganglions (DRGs). TRPV1 activation promotes extracellular Ca^{2+} influx and then activates calmodulin-dependent protein kinase II (CaMKII) and transcription factor cAMP-responsive element-binding protein (CREB), leading to the transcriptional activation of the pain-related molecule calcitonin gene-related peptide (CGRP).[108 109] Similarly, acid-sensing ion channel 3 (ASIC3) is upregulated in mono-iodoacetate-induced OA model and is associated with hyperalgesia caused by increased Ca^{2+} influx.[110] Netrin-1 secreted by osteoclasts induces sensory innervation and pain in OA through its receptor deleted in colon cancer (DCC).[10] Preosteoclasts produce nerve growth factor (NGF), serving as key drivers of subchondral nerve innervation during OA development.[61] In addition, PGE2 is synthesised by osteoblasts in response to low bone density and contributes to skeletal allodynia in OA mice by upregulating the voltage-gated sodium channel $Na_v1.8$ and increasing Na^+ influx in subchondral nociceptive neurons.[11]

Pain medications recommended in the current guidelines for OA include non-steroidal anti-inflammatory drugs, paracetamol, opioids and corticosteroids administered via the oral, topical or intraarticular route. Several new pain treatments are currently moving forward in preclinical and clinical evaluation processes, potentially marking the beginning of a new era in the management of OA-related pain. Tanezumab (a human monoclonal antibody against NGF) is significantly superior to placebo in reducing pain and improving joint function with fewer adverse events based on a meta-analysis of 10 studies.[111 112] Evidence suggests that a small molecule conjugate linking the TGF-βR inhibitor TLY-2109761 and alendronate substantially reduces excessive PGE2 production by osteoblasts and alleviates OA-induced pain in OA mice by restoring aberrant bone remodelling.[11] In addition, nociceptive signals were blunted in subchondral sensory neurons in OA mice by the administration of a cyclooxygenase 2 (COX2) inhibitor, the $Na_v1.8$ inhibitor A-803467 and an EP4 receptor antagonist.[11 113 114] Furthermore, Ca^{2+} influx into the cytoplasm in sensory neuron was inhibited by the TRPV1 antagonist SB366791 and the ASIC3 antagonist APETx2 to reduce acid-induced pain in a murine model of bone cancer pain and a rat model of OA, respectively.[110 115] Collectively, further exploration of how the subchondral bone microenvironment is related to OA pain may be an excellent approach to develop specific drugs useful for the treatment of OA (table 2).

CONCLUSION AND PERSPECTIVE

The bone–cartilage unit composed of subchondral bone and cartilage plays a significant role in joint homeostasis and OA development. During OA progression, the two joint compartments of the functional unit experience abnormal alterations in tissue structure and cellular activity. Therefore, therapeutic strategies targeting one of the abnormal joint compartments could restrain the progression of the pathology of the whole joint. Furthermore, this strategy may be an effective disease-modifying method to block pathological interactions between the two joint compartments through pharmacological interventions. More extensive cellular and molecular studies of bone–cartilage interface crosstalk will help us to better understand the pathophysiology of OA and modify existing OA therapies. In particular, the microenvironment in subchondral bone serves as the predominant regulator of the development of OA. Therefore, future studies should focus on how pathological cellular interactions in the subchondral bone microenvironment promote subchondral bone destruction and OA pain and the development of novel drugs to treat OA by targeting the subchondral bone microenvironment.

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EPIDEMIOLOGICAL SCIENCE

Concomitant use of oral glucocorticoids and proton pump inhibitors and risk of osteoporotic fractures among patients with rheumatoid arthritis: a population-based cohort study

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ABSTRACT

Background Patients with rheumatoid arthritis (RA) commonly use oral glucocorticoids (GCs) and proton pump inhibitors (PPIs), both associated with osteoporotic fractures. We investigated the association between concomitant use of oral GCs and PPIs and the risk of osteoporotic fractures among patients with RA.

Methods This was a cohort study including patients with RA aged 50+ years from the Clinical Practice Research Datalink between 1997 and 2017. Exposure to oral GCs and PPIs was stratified by the most recent prescription as current use (<6 months), recent use (7–12 months) and past use (>1 year); average daily and cumulative dose; and duration of use. The risk of incident osteoporotic fractures (including hip, vertebrae, humerus, forearm, pelvis and ribs) was estimated by time-dependent Cox proportional-hazards models, statistically adjusted for lifestyle parameters, comorbidities and comedications.

Results Among 12 351 patients with RA (mean age of 68 years, 69% women), 1411 osteoporotic fractures occurred. Concomitant current use of oral GCs and PPIs was associated with a 1.6-fold increased risk of osteoporotic fractures compared with non-use (adjusted HR: 1.60, 95% CI: 1.35 to 1.89). This was statistically different from a 1.2-fold increased osteoporotic fracture risk associated with oral GC or PPI use alone. Most individual fracture sites were significantly associated with concomitant use of oral GCs and PPIs. Among concomitant users, fracture risk did not increase with higher daily dose or duration of PPI use.

Conclusions There was an interaction in the risk of osteoporotic fractures with concomitant use of oral GCs and PPIs. Fracture risk assessment could be considered when a patient with RA is co-prescribed oral GCs and PPIs.

Key messages

What is already known about this subject?

- Oral glucocorticoids (GCs) and proton pump inhibitors (PPIs) are among the commonly prescribed medications for patients with rheumatoid arthritis (RA).
- Oral GCs increase the risk of osteoporotic fractures by well-established biological mechanisms, including effects on bone, muscle strength and vision.
- The association between PPIs and fracture risk is less well established, although biological mechanisms such as hypochlorhydria and reduced calcium absorption have been proposed.

What does this study add?

- This is the first study to evaluate the association between concomitant use of oral GCs and PPIs and the risk of osteoporotic fractures in patients with RA, using a large primary care database.

How might this impact on clinical practice or future developments?

- As we observed a 1.6-fold increased risk of osteoporotic fractures with concomitant use of oral GCs and PPIs in patients with RA, fracture risk assessment could be considered for these patients.

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INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic musculoskeletal inflammatory disease with many complications, including an elevated risk of osteoporotic (OP) fractures.[1–3] The contributors to increased fracture risk include the inflammatory process of RA and the pharmacological treatment of the disease, most importantly oral glucocorticoids (GCs). About one-quarter of patients with RA in the UK are current users of oral GCs.[1] Patients with RA taking oral GCs have reduced bone mineral density (BMD) at the hip and vertebrae and up to a 35% increased 5-year fracture risk.[1 4] This higher fracture risk with GCs is independent of the disease process and by known mechanisms, such as decreased bone formation, elevated bone resorption and ultimately reduced bone density.[5–9]

Apart from GCs, patients with RA frequently use other medications that could also be associated with fragility fractures. Non-steroidal anti-inflammatory drugs (NSAIDs) are routinely prescribed for patients with RA as analgesics, and proton pump inhibitors (PPIs) may be co-prescribed to reduce the gastrointestinal side effects. A randomised, double-blind,

crossover trial showed that fractional ^{45}calcium absorption was significantly decreased among elderly women using omeprazole (3.5%) versus placebo (9.1%), possibly because of hypochlorhydria.[10] Observational studies have reported conflicting results. Some reported an increased risk of hip and vertebral fractures with PPI use, suggesting a causal effect,[11–15] whereas others could not match the shape of the hazard function of PPI-induced fracture risk to calcium absorption hypothesis.[16 17] Other mechanisms such as an increased fall risk due to hypomagnesaemia or explanations such as unmeasured confounding were also proposed to explain this association.[16–20]

A population-based study reported a 2.4-fold increased risk of hip fracture among concomitant users of both PPIs and high-dose oral GCs (≥15 mg prednisolone equivalent dose (PED)).[16] But, to our knowledge, no studies have evaluated the effects of simultaneous use of both drugs on fracture risk in patients with RA, particularly in elderly patients who are regular users of PPIs.[21 22] Thus, we sought to investigate the association between concomitant use of oral GCs and PPIs and the risk of OP fractures among patients with RA.

METHODS

Data source

This was a retrospective cohort study based on the Clinical Practice Research Datalink (CPRD) GOLD database (http://www.cprd.com). The CPRD is one of the largest databases of primary care data in the world, which contained medical records of 674 practices in the UK in 2013, representing 4.4 million active patients, which equalled 6.9% of the total population.[23] It includes data on patient demographics, clinical diagnoses, prescription details, laboratory test results, specialist referrals and major outcomes since 1987, with continuing data collection. The CPRD has been well validated for a wide range of diseases, including hip and vertebral fractures.[24 25]

Study population

The study cohort included adults aged 50+ years and diagnosed with RA between 1 January 1997 and 31 December 2017. We used a validated algorithm to identify definite RA cases in the CPRD, which can detect 86% of the true RA cases (online supplementary table 1).[26 27] The date of the first RA diagnosis during valid data collection defined the index date. Patients were followed until the occurrence of the outcome, the end of the study period, a patient's transfer out of practice, death or the end of data collection, whichever came first. Following a new-user design, patients with a history of GC/PPI use during the 1 year before the index date and those with an OP fracture prior to the index date were excluded.

Exposure and outcome

Oral GCs and PPIs were the exposures of interest. From the RA index date, follow-up was divided into consecutive 30-day periods and exposure status was assessed time-dependently at the start of each period. A period was defined as current, recent or past use when the most recent prescription of oral GCs/PPIs was issued within 6 months, 7–12 months and >12 months before a period, respectively.[7 11 12 16 28] Follow-up time was defined as non-use if no oral GC/PPI had ever been prescribed. Patients were allowed to move between exposure states during follow-up. Once a non-user had taken oral GCs/PPIs, he could never become a non-user again.

To evaluate a dose–response relationship and to replicate previous similar studies,[11 17 28] current use of both drugs was further stratified in average daily and cumulative dose, and duration of treatment. All oral GC and PPI prescriptions were retrieved, and the prescribed quantity was extracted and converted into PED for GCs and omeprazole equivalent dose (OED) for PPIs using the WHO Anatomical Therapeutic Chemical classification system .[29] Values for missing data on prescribed quantity were assigned the median value of all prescriptions. The cumulative amount of the drug prescribed in each follow-up period was estimated by summing all consecutive prescriptions since the index date. The average daily dose in each follow-up period was calculated by dividing the cumulative amount prescribed by the treatment time (ie, the time between the first oral GC/PPI prescription and the start date of a period of current use). Continuous duration of PPI use was determined at each period of current use using the prescribed quantity and written dosage information, allowing a gap of 30 days after the expected end date of a prescription.[30] The outcome in this study was a first OP fracture after the RA index date, which included hip, clinically symptomatic vertebral, humerus, forearm, pelvic and rib fractures.[1 28 31 32]

Potential confounders

Body mass index (BMI), smoking status and alcohol use were determined at the index date. Age and history of comorbidities and comedications were determined time-dependently. Comorbidities included asthma, chronic obstructive pulmonary disease, ischaemic heart disease (including myocardial infarction), cerebrovascular disease, congestive heart failure, anaemia, peripheral vascular disease, gastro-oesophageal reflux disease, peptic ulcer disease, inflammatory bowel diseases (Crohn's disease and ulcerative colitis), coeliac disease, hyperthyroidism, hypothyroidism, type 1 and 2 diabetes mellitus, chronic renal failure, ankylosing spondylitis, dementia, Parkinson's disease, major infections (ie, sepsis, meningitis, and upper and lower respiratory tract infections) and malignant neoplasms (excluding non-melanoma skin cancers).[33] Falls were measured in the 7–12 months prior to a period. Use of comedications in the 6 months prior included antihypertensives, anticoagulants, calcium/vitamin D, bisphosphonates, hormone replacement therapy, anticonvulsants, hypnotics/anxiolytics, antidepressants and antipsychotics. The following proxy indicators of RA severity were included: use of non-selective NSAIDs, cyclo-oxygenase-2 selective inhibitors, paracetamol, tramadol, opioids (stronger than tramadol) or conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) in the past 6 months.

Statistical analysis

Time-dependent Cox proportional-hazards models estimated the risk of OP fracture in patients with RA with concomitant current use of oral GCs and PPIs versus non-use. Also, the use of oral GCs alone and PPIs alone, and the recent and past use of oral GCs and PPIs (regardless of the use of the other drug) were compared with non-use. Individual exposure categories were statistically compared with a Wald test to detect between-group significance. Stratified analyses were conducted for various OP fracture sites. Potential confounders were incorporated into the model if the beta coefficient of the association changed by >5% or based on expert opinion.

Secondary analyses focused on average daily and cumulative dose of current GC use in relation to average daily dose and continuous duration of PPI use. Furthermore, three sensitivity analyses were performed. First, calcium/vitamin D and bisphosphonates were added to the model as confounders. They were not

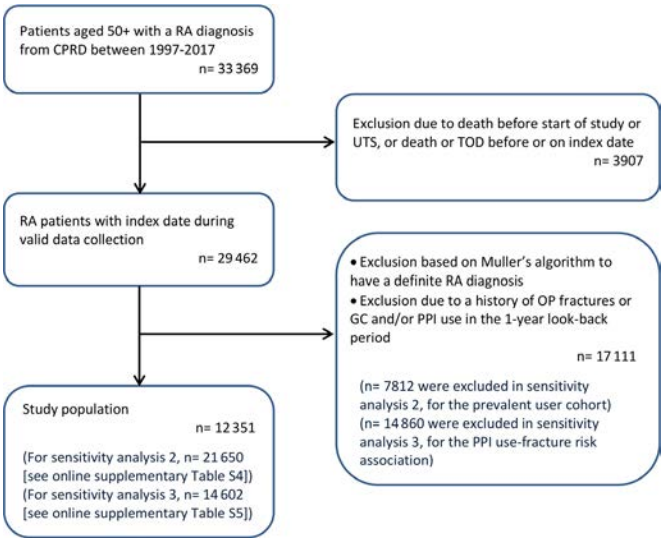

Figure 1 Flowchart on establishment of patient population. CPRD, Clinical Practice Research Datalink; GC, glucocorticoid; OP,osteoporotic; PPI, proton pump inhibitor; RA, rheumatoid arthritis; TOD, transfer out of database date (ie, date the patient was transferred out of the practice); UTS, up to standard time (ie, date at which the practice data are deemed to be of research quality).

considered in the main analysis because of the accompaniment of their prescriptions with those of oral GCs and as we expected them to lie in the causal pathway of the intended association as mediators.[34–36] Second, the main association was re-evaluated by including the prevalent users of GCs and PPIs. Finally, the association between PPI use and OP fractures was assessed among the primary cohort of patients with RA, by excluding only those with PPI use during the 1 year before the index date. Data were analysed using SAS V.9.4 (SAS Institute).

RESULTS

The study population included 12 351 patients with RA (figure 1). The mean age of concomitant users of oral GCs and PPIs at the index date was 67.5 years, 1.5 years younger than non-users (table 1). The mean duration of follow-up was 9.1 years for concomitant users and 5.1 years for non-users. About two-thirds of patients with RA were women (concomitant users: 67%; non-users: 70%). More than one-third of concomitant users were overweight, whereas 34% of non-users had a normal BMI. In the 6 months before the index date, 54% of concomitant users and 48% of non-users had taken non-selective NSAIDs. The average duration of drug use was 3.3 years for concomitant and single GC users, and 4.1 years for single PPI users.

Concomitant current use of oral GCs and PPIs in patients with RA was associated with a 1.6-fold increased risk of OP fractures compared with non-use of both drugs (adjusted HR (adj. HR): 1.60, 95% CI: 1.35 to 1.89; table 2). Both oral GC and PPI use alone had a 1.2-fold increased risk of OP fracture (adj. HR: 1.23, 95% CI: 1.03 to 1.47 (oral GC use alone); adj. HR: 1.22, 95% CI: 1.05 to 1.42 (PPI use alone)). The OP fracture risk associated with the current use of oral GCs or PPIs alone was statistically different from concomitant use. There was no significant increase in OP fracture risk in those patients who had stopped taking oral GCs or PPIs for more than 6 months (recent and past users) versus non-use. Considering calcium/vitamin D and bisphosphonates as confounders reduced the association to a 1.4-fold increased fracture risk for concomitant users and to a statistically non-significant risk for oral GC use alone versus non-use (online supplementary table 3).

Table 3 shows that among patients with RA, most OP fracture sites were statistically significantly associated with concomitant current use of oral GCs and PPIs versus non-use. With concomitant current use of oral GCs and PPIs, we observed a 1.5-fold increased risk of hip fracture, a 2.8-fold increased risk of clinical vertebral fracture, a 2.5-fold increased risk of pelvic fracture and a 4-fold increased risk of rib fracture. Risks of fracture of the humerus or forearm were not increased.

Table 4 shows the stratification of concomitant oral GC and PPI use by average daily doses of GCs and then substratification by average daily doses and continuous duration of PPI use. There was no increased fracture risk with increasing PPI daily doses. Under all strata of GC use, short-term PPI use (≤1 year) was associated with higher fracture risk, but there was no association between long-term PPI use (>1 year) and OP fractures. When concomitant use of GCs and PPIs was stratified by cumulative GC use and then substratified by PPI use, similar associations were observed (online supplementary table 2).

The second sensitivity analysis including prevalent users of GCs and PPIs (N=21 650) resulted in similar estimates to the main analyses (online supplementary table 4). In the third sensitivity analysis (N=14 602), current PPI use was associated with a 1.3-fold increased risk of OP fractures (adj. HR: 1.30, 95% CI: 1.15 to 1.47) versus non-use (online supplementary table 5).

DISCUSSION

Concomitant use of oral GCs and PPIs was associated with an increased risk of OP fractures compared with non-use in patients with RA. This was significantly higher when compared with the single use of oral GCs or PPIs. Increased fracture risk associated with concomitant GC and PPI use was observed for fractures of the hip, clinical vertebrae, pelvis and ribs, but not for those of the humerus or forearm. Among concomitant users, there was no increased OP fracture risk with higher daily dose or longer duration of PPI use.

This is the first study, to our knowledge, that looked into the association between concomitant use of GCs and PPIs and the risk of OP fracture in patients with RA . A Dutch population-based study found a 1.3-fold to 2.4-fold increased risk of hip/femur fracture with concomitant use of PPIs and various daily doses of oral GCs.[16] This is in line with our finding for the concomitant current use of GCs and PPIs (adj. HR: 1.60) and most of the strata of concomitant use in table 4. However, their reference group was different and limited to never PPI users. Moreover, they focused on 18+ general population, whereas we included patients with RA aged 50+ years with higher baseline fracture risks.

Our results regarding the higher fracture risk with PPI use are partly in line with several previous observational studies.[11 12 15–17] A meta-analysis of observational studies in non-RA patients reported increased risk of hip and spine fracture with PPI use (relative risks of 1.30 and 1.56, respectively),[14] which is comparable with adj. HR of 1.30 for current PPI use and OP fractures in our study. However, a recent study in patients with RA did not reveal a higher risk of OP fractures with PPI use, which was attributed to higher use of bisphosphonates among PPI users.[22] Previous studies found stronger associations with higher daily doses of PPIs[11] or with 7 years of PPI use and fracture risk,[12] whereas another older study that used the same data source but a different reference group did not report any dose–response or duration–response relationships at all.[17] Our findings (ie, no

Table 1 Baseline characteristics of study population at index date, stratified by oral GC and PPI therapy status during follow-up (N=12 351)

	Concomitant users of oral GCs and PPIs* (N=4254)		Users of oral GCs alone† (N=2136)		Users of PPIs alone‡ (N=2823)		Non-users (N=3138)	
	N	%	N	%	N	%	N	%
Mean duration of follow-up (years, SD)	9.1	5.0	7.5	4.9	8.4	5.0	5.1	4.3
Age (years)§								
Mean, SD	67.5	8.4	68.3	8.8	67.5	8.5	69.0	9.2
50–59	802	18.9	390	18.3	540	19.1	550	17.5
60–69	1763	41.4	813	38.1	1190	42.2	1102	35.1
70–79	1328	31.2	699	32.7	842	29.8	1055	33.6
80+	361	8.5	234	11.0	251	8.9	431	13.7
Number of women	2837	66.7	1443	67.6	2003	71.0	2190	69.8
BMI (kg/m^2)§								
Mean, SD	26.4	5.1	26.2	5.0	26.2	5.1	25.9	5.1
<20.0	304	7.1	146	6.8	200	7.1	222	7.1
20.0–24.9	1384	32.5	735	34.4	937	33.2	1079	34.4
25.0–29.9	1482	34.8	698	32.7	981	34.8	965	30.8
30.0–34.9	586	13.8	279	13.1	377	13.4	328	10.5
≥35.0	234	5.5	109	5.1	142	5.0	151	4.8
Missing	264	6.2	169	7.9	186	6.6	393	12.5
Smoking status§								
Non	1560	36.7	805	37.7	1158	41.0	1252	39.9
Current	988	23.2	522	24.4	571	20.2	646	20.6
Past	1670	39.3	770	36.0	1058	37.5	1104	35.2
Missing	36	0.8	39	1.8	36	1.3	136	4.3
Alcohol use§								
No	1249	29.4	559	26.2	780	27.6	795	25.3
Yes	2720	63.9	1380	64.6	1819	64.4	1969	62.7
Missing	285	6.7	197	9.2	224	7.9	374	11.9
History of comorbidities								
Asthma	573	13.5	277	13.0	170	6.0	207	6.6
COPD	321	7.5	161	7.5	65	2.3	102	3.3
Ischaemic heart disease (including myocardial infarction)	503	11.8	234	11.0	278	9.8	323	10.3
Cerebrovascular disease	234	5.5	109	5.1	139	4.9	152	4.8
Congestive heart failure	98	2.3	70	3.3	61	2.2	115	3.7
Anaemia	520	12.2	262	12.3	338	12.0	399	12.7
Peripheral arterial disease	200	4.7	103	4.8	132	4.7	139	4.4
Gastro-oesophageal reflux disease	198	4.7	94	4.4	130	4.6	110	3.5
Peptic ulcer disease	38	0.9	15	0.7	21	0.7	15	0.5
Coeliac disease	10	0.2	5	0.2	9	0.3	6	0.2
Inflammatory bowel disease (Crohn's disease and ulcerative colitis)	45	1.1	19	0.9	28	1.0	19	0.6
Hyperthyroidism	24	0.6	13	0.6	16	0.6	15	0.5
Hypothyroidism	289	6.8	149	7.0	206	7.3	213	6.8
Type 1 diabetes mellitus	25	0.6	17	0.8	17	0.6	15	0.5
Type 2 diabetes mellitus	230	5.4	101	4.7	164	5.8	172	5.5
Chronic renal failure	144	3.4	68	3.2	81	2.9	110	3.5
Ankylosing spondylitis	6	0.1	<5	<0.3	5	0.2	7	0.2
Dementia	17	0.4	11	0.5	19	0.7	28	0.9
Parkinson's disease	10	0.2	4	0.2	9	0.3	20	0.6
Malignant neoplasms (excluding non-melanoma skin cancers)	371	8.7	173	8.1	248	8.8	244	7.8
Major infections¶	812	19.1	397	18.6	468	16.6	452	14.4
Falls (in the past 7–12 months)	38	0.9	10	0.5	16	0.6	21	0.7
Comedication use (in the past 6 months)								
Antihypertensives	1383	32.5	718	33.6	905	32.1	1117	35.6
Anticoagulants	118	2.8	70	3.3	45	1.6	104	3.3
Calcium/vitamin D	290	6.8	105	4.9	133	4.7	190	6.1
Bisphosphonates	260	6.1	87	4.1	108	3.8	119	3.8

Continued

Table 1 Continued

	Concomitant users of oral GCs and PPIs* (N=4254)		Users of oral GCs alone† (N=2136)		Users of PPIs alone‡ (N=2823)		Non-users (N=3138)	
	N	%	N	%	N	%	N	%
Hormone replacement therapy	184	4.3	65	3.0	107	3.8	81	2.6
Anticonvulsants	51	1.2	29	1.4	37	1.3	46	1.5
Hypnotics/anxiolytics	356	8.4	168	7.9	184	6.5	189	6.0
Antidepressants	498	11.7	237	11.1	275	9.7	290	9.2
Antipsychotics	36	0.8	19	0.9	17	0.6	40	1.3
Disease severity indicators								
Non-selective NSAIDs	2309	54.3	1202	56.3	1514	53.6	1518	48.4
COX-2 selective inhibitors	409	9.6	205	9.6	255	9.0	191	6.1
Paracetamol	2117	49.8	987	46.2	1147	40.6	1328	42.3
Tramadol	263	6.2	113	5.3	148	5.2	138	4.4
Opioids (stronger than tramadol)	241	5.7	105	4.9	114	4.0	118	3.8
csDMARDs	1323	31.1	637	29.8	915	32.4	1091	34.8

*Concomitant users of oral GCs and PPIs are patients who had at least one co-prescription of an oral GC and PPI during follow-up.
†Users of oral GCs alone are patients who had at least one prescription of an oral GC during follow-up without having prescribed PPI and excluding concomitant users.
‡Users of PPIs alone are patients who had at least one prescription of a PPI during follow-up without having prescribed oral GCs and excluding concomitant users and users of oral GCs alone.
§At index date.
¶Major infections included sepsis, meningitis, and upper and lower respiratory tract infections.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; COX-2, cyclo-oxygenase-2; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; GCs, glucocorticoids; NSAIDs, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors.

specific trend with longer duration or higher daily doses of PPI use) are comparable to the latter study.

Our findings in the single GC use group were generally consistent with the literature. Previous observational studies have reported increased OP fracture risks in patients with RA with current GC use between 43% and 70%, higher than the 23% increased risk that we found.[1 31] We used a different reference group (non-users of both GCs and PPIs), which may also explain the unexpected lack of statistical significance for a higher risk of clinical vertebral fracture with current GC use alone.

The magnitude of the association between concomitant GC and PPI use and the risk of OP fracture may indicate an additive effect of the individual drugs rather than a synergistic effect. This was suggested by a significantly higher fracture risk with concomitant GC and PPI use compared with monotherapy with either drug and as the observed HRs seem to be additive. This may be related to different biological mechanisms of GCs and PPIs acting on osteoporosis or falling. The effect of GCs on bone is mostly via decreased bone formation and interference with active bone remodelling sites.[1 6 8 9] But additionally, GCs might increase the fracture risk by inducing muscle atrophy or cataract especially with higher doses and in long-term use.[37–39] Previous studies have shown that the onset and offset of the effects of GCs on fracture risk are rather rapid, which is supported by our results.[7 31 40] Similar to GCs, the positive association of fracture risk with PPI use quickly subsided when the patient discontinued

Table 2 OP fracture risk by concomitant use of oral GCs and PPIs in patients with rheumatoid arthritis

By recency of use	Number of OP fractures (N=1411)*	IR per 1000 Pys	Age/sex-adjusted HR (95% CI)	Fully adjusted HR† (95% CI)
Non-use of GCs and PPIs	325	10.5	Reference	Reference
Current use‡				
GCs and PPIs concomitantly	264	24.4	**1.93 (1.65 to 2.27)**	**1.60 (1.35 to 1.89)**
GCs alone	178	15.5	**1.34 (1.12 to 1.59)**	**1.23 (1.03 to 1.47)**§
PPIs alone	324	16.7	**1.32 (1.14 to 1.54)**	**1.22 (1.05 to 1.42)**§
Recent GC use‡ ¶	34	11.0	0.87 (0.62 to 1.23)	0.82 (0.58 to 1.16)
Recent PPI use‡¶	49	16.0	1.21 (0.90 to 1.62)	1.17 (0.87 to 1.57)
Past GC use‡ ¶	339	15.6	**1.16 (1.01 to 1.33)**	1.13 (0.98 to 1.29)
Past PPI use‡ ¶	219	13.5	0.96 (0.82 to 1.13)	0.94 (0.80 to 1.10)

Statistically significantly increased HRs are shown in bold.
*1411 OP fracture events among all included patients with RA. The number of events in exposure groups do not sum to this total due to the overlap between recent and past use of GCs and PPIs.
†Adjusted at baseline for sex, body mass index, smoking status and alcohol use; during follow-up for age, a history of ankylosing spondylitis, chronic obstructive pulmonary disease, dementia, falls (in the past 7–12 months) and inflammatory bowel disease; and use in the past 6 months of antidepressants, paracetamol, non-selective non-steroidal anti-inflammatory drugs, cyclo-oxygenase-2 selective inhibitors, tramadol, opioids and conventional synthetic disease-modifying antirheumatic drugs.
‡Current, recent and past use refer to the last prescription within 6 months, 7–12 months and >12 months before a period, respectively.
§Statistically different from concomitant GC and PPI use, Wald test p<0.05.
¶Regardless of the use of the other drug.
GCs, glucocorticoids; IR, incidence rate; OP, osteoporotic; PPIs, proton pump inhibitors; Pys, person years.

Table 3 OP fracture risk by concomitant use of oral GCs and PPIs in patients with rheumatoid arthritis, stratified by fracture type

	Hip (N=541)		Clinical vertebral (N=224)		Humerus (N=372)		Forearm (N=302)		Pelvis (N=116)		Rib (N=90)	
By recency of use	IR per 1000 Pys	Fully adjusted HR* (95% CI)	IR per 1000 Pys	Fully adjusted HR† (95% CI)	IR per 1000 Pys	Fully adjusted HR‡ (95% CI)	IR per 1000 Pys	Fully adjusted HR§ (95% CI)	IR per 1000 Pys	Fully adjusted HR¶ (95% CI)	IR per 1000 ys	Fully adjusted HR** (95% CI)
Non-use of GCs and PPIs	3.8	Reference	1.0	Reference	2.9	Reference	2.5	Reference	0.6	Reference	0.6	Reference
Current use††												
GCs and PPIs concomitantly	9.0	**1.45 (1.11 to 1.91)**	5.4	**2.84 (1.87 to 4.32)**	5.8	1.29 (0.93 to 1.78)	2.9	0.87 (0.57 to 1.32)	2.9	**2.47 (1.41 to 4.34)**	1.7	**4.03 (2.13 to 7.63)**
GCs alone	6.0	1.26 (0.95 to 1.66)	1.8	1.31 (0.79 to 2.16)‡‡	3.3	0.99 (0.69 to 1.43)	3.2	1.17 (0.81 to 1.70)	0.9	1.07 (0.54 to 2.14)‡‡	1.2	**2.28 (1.17 to 4.46)**
PPIs alone	5.9	1.10 (0.86 to 1.41)	3.1	**1.78 (1.20 to 2.65)**‡‡	4.5	1.17 (0.88 to 1.55)	3.3	0.98 (0.71 to 1.37)	1.6	**1.93 (1.11 to 3.34)**	0.8	1.24 (0.66 to 2.34)‡‡
Recent GC use†† §§	5.9	1.25 (0.78 to 2.01)	1.2	0.59 (0.22 to 1.63)	1.5	0.42 (0.17 to 1.03)	3.1	1.00 (0.52 to 1.91)	1.2	0.97 (0.35 to 2.72)	0.6	1.21 (0.29 to 5.14)
Recent PPI use†† §§	5.3	1.00 (0.60 to 1.65)	2.8	1.73 (0.85 to 3.54)	3.4	0.93 (0.50 to 1.72)	3.7	1.09 (0.60 to 2.00)	0.9	1.16 (0.35 to 3.87)	1.5	2.17 (0.83 to 5.62)
Past GC use†† §§	5.5	1.08 (0.86 to 1.35)	2.4	1.12 (0.78 to 1.59)	3.7	0.98 (0.75 to 1.27)	3.6	1.19 (0.90 to 1.58)	0.8	0.64 (0.37 to 1.11)	1.2	**2.43 (1.39 to 4.22)**
Past PPI use†† §§	5.4	0.98 (0.76 to 1.27)	1.9	1.11 (0.71 to 1.74)	2.7	0.72 (0.51 to 1.01)	3.0	0.86 (0.61 to 1.21)	0.9	1.17 (0.62 to 2.20)	0.6	0.86 (0.43 to 1.75)

Statistically significantly increased HRs are shown in bold.
*Adjusted at baseline for sex, BMI, smoking status and alcohol use; during follow-up for age, a history of anaemia, AS, COPD, dementia, falls (in the past 7–12 months) and IBD; and use in the past 6 months of antidepressants, hypnotics/anxiolytics, paracetamol, non-selective NSAIDs, COX-2 selective inhibitors, tramadol, opioids and csDMARDs.
†Adjusted at baseline for sex, BMI, smoking status and alcohol use; during follow-up for age, a history of COPD, falls (in the past 7–12 months) and IBD; and use in the past 6 months of antidepressants, anticonvulsants, paracetamol, non-selective NSAIDs, COX-2 selective inhibitors, tramadol, opioids and csDMARDs.
‡Adjusted at baseline for sex, BMI, smoking status and alcohol use; during follow-up for age, a history of anaemia, AS, COPD, dementia, falls (in the past 7–12 months) and IBD; and use in the past 6 months of antidepressants, paracetamol, non-selective NSAIDs, COX-2 selective inhibitors, tramadol, opioids and csDMARDs.
§Adjusted at baseline for sex, BMI, smoking status and alcohol use; during follow-up for age, a history of anaemia, type 2 diabetes mellitus, COPD, dementia, falls (in the past 7–12 months), gastro-oesophageal reflux disease and IBD; and use in the past 6 months of antidepressants, anticoagulants, anticonvulsants, antihypertensives, paracetamol, non-selective NSAIDs, COX-2 selective inhibitors, tramadol, opioids and csDMARDs.
¶Adjusted at baseline for sex, during follow-up for age and use in the past 6 months of antidepressants, paracetamol and opioids.
**Adjusted at baseline for sex and during follow-up for age.
††Current, recent and past use refer to the last prescription within 6 months, 7–12 months and >12 months before a period, respectively.
‡‡Statistically different from concomitant GC and PPI use within the same fracture type, Wald test p<0.05.
§§Regardless of the use of the other drug.
AS, ankylosing spondylitis; BMI, body mass index; COPD, chronic obstructive pulmonary disease; COX-2, cyclo-oxygenase-2; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; GCs, glucocorticoids; ; IBD, inflammatory bowel disease; IR, incidence rate; NSAIDs, non-steroidal anti-inflammatory drugs; OP, Osteoporotic; PPIs, proton pump inhibitors; Pys, person years.

Table 4 OP fracture risk by average daily dose of oral GC use in patients with rheumatoid arthritis, stratified by average daily dose and continuous duration of PPI use

By recency of use	OP fractures (N=1411)*	IR per 1000 Pys	Age/sex-adjusted HR (95% CI)	Fully adjusted HR† (95% CI)
Non-use of GCs and PPIs	325	10.5	Reference	Reference
Current use of GCs and PPIs concomitantly‡	264	24.4	**1.93 (1.65 to 2.27)**	**1.60 (1.35 to 1.89)**
❶*Low GC use (DD ≤7.5 mg PED/day)*				
+ Low-dose PPI use (DD <20 mg OEDs/day)	142	23.2	**1.75 (1.44 to 2.13)**	**1.42 (1.16 to 1.74)**
+ Medium-dose PPI use (DD 20–35 mg OEDs/day)	39	24.9	**1.93 (1.39 to 2.69)**	**1.54 (1.10 to 2.16)**
+ High-dose PPI use (DD >35 mg OEDs/day)	8	34.2	**2.72 (1.35 to 5.47)**	**2.10 (1.04 to 4.24)**
+ Short-term continuous PPI use (≤1 year)	89	25.7	**2.00 (1.59 to 2.52)**	**1.60 (1.26 to 2.04)**
+ Long-term continuous PPI use (>1 year)	71	20.3	**1.49 (1.15 to 1.93)**	1.18 (0.91 to 1.53)
+ No continuous duration of PPI§	29	30.4	**2.36 (1.62 to 3.45)**	**2.00 (1.36 to 2.93)¶**
❷*Medium GC use (DD 7.6–14.9 mg PED/day)*				
+ Low-dose PPI use (DD <20 mg OEDs/day)	43	25.0	**2.22 (1.62 to 3.04)**	**1.76 (1.27 to 2.43)**
+ Medium-dose PPI use (DD 20–35 mg OEDs/day)	19	27.7	**2.41 (1.52 to 3.82)**	**1.92 (1.20 to 3.05)**
+ High-dose PPI use (DD >35 mg OEDs/day)	<5	17.2	1.46 (0.36 to 5.86)	1.26 (0.31 to 5.07)
+ Short-term continuous PPI use (≤1 year)	36	30.2	**2.70 (1.92 to 3.80)**	**2.20 (1.55 to 3.11)**
+ Long-term continuous PPI use (>1 year)	23	20.9	**1.78 (1.17 to 2.72)**	1.37 (0.89 to 2.10)
+ No continuous duration of PPI§	5	22.3	2.00 (0.83 to 4.84)	1.67 (0.69 to 4.03)
❸*High GC use (DD ≥15.0 mg PED/day)*				
+ Low-dose PPI use (DD <20 mg OEDs/day)	5	21.1	1.92 (0.79 to 4.64)	1.58 (0.65 to 3.81)
+ Medium-dose PPI use (DD 20–35 mg OEDs/day)	<5	38.8	**3.77 (1.41 to 10.09)**	**3.05 (1.13 to 8.18)**
+ High-dose PPI use (DD >35 mg OEDs/day)	<5	41.1	3.83 (0.95 to 15.37)	3.30 (0.82 to 13.26)
+ Short-term continuous PPI use (≤1 year)	9	34.1	**3.21 (1.66 to 6.21)**	**2.72 (1.40 to 5.27)**
+ Long-term continuous PPI use (>1 year)	<5	11.3	0.99 (0.14 to 7.08)	0.72 (0.10 to 5.15)
+ No continuous duration of PPI§	<5	27.1	2.65 (0.37 to 18.90)	2.38 (0.33 to 16.97)

Statistically significantly increased HRs are shown in bold.
*1411 OP fracture events among all included patients with RA. The number of fractures in exposure groups do not sum to this total due to not reporting the current only use and recent and past use of GCs and PPIs.
†Adjusted at baseline for sex, body mass index, smoking status and alcohol use; during follow-up for age, a history of anaemia, ankylosing spondylitis, chronic obstructive pulmonary disease, dementia, falls (in the past 7–12 months) and inflammatory bowel disease; use in the past 6 months of antidepressants, paracetamol, non-selective non-steroidal anti-inflammatory drugs, cyclo-oxygenase-2 selective inhibitors, tramadol, opioids and conventional synthetic disease-modifying antirheumatic drugs; and current only use and recent and past use of oral GCs and PPIs.
‡Concomitant current use refers to the most recent prescription of both oral GCs and PPIs in the 6 months before the start of a period.
§This represents fracture events that happened during a current period of PPI use but not eligible for a continuous duration of use calculation (ie, up to 6 months after the last PPI prescription, but after 1-month threshold gap of our definition for the continuous duration of PPI use).
¶Statistically different from long-term continuous PPI use within the same category, Wald test p<0.05.
DD, average daily dose; GCs, glucocorticoids; IR, incidence rate; OED, omeprazole equivalent dose; OP, osteoporotic; PED, prednisolone equivalent dose; PPIs, proton pump inhibitors; Pys, person years.

the treatment (after 6 months). But for PPIs, underlying pharmacological effects on fracture are not well understood.[41 42] The US Food and Drug Administration published a drug safety communication for a possible increased fracture risk with PPI use in 2011, which remained unchanged to date and was based on evidence from observational studies.[43] This was later criticised for not being supported by a clear biological mechanism.[44]

Various pharmacological mechanisms have been proposed to explain the PPI use and fracture risk association. Reduced intestinal absorption of calcium was previously suggested due to induced hypochlorhydria by PPI therapy and the effect on bone quality.[10] However, a more recent trial found no BMD changes after 52 weeks and non-significant changes in bone turnover markers after 26 weeks with dexlansoprazole or esomeprazole use.[45] An alternative mechanism is an increased falling risk due to muscle weakness and drowsiness, caused by malabsorption of magnesium or vitamin B12.[18–20 46 47] Long-term PPI therapy (≥1 year) in elderly women was shown to significantly reduce serum vitamin B12 levels and double the 5-year risk of injurious falling-related and fracture-related hospitalisation.[46] But the design of this study did not consider proper timing of the exposure and outcome, which limits its interpretation. A third mechanism is effects on osteoclasts to increase bone resorption by PPIs.[48] Finally, methodological explanations for the observed associations include selection bias and/or unknown confounding.[16 17 44] Significant association only with short-term PPI use and no specific trend with increasing daily doses do not fit into any of the proposed mechanisms mentioned above. As we used different strategies in design and analysis to avoid potential sources of bias and to adjust for confounding, and when the GC findings are supported by previous literature with well-known biological mechanisms, the mere explanation of the PPI results by unmeasured confounding would be difficult. Hence, more research is recommended to elaborate on the exact biological mechanism of PPIs on bone.

This study had several strengths. We used data from the CPRD, which is one of the world's largest primary care databases. Our study had a substantial mean duration of follow-up (9.1 years for concomitant users). To bring more insight into the observed association, we stratified GC and PPI use by recency of use, average daily and cumulative dose, and duration of treatment. Furthermore, all analyses were performed time-dependently, incorporating all follow-up times, to avoid time-related biases. There were also several limitations. Biological

therapies, especially during hospitalisation, and some RA severity indices (eg, the disease activity score using 28 joints (DAS-28)) were not adequately captured in the CPRD as a general practice database, which might have introduced confounding by indication or disease severity. Patients with higher disease activity may have an elevated risk of fracture and be more prone to receive oral GCs/PPIs. Also, an improved clinical status might have led to both discontinuation of drug(s) and lower fracture rates. To partly overcome this, we statistically adjusted our analyses for six indicators of RA severity, including analgesics and csDMARDs. We cannot confirm the actual use of medications as we only had prescribing information, and GCs and PPIs are often prescribed on an as-needed basis. The over-the-counter use of PPIs was also not captured. However, with an average duration of use of >3 years, repeated prescriptions are indicators of actual use. Finally, the number of vertebral fractures might be underestimated, as some of them might not immediately come into clinical attention.[49 50] This might virtually increase the HRs for vertebral fractures due to detection bias.[35]

In conclusion, there was an interaction in the risk of OP fracture with concomitant use of oral GCs and PPIs. This increased risk seems to emerge from separate mechanisms of action of GCs and PPIs on bone or falling risk. Considering the increasing life expectancies and high consumption of PPIs among elderly patients, fracture risk assessment could be considered when a patient with RA is co-prescribed oral GCs and PPIs.

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CLINICAL SCIENCE

Switching between Janus kinase inhibitor upadacitinib and adalimumab following insufficient response: efficacy and safety in patients with rheumatoid arthritis

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ABSTRACT

Objectives To evaluate efficacy and safety of immediate switch from upadacitinib to adalimumab, or vice versa, in patients with rheumatoid arthritis with non-response or incomplete-response to the initial therapy.

Methods SELECT-COMPARE randomised patients to upadacitinib 15 mg once daily (n=651), placebo (n=651) or adalimumab 40 mg every other week (n=327). A treat-to-target study design was implemented, with blinded rescue occurring prior to week 26 for patients who did not achieve at least 20% improvement in both tender and swollen joint counts ('non-responders') and at week 26 based on Clinical Disease Activity Index (CDAI) >10 ('incomplete-responders') without washout.

Results A total of 39% (252/651) and 49% (159/327) of patients originally randomised to upadacitinib and adalimumab were rescued to the alternate therapy. In both switch groups (adalimumab to upadacitinib and vice versa) and in non-responders and incomplete-responders, improvements in disease activity were observed at 3 and 6 months following rescue. CDAI low disease activity was achieved by 36% and 47% of non-responders and 45% and 58% of incomplete-responders switched to adalimumab and upadacitinib, respectively, 6 months following switch. Overall, approximately 5% of rescued patients experienced worsening in disease activity at 6 months postswitch. The frequency of adverse events was similar between switch groups.

Conclusions These observations support a treat-to-target strategy, in which patients who fail to respond initially (or do not achieve sufficient response) are switched to a therapy with an alternate mechanism of action and experience improved outcomes. No new safety findings were observed despite immediate switch without washout.

Key messages

What is already known about this subject?

- In patients with rheumatoid arthritis, a treat-to-target strategy is recommended, in which therapy is optimised every 3–6 months until remission, or low disease activity, is achieved. Recent treatment recommendations suggest the addition of a biological or targeted-synthetic disease-modifying antirheumatic drug in patients who do not achieve treatment goals, and switches between mechanisms of action occur commonly in clinical practice.
- The SELECT-COMPARE study followed treat-to-target principles. Patients were blindly switched from upadacitinib, a Janus kinase (JAK) inhibitor, to adalimumab, a tumour necrosis factor (TNF) inhibitor, and vice versa following insufficient response to the initial therapy. Previously reported high-level efficacy data from this study showed that patients switched to either agent experienced improved response following switch.

What does this study add?

- This observation from SELECT-COMPARE provides clinically relevant and detailed switch efficacy data in the subgroups of patients who switched due to initial non-response or incomplete-response. Following a blinded switch in mechanism of action, more patients were able to achieve treatment goals of remission and low disease activity, in both the non-responder and incomplete-responder groups. This study also reports minimal risk of flare following a switch in treatment.
- Additionally, not previously reported, unique and important details on the safety of an immediate switch from a JAK inhibitor to a TNF inhibitor are also provided. This study revealed no new safety signals despite an immediate switch in mechanism of action without washout.

INTRODUCTION

It is recommended that the rheumatoid arthritis (RA) treatment paradigm use a treat-to-target strategy in which therapy is optimised every 3–6 months until clinical remission, or at minimum, low disease activity (LDA) is achieved.[1–5] For patients who do not achieve these goals with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), both American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) guidelines suggest the addition of a biological DMARD (bDMARD) or a targeted synthetic DMARD (tsDMARD).[2 3] If patients continue to exhibit unacceptable disease

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Key messages

How might this impact on clinical practice or future developments?

- Findings here indicate that an immediate switch in mechanism of action (from a JAK inhibitor to a TNF inhibitor and vice versa) following treat-to-target principles is feasible with minimal risk of flare regardless of whether patients are switched due to non-response or incomplete-response without an increase in clinically meaningful adverse events.

activity, a switch to a different bDMARD, or to a tsDMARD, is recommended. Although therapeutic options continue to increase, many patients with RA do not achieve stringent treatment goals. Therefore, data on the effectiveness and safety of switching between different mechanisms of action (MoAs) have become increasingly important. Results from controlled trials suggest that patients with insufficient response to a bDMARD may respond to a Janus kinase inhibitor (JAKi).[6–9] In contrast, there is limited evidence regarding the efficacy and safety of switching patients to a tumour necrosis factor inhibitor (TNFi) following insufficient response to a JAKi.[10]

Upadacitinib, an oral JAKi, has been studied across various patient populations in RA, including methotrexate (MTX)-inadequate responders in SELECT-COMPARE.[11–13] The most recent EULAR recommendations for RA treatment address the shifting therapeutic paradigm.[3] This study employed a unique rescue strategy, permitting blinded rescue from upadacitinib to adalimumab, and vice versa, in the subgroup of patients who did not achieve treatment targets with their initial therapy. Although preliminary data on an immediate switch from either a TNFi to a JAKi or vice versa have been reported,[10 11] the safety of an immediate switch and the efficacy of a switch from a JAKi to a TNFi in patients who either do not have an initial response or experience an insufficient response have not been fully described. The present observational analysis describes the efficacy and safety results of the application of this treat-to-target strategy and expands significantly on the limited results reported previously.[10]

PATIENTS AND METHODS

Patients

Eligibility criteria have been described previously.[11] Briefly, adult patients with RA with ≥6 swollen and ≥6 tender joints, a high-sensitivity C-reactive protein (hsCRP) level ≥5 mg/L, and evidence of erosive disease and/or seropositivity for either rheumatoid factor or anticyclic citrullinated peptide antibodies were enrolled.

Study design

Patients were randomised to double-blinded upadacitinib 15 mg once daily, placebo or adalimumab 40 mg every other week with background MTX (online supplemental figure S1). Blinded rescue (upadacitinib to adalimumab, adalimumab to upadacitinib and placebo to upadacitinib) occurred prior to week 26 (weeks 14, 18 or 22) for patients who did not achieve ≥20% improvement from baseline in both tender and swollen joint count based on 68 joints (TJC68) or 66 joints (SJC66) (defined as 'non-responders' (NR)). An additional blinded switch occurred at week 26 for patients who did not achieve Clinical Disease Activity Index (CDAI) LDA (≤10; defined as 'incomplete-responders' (IR)). Rescue was immediate and without washout according to the following schedule: (1) switching to upadacitinib: last dose of adalimumab was administered 2 weeks prior to starting upadacitinib; (2) switching to adalimumab: adalimumab was injected 1 day after the last dose of upadacitinib. Each patient could only be switched once. Further details on the blinded rescue are provided in the online supplemental text. The observations of efficacy and safety of patients switching between upadacitinib and adalimumab (and vice versa) are presented here.

The study was conducted in accordance with the International Conference on Harmonization guidelines, applicable regulations and the Declaration of Helsinki. All patients provided written informed consent.

Assessments

Efficacy was evaluated up to 6 months (±2 weeks) postswitch using validated outcome measures including ACR response criteria (ACR20/50/70 (improvement of ≥20%, 50% and 70% in ACR criteria)); CDAI LDA (≤10) and remission (≤2.8); 28-joint Disease Activity Score based on C-reactive protein (DAS28(CRP))≤3.2 and<2.6 and change from baseline in Health Assessment Questionnaire-Disability Index (HAQ-DI), patient assessment of pain (0–100 mm visual analogue scale), TJC68, SJC66, Patient's Global Assessment of Disease Activity (PtGA), Physician's Global Disease Activity (PhGA) and hsCRP. Response criteria and change from baseline were evaluated as change from original baseline value at randomisation.

Disease worsening after switch was determined based on DAS28(CRP) increase >0.6 or >1.2 from rescue, evaluated at 3 and 6 months following rescue.[14]

Treatment-emergent adverse events (TEAEs) were evaluated 0–3 months postswitch to assess the safety of an immediate switch and, separately, 4–6 months following switch.[10 11] To better understand the safety preswitch and postswitch, TEAEs were evaluated for the same patients both before and after switch. In this analysis, a matching follow-up period was used to ensure a consistent evaluation across patients who were rescued at different time points. Finally, TEAEs were also evaluated in patients who switched and those who remained on continuous therapy using matching time periods.

Statistical analysis

For these observations, descriptive statistics are summarised for the NR and IR treatment groups following switch. As rescue groups were not randomised for this subset of patients, no direct statistical comparison was made between groups. The study was not designed to compare efficacy or safety between the switch treatment arms. Data are reported as observed with no imputation for missing data. Adverse event data are reported as n (%) with 95% CIs.

Sensitivity analyses were conducted to exclude the few patients in the IR group who were rescued at week 26 despite the achievement of CDAI LDA. Spearman correlation and a univariate logistic regression analysis were used to assess the association between baseline disease characteristics and 'double non-response', defined as patients who required rescue (at any time point) and still failed to achieve CDAI LDA at both 3 and 6 months postswitch.

RESULTS

Of the 651 patients randomised to upadacitinib and 327 patients randomised to adalimumab, 38.7% (252/651) and 48.6% (159/327), respectively, were rescued to the alternate therapy prior to week 26 due to NR or at week 26 due to IR (figure 1). Across both treatment groups, roughly equal proportions of patients were rescued due to NR and IR.

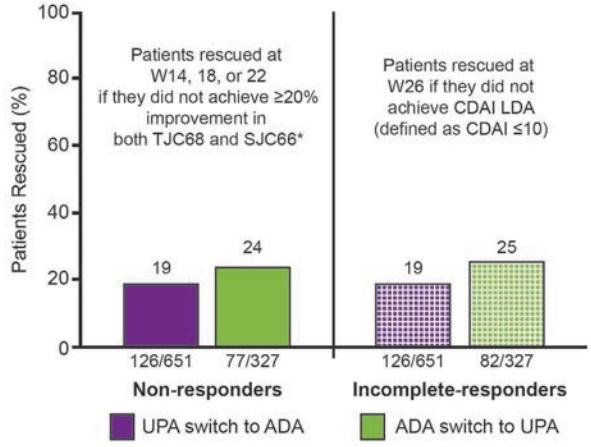

Figure 1 Proportion of patients rescued. *12% (78/651), 5% (29/651) and 3% (19/651) of patients were rescued from UPA to ADA at W14, W18 and W22, respectively. 17% (56/327), 4% (14/327) and 2% (7/327) were rescued from ADA to UPA at W14, W18 and W22, respectively. ADA, adalimumab; CDAI, Clinical Disease Activity Index; LDA, low disease activity; SJC66, swollen joint count-66 joints; TJC68, tender joint count-68 joints; UPA, upadacitinib; W, week.

Baseline demographics were generally similar between patients who were switched and the overall study population.[11] There was improvement in disease activity assessments from baseline to the time of switch, and the improvements were greater in the IR patients compared with patients in the NR group (online supplemental table S1).

Non-responders

A switch in MoA had a beneficial effect on clinical responses in both groups. Six months after rescue, 59.3% (67/113)/25.9% (29/112)/12.3% (14/114) of patients achieved ACR20/50/70 responses following rescue to adalimumab and 74.6% (53/71)/49.3% (34/69)/23.6% (17/72) following rescue to upadacitinib (figure 2). CDAI LDA and remission were achieved by 36.0% (41/114) and 5.3% (6/114) of patients after rescue to adalimumab and 47.1% (33/70) and 14.3% (10/70) of patients after rescue to upadacitinib (figure 3). Six months after rescue to adalimumab, DAS28(CRP) ≤3.2 and DAS28(CRP) <2.6 were achieved by 34.9% (38/109) and 19.3% (21/109) of patients; 54.3% (38/70) and 31.4% (22/70) of patients achieved DAS28(CRP) ≤3.2 and DAS28(CRP) <2.6 at 6 months after rescue to upadacitinib (figure 3). There were also improvements from baseline in function (HAQ-DI), joint counts (TJC68/SJC66), patient and physician global assessments (PtGA, PhGA, pain) and hsCRP following rescue to the alternate agent (online supplemental figure S2).

Some NR experienced increases in disease activity following rescue: at 6 months postrescue, 12.4% (13/105) and 7.4% (5/68) of those switched to adalimumab and upadacitinib, respectively, had an increase in DAS28(CRP) >0.6. Eight (4.6%; 8/173) of all NR (6 rescued to adalimumab and 2 rescued to upadacitinib) experienced a worsening in DAS28(CRP) >1.2 (figure 4).

Incomplete-responders

Six months after switch to adalimumab, 77.3% (92/119)/46.7% (56/120)/18.5% (22/119) of IR achieved ACR20/50/70 responses; of those switched to upadacitinib, 86.7% (65/75)/62.5% (45/72)/39.2% (29/74) achieved ACR20/50/70 at 6 months following switch (figure 2). As expected, given that incomplete-responders by definition had at least 20% improvement in TJC and SJC at the rescue visits prior to week 26, most had achieved an ACR20 response at switch. At 6 months following switch, CDAI LDA and remission were achieved by 45.0% (54/120) and 5.0% (6/120) of patients switched to adalimumab and 57.9% (44/76) and 15.8% (12/76) of patients switched to upadacitinib (figure 3). Among patients switched to adalimumab, 43.8% (53/121) and 23.1% (28/121) achieved DAS28(CRP) ≤3.2 and DAS28(CRP) <2.6 at 6 months

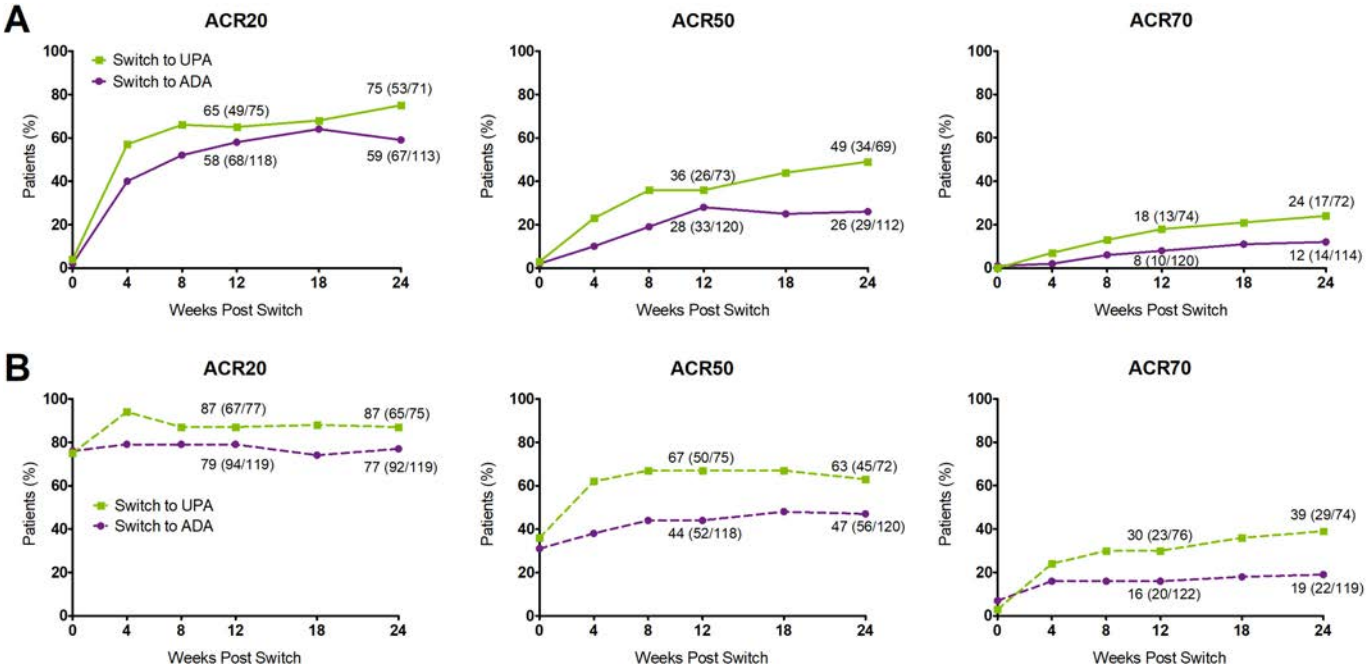

Figure 2 Percentage of non-responders (A) and incomplete-responders (B) achieving ACR20/50/70 at 3 and 6 months postswitch. All data points are provided in online supplemental table S6. ACR20/50/70, improvement of at least 20%, 50% and 70% in American College of Rheumatology criteria from baseline; ADA, adalimumab; mo, month; UPA, upadacitinib.

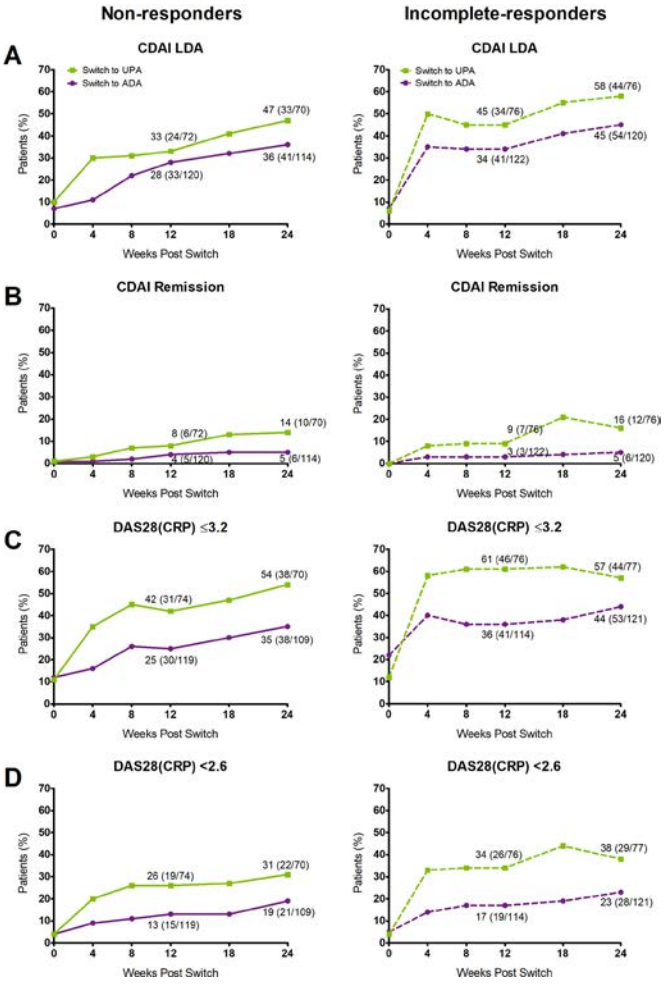

Figure 3 Percentage of non-responders and incomplete-responders achieving CDAI LDA (A) and remission (B), and DAS28(CRP)≤3.2 (C) and <2.6 (D) at 3 and 6 months postswitch. All data points are provided in online supplemental table S7. ADA, adalimumab; CDAI, Clinical Disease Activity Index; DAS28(CRP), 28-joint Disease Activity Score based on C-reactive protein; LDA, low disease activity; UPA, upadacitinib.

postswitch; 57.1% (44/77) and 37.7% (29/77) of patients switched to upadacitinib achieved DAS28(CRP) ≤3.2 and<2.6 (figure 3). In addition, when switched to the alternate therapy, an improvement from baseline was observed in HAQ-DI, TJC68/SJC66, PtGA, PhGA, pain and hsCRP (online supplemental figure S2).

Following switch, some IR experienced a worsening in disease. At 6 months postrescue, 19.8% (24/121) and 3.9% (3/77) of patients switched to adalimumab and upadacitinib, respectively, experienced an increase in DAS28(CRP) >0.6. (figure 4). At the same time point, 9 (4.5%; 9/198) of all IR (8 switched to adalimumab and 1 switched to upadacitinib) experienced a clinically relevant worsening of DAS28(CRP) >1.2.[14]

In the IR group, 7.1% (9/126) of patients switched to adalimumab and 6.1% (5/82) of patients switched to upadacitinib were switched despite achievement of CDAI LDA. Results were unchanged when these patients were excluded from the analyses (online supplemental table S2).

Double non-response

Correlation and logistic regression analyses were conducted to evaluate potential factors associated with double non-response. In total, 210 patients (21.2% (138/651) and 22.0% (72/327) of patients initially randomised to upadacitinib or adalimumab, respectively) were double non-responders. Both analyses showed a weak association between higher disease activity and functional impairment at baseline and double non-response (online supplemental tables S3 and S4). No other discriminators were observed.

Safety

Following immediate switch in treatment without washout, the proportion of patients experiencing any TEAE was similar regardless of whether patients switched to adalimumab or upadacitinib (table 1). The frequency of infections, including serious infections, and herpes zoster was also similar between switch groups. No active tuberculosis, non-melanoma skin cancer, adjudicated major adverse cardiovascular event, or deaths were reported. Additionally, no differences in the proportion of TEAEs were observed when the same patient groups were evaluated prior to and following rescue (table 2). Similarly, no meaningful differences in TEAEs were observed in patients who switched therapy compared with those who remained on continuous therapy (online supplemental table S5).

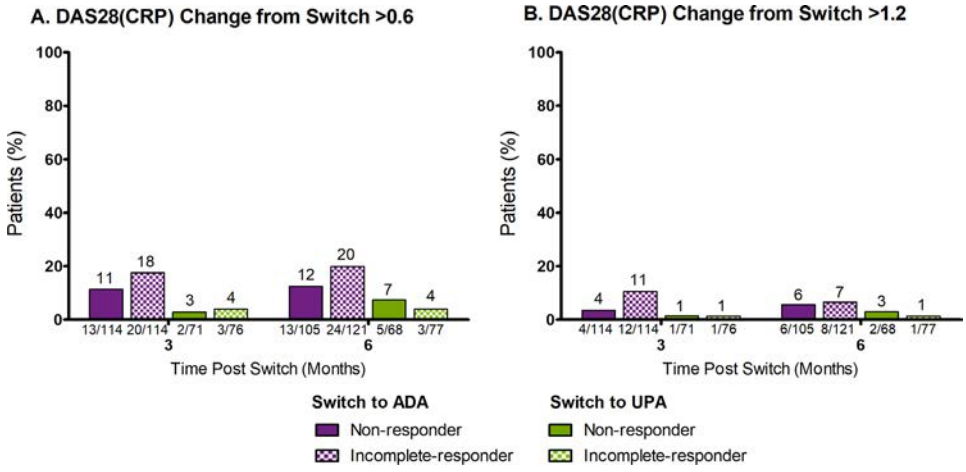

Figure 4 Percentage of non-responders and incomplete-responders with DAS28(CRP) change from switch >0.6 (A) and >1.2 (B). ADA, adalimumab; DAS28(CRP), 28-joint Disease Activity Score based on C-reactive protein; UPA, upadacitinib.

Table 1 Number and percentage of patients experiencing TEAEs 0–3 months and 4–6 months post-treatment switch

	0–3 months postswitch		4–6 months postswitch	
Adverse events no. (%) (95% CI)	UPA 15 mg to ADA (n=252)	ADA to UPA 15 mg (n=159)	UPA 15 mg to ADA (n = 252)	ADA to UPA 15 mg (n=159)
Any AE	125 (49.6) (43.5 to 55.7)	64 (40.3) (33.0 to 48.0)	90 (35.7) (30.1 to 41.8)	58 (36.5) (29.4 to 44.2)
Serious AE	6 (2.4) (1.1 to 5.1)	6 (3.8) (1.7 to 8.0)	11 (4.4) (2.5 to 7.7)	9 (5.7) (3.0 to 10.4)
AE leading to D/C	7 (2.8) (1.4 to 5.6)	3 (1.9) (0.6 to 5.4)	8 (3.2) (1.6 to 6.1)	5 (3.1) (1.4 to 7.2)
Deaths	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 1.5)	0 (0.0 to 2.4)
Infection	41 (16.3) (12.3 to 21.3)	30 (18.9) (13.6 to 25.7)	46 (18.3) (14.0 to 23.5)	29 (18.2) (13.0 to 25.0)
Serious infection*	2 (0.8) (0.2 to 2.9)	4 (2.5) (1.0 to 6.3)	3 (1.2) (0.4 to 3.4)	2 (1.3) (0.4 to 4.5)
Opportunistic infection	0 (0.0 to 1.5)	1 (0.6) (0.1 to 3.5)	0 (0.0 to 1.5)	1 (0.6) (0.1 to 3.5)
Herpes zoster	1 (0.4) (0.1 to 2.2)	2 (1.3) (0.4 to 4.5)†	2 (0.8) (0.2 to 2.9)	2 (1.3) (0.4 to 4.5)
TB‡	0 (0.0 to 1.5)	0 (0.0 to 2.4)	7 (2.8) (1.4 to 5.6)	3 (1.9) (0.6 to 5.4)
Malignancy (excluding NMSC)	0 (0.0 to 1.5)	1 (0.6) (0.1 to 3.5)	1 (0.4) (0.1 to 2.2)	0 (0.0 to 2.4)
NMSC	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 1.5)	0 (0.0 to 2.4)
GI perforation§	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 1.5)	1 (0.6) (0.1 to 3.5)
Adjudicated MACE	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 1.5)	0 (0.0 to 2.4)
Adjudicated VTE¶	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 1.5)	1 (0.6) (0.1 to 3.5)
Hepatic disorder**	8 (3.2) (1.6 to 6.1)	4 (2.5) (1.0 to 6.3)	8 (3.2) (1.2 to 6.1)	2 (1.3) (0.4 to 4.5)
Anaemia	3 (1.2) (0.4 to 3.4)	2 (1.3) (0.4 to 4.5)	1 (0.4) (0.1 to 2.2)	2 (1.3) (0.4 to 4.5)
Neutropaenia	4 (1.6) (0.6 to 4.0)	2 (1.3) (0.4 to 4.5)	2 (0.8) (0.2 to 2.9)	1 (0.6) (0.1 to 3.5)
Lymphopaenia	1 (0.4) (0.1 to 2.2)	1 (0.6) (0.1 to 3.5)	1 (0.4) (0.1 to 2.2)	0 (0.0 to 2.4)
CPK elevation	2 (0.8) (0.2 to 2.9)	0 (0.0 to 2.4)	1 (0.4) (0.1 to 2.2)	1 (0.6) (0.1 to 3.5)

0–3 months postswitch is from first dose of study drug to day 91; 4–6 months postswitch is from day 92 to 183.
Values are the number (%) of patients with events. CIs are calculated using the Wilson method.
*0–3 months postswitch UPA to ADA: pneumonia, tonsillitis, ADA to UPA: upper respiratory tract infection, herpes zoster, cellulitis and one patient with oral herpes, sepsis and pneumonia. 4–6 months postswitch UPA to ADA: diverticulitis, uveitis, pyelonephritis, ADA to UPA: latent TB, pneumonia.
†46-year-old patient without a history of herpes zoster vaccination and on background glucocorticoid and methotrexate therapy developed a serious herpes zoster infection in the face affecting one dermatome. Upadacitinib could be restarted after successful antiviral treatment with acyclovir.
‡All cases were latent TB.
§GI perforations were identified through Standardised Medical Dictionary for Regulatory Activities query. The one event in a patient rescued to upadacitinib was not a spontaneous GI perforation but an event of anal fistula.
¶One patient experienced a venous thromboembolism (pulmonary embolism) 4–6 months after switch to upadacitinib; this patient had risk factors (smoker, previous deep vein thrombosis) and upadacitinib was permanently discontinued.
**Majority of hepatic disorders were asymptomatic alanine aminotransferase/aspartate aminotransferase elevations.
ADA, adalimumab; AE, adverse event; CPK, creatine phosphokinase; D/C, discontinuation; GI, gastrointestinal; MACE, major adverse cardiovascular event; NMSC, non-melanoma skin cancer; TB, tuberculosis; TEAE, treatment-emergent adverse event; UPA, upadacitinib; VTE, venous thromboembolism.

Table 2 Number and percentage of patients experiencing TEAEs in patients prior to and following treatment switch

	UPA 15 mg to ADA (n = 252)		ADA to UPA 15 mg (n = 159)	
Adverse events, no. (%) (95% CI)	Prior to switch (UPA 15 mg)	After switch (ADA)	Prior to switch (ADA)	After switch (UPA 15 mg)
Any AE	163 (64.7) (58.6 to 70.3)	152 (60.3) (54.2 to 66.2)	91 (57.2) (49.5 to 64.7)	85 (53.5) (45.7 to 61.0)
Serious AE	10 (4.0) (2.2 to 7.2)	12 (4.8) (2.7 to 8.1)	3 (1.9) (0.6 to 5.4)	10 (6.3) (3.5 to 11.2)
AE leading to D/C	NA	12 (4.8) (2.7 to 8.1)	NA	8 (5.0) (2.6 to 9.6)
Deaths	0 (0.0 to 1.5)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 2.4)
Infection	79 (31.3) (26.0 to 37.3)	65 (25.8) (20.8 to 31.5)	36 (22.6) (16.8 to 29.8)	38 (23.9) (17.9 to 31.1)
Serious infection	5 (2.0) (0.9 to 4.6)	4 (1.6) (0.6 to 4.0)	1 (0.6) (0.1 to 3.5)	4 (2.5) (1.0 to 6.3)
Opportunistic infection	3 (1.2) (0.4 to 3.4)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	1 (0.6) (0.1 to 3.5)
Herpes zoster	2 (0.8) (0.2 to 2.9)	2 (0.8) (0.2 to 2.9)	0 (0.0 to 2.4)	3 (1.9) (0.6 to 5.4)
TB	0 (0.0 to 1.5)	7 (2.8) (1.4 to 5.6)	0 (0.0 to 2.4)	1 (0.6) (0.1 to 3.5)
Malignancy (excluding NMSC)	0 (0.0 to 1.5)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	1 (0.6) (0.1 to 3.5)
NMSC	0 (0.0 to 1.5)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 2.4)
GI perforation	0 (0.0 to 1.5)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 2.4)
Adjudicated MACE	0 (0.0 to 1.5)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 2.4)
Adjudicated VTE	2 (0.8) (0.2 to 2.9)	0 (0.0 to 1.5)	0 (0.0 to 2.4)	0 (0.0 to 2.4)
Hepatic disorder	17 (6.7) (4.3 to 10.6)	13 (5.2) (3.0 to 8.6)	6 (3.8) (1.7 to 8.0)	5 (3.1) (1.4 to 7.2)
Anaemia	2 (0.8) (0.2 to 2.9)	5 (2.0) (0.9 to 4.6)	4 (2.5) (1.0 to 6.3)	3 (1.9) (0.6 to 5.4)
Neutropaenia	6 (2.4) (1.1 to 5.1)	6 (2.4) (1.1 to 5.1)	2 (1.3) (0.4 to 4.5)	2 (1.3) (0.4 to 4.5)
Lymphopaenia	2 (0.8) (0.2 to 2.9)	2 (0.8) (0.2 to 2.9)	2 (1.3) (0.4 to 4.5)	1 (0.6) (0.1 to 3.5)
CPK elevation	12 (4.8) (2.7 to 8.1)	3 (1.2) (0.4 to 3.4)	0 (0.0 to 2.4)	4 (2.5) (1.0 to 6.3)

Before switch time period is defined as day one to date of switch; post switch time period is defined as the day after switch to 99, 127, 155, and 183 days after switch for the patients who switched at weeks 14, 18, 22, and 26, respectively.
Values are the number (%) of patients with events. CIs are calculated using the Wilson method.
ADA, adalimumab; AE, adverse event; CPK, creatine phosphokinase; D/C, discontinuation; GI, gastrointestinal; MACE, major adverse cardiovascular event; NA, not applicable; NMSC, non-melanoma skin cancer; TB, tuberculosis; TEAE, treatment-emergent adverse event; UPA, upadacitinib; VTE, venous thromboembolism.

DISCUSSION

Recent advances in drug development have led to approval of multiple therapeutic options, including oral therapies, for RA. Recommendations from professional societies have noted the importance of the availability of a variety of MoAs and discuss switching MoAs in the event of insufficient response.[2 3] While there are multiple reports of switching to an alternative agent following inadequate response to a TNFi, there are no other data on an immediate switch in MoA following insufficient response to a JAKi. This poses a challenge for clinicians making evidence-based treatment decisions. The unique trial design of SELECT-COMPARE, the first fully blinded study to report switch data between a JAKi and a TNFi, permitted assessment of these questions in a setting with defined criteria and provides the first data showing clinical outcomes in patients who failed to respond to a JAKi and subsequently switched to a TNFi. In contrast to another JAKi trial where patients were not rescued from the JAKi to adalimumab, SELECT-COMPARE provides data with a rescue in both directions based on objective and predefined criteria.[15] Ultimately, the observations from SELECT-COMPARE provide valuable outcomes for providers using treat-to-target principles for their patients who continue to manifest active disease despite treatment with a TNFi and highlights the importance of diverse MoAs.

In the present analysis, many patients with initial NR or IR to either upadacitinib or adalimumab experienced meaningful improvement in clinical and functional outcomes following rescue to the alternate therapy, suggesting that a switch to either MoA may be beneficial for patients with RA not previously meeting treatment goals. Clinically relevant improvements from baseline across different disease measures were consistently seen in both groups, although numerically better improvement was generally observed in IR versus NR patients. The data observed for patients switching from adalimumab to upadacitinib were in line with previously reported data from the SELECT-BEYOND trial, which evaluated upadacitinib in bDMARD-inadequate responder patients.[6] Similarly, the outcomes observed for patients switching from upadacitinib to adalimumab were consistent with those reported in the EXXELERATE study where patients were switched to adalimumab following inadequate response to an alternate TNFi.[16] Studies involving bDMARD-inadequate responder patients, such as SELECT-BEYOND, used prolonged intervals between the stop of biological therapy and initiation of JAKi for perceived safety concerns; the observations in SELECT-COMPARE provide direct switch data with no washout period suggesting that an immediate switch did not lead to increased safety concerns. The safety of immediate switch between adalimumab and upadacitinib seen here is in line with prior safety experience of an immediate switch between two TNFis seen in EXXELERATE.[16]

As with all therapies, there was a proportion of patients who either had little if any initial response or who improved but failed to achieve disease targets. A potential concern with switching therapies in the latter group is whether their disease will worsen on a change in therapy. In the present analysis, relatively few patients experienced a clinically significant worsening in disease following switch. Six months following rescue for either incomplete-response or non-response, only approximately 5% of patients rescued to either therapy (adalimumab to upadacitinib or vice versa) experienced a worsening in disease as defined by an increase in DAS28(CRP) >1.2. In SELECT-COMPARE, this flare risk is largely outweighed by the observed efficacy outcomes. Approximately one-half of patients who had a clinically relevant response with a significant decrease in CDAI, but did not achieve CDAI LDA, were able to achieve this stringent endpoint with a switch in MoA. These outcomes support a treat-to-target strategy, and address a common question asked in clinical practice regarding the likelihood of a patient achieving treatment goals with a switch in therapy versus the chances of them experiencing disease worsening. The current analysis would suggest that a switch is much more likely to be successful than the risk of a flare.

There were patients who did not respond to either therapy. Based on exploratory analyses examining baseline demographics, predictors for double non-response could not be identified; additional research is needed to elucidate predictors of patients who will fail to respond to either therapy.

Although it may appear that the proportion of patients rescued in this study (in total, 252 patients (39%) rescued to adalimumab and 159 patients (49%) rescued to upadacitinib) is greater than rescue rates observed in other trials, this was largely due to the unique rescue scheme used in SELECT-COMPARE. Treatment switch was permitted at four time points and included rescue based on the stringent metric of CDAI LDA at week 26. Other studies typically only permitted rescue at a single time point.[17–19] In these studies, the rates of rescue are consistent with those described at week 14 for SELECT-COMPARE.

This analysis also provides clinically relevant, blinded data on the safety of an immediate switch in therapy from a biological to a JAKi without washout. Given a mean terminal half-life of approximately 2 weeks for adalimumab,[20 21] pharmacodynamic (PD) effects might persist to a certain degree after discontinuation of adalimumab until complete washout within several weeks. On the other hand, upadacitinib has a shorter half-life of 9–14 hours and immediate PD effects (eg, those based on IL-6 signalling) are expected to disappear within a day.[22] Complete washout of upadacitinib is expected within a few days; however, delayed PD effect may last beyond this. While there is potential for differences in the overlap of inhibition between the two switch arms, overall efficacy appears consistent through 6 months with no fluctuations in response. Clinically, consideration for half-life and PD effects may need to be given for individual patients. Importantly, in terms of safety, although limited by sample size, based on available data from over 400 patients, no additional safety concerns were observed in either treatment group (upadacitinib to adalimumab and vice versa) despite this immediate switch. In particular, and perhaps most pertinent considering overlapping PD effects, no differences in frequency of infections were observed between treatment groups at both 0–3 and 4–6 months postrescue. Overall consistent findings were observed in the adverse event profile of patients evaluated prior to and following switch.

This study is not without limitations. Due to the observational nature of this analysis, both the safety and efficacy evaluations are ultimately limited by the number of patients who met rescue criteria; as such, the present analysis was not designed or powered for statistical comparisons between switch groups (either the two switch arms, or NR versus IR) and the results should be interpreted as observational. Additionally, this analysis from a clinical trial population may not be generalisable to all patients in clinical practice; further real-world studies are needed to confirm these results. While the study used aspects of a treat-to-target strategy, the rescue was based on predefined criteria at specified timepoints, and did not allow providers the opportunity to adjust therapy more freely in accordance with their clinical judgement, as may be more typical of a true treat-to-target strategy. Patients were not rerandomised at rescue

but were switched to the alternate treatment in a double-blind fashion using an interactive response technology system.

In summary, SELECT-COMPARE used a treat-to-target strategy with blinded rescue and provides the first data to suggest patients switching from a JAKi to a TNFi may experience an improved response following rescue. Patients with initial NR or IR to either upadacitinib 15 mg once daily or adalimumab 40 mg every other week, both in combination with MTX, showed benefit in both clinical and functional outcomes when switched to the alternate therapy. Numerous patients who had a significant clinical response but did not reach CDAI LDA were able to reach this target with a switch in MoA. Despite an immediate switch in MoA, without washout, no new safety signals were observed in either treatment group.[23]

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Contributors RMF, YL and I-HS were involved in the design of the study. RMF, RB, SH, GTDT, FEVdB, CZ and LB were involved in the acquisition of data. All authors were involved in the analysis and interpretation of the data, drafting the manuscript and revising it for critically important intellectual content and reviewing and approving the final version of the manuscript.

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Patient consent for publication Not required.

Ethics approval Study-related documents were approved by institutional ethics committees and review boards.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymised, individual and trial-level data (analysis datasets) as well as other information (eg, protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

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TRANSLATIONAL SCIENCE

Parsing multiomics landscape of activated synovial fibroblasts highlights drug targets linked to genetic risk of rheumatoid arthritis

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ABSTRACT

Objectives Synovial fibroblasts (SFs) are one of the major components of the inflamed synovium in rheumatoid arthritis (RA). We aimed to gain insight into the pathogenic mechanisms of SFs through elucidating the genetic contribution to molecular regulatory networks under inflammatory condition.

Methods SFs from RA and osteoarthritis (OA) patients (n=30 each) were stimulated with eight different cytokines (interferon (IFN)-α, IFN-γ, tumour necrosis factor-α, interleukin (IL)-1β, IL-6/sIL-6R, IL-17, transforming growth factor-β1, IL-18) or a combination of all 8 (8-mix). Peripheral blood mononuclear cells were fractioned into five immune cell subsets ($CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer (NK) cells, monocytes). Integrative analyses including mRNA expression, histone modifications (H3K27ac, H3K4me1, H3K4me3), three-dimensional (3D) genome architecture and genetic variations of single nucleotide polymorphisms (SNPs) were performed.

Results Unstimulated RASFs differed markedly from OASFs in the transcriptome and epigenome. Meanwhile, most of the responses to stimulations were shared between the diseases. Activated SFs expressed pathogenic genes, including *CD40* whose induction by IFN-γ was significantly affected by an RA risk SNP (rs6074022). On chromatin remodelling in activated SFs, RA risk loci were enriched in clusters of enhancers (super-enhancers; SEs) induced by synergistic proinflammatory cytokines. An RA risk SNP (rs28411362), located in an SE under synergistically acting cytokines, formed 3D contact with the promoter of *metal-regulatory transcription factor-1 (MTF1)* gene, whose binding motif showed significant enrichment in stimulation specific-SEs. Consistently, inhibition of MTF1 suppressed cytokine and chemokine production from SFs and ameliorated mice model of arthritis.

Conclusions Our findings established the dynamic landscape of activated SFs and yielded potential therapeutic targets associated with genetic risk of RA.

Key messages

What is already known about this subject?

- In rheumatoid arthritis (RA), a variety of dysregulated molecules from immune cells and mesenchymal cells drive disease progression. Synovial fibroblasts (SFs), the most abundant resident mesenchymal cells in the inflamed synovium, produce a variety of pathogenic molecules including interleukin 6.
- Genome-wide association studies have identified more than 100 RA susceptibility loci. To gain insight into the pathogenic mechanisms of SFs, understanding the genetic contribution to molecular regulatory networks under inflammatory condition is crucial.

What does this study add?

- Integrated analyses of activated SFs revealed an overview of relationships between pathogenic gene expressions, epigenomic modulations and RA susceptibility loci.
- Chromatin remodelling induced by synergistic proinflammatory cytokines were associated with RA heritability, and some transcription factors (metal-regulatory transcription factor-1, runt-related transcription factor 1) could be crucial for the structural rearrangement and the formation of inflammatory arthritis.

How might this impact on clinical practice or future developments?

- Our findings established the dynamic landscape of activated SFs and yielded potential therapeutic targets associated with genetic risk of RA.

INTRODUCTION

Rheumatoid arthritis (RA) causes persistent synovitis leading to disabling joint destruction. Current treatment strategies that target cytokines (eg, tumour necrosis factor-α (TNF-α), interleukin (IL)-6), cell surface proteins (eg, CD20, CD80/86) or signalling molecules (eg, Janus kinase) have brought a paradigm shift in RA treatment. However, achieving sustained remission is still challenging even with such agents.[1] Although the concepts of targeting multiple molecules have been proposed, combination or bispecific antibodies (anti-TNF-α and anti-IL-1β or anti-IL-17) failed to improve therapeutic efficacy.[2] These findings imply that some unknown factors play critical roles in the progression of synovitis.

In the pathogenesis of RA, the activities of a variety of dysregulated molecules in immune cells and mesenchymal cells are orchestrated by genetic and environmental factors.[3] To date, more than 100 RA susceptibility loci have been identified in genome-wide association studies (GWAS).[4] Recent genetic studies of autoimmune diseases have reported that the majority (>90%) of these risk variants are located in non-coding regions and regulate gene expression in a cell type-specific manner,[5] partly in an environment-specific fashion.[6] An integrated understanding of the risk variants' contribution to gene regulatory networks is crucial to gain insight into the pathogenic mechanisms of RA.

Synovial fibroblasts (SFs), the most abundant resident mesenchymal cells in the synovium, are major local effectors in the initiation and perpetuation of destructive joint inflammation through their production of a variety of pathogenic molecules including IL-6.[3] Previous multiomics data of unstimulated SFs have proposed activated pathways in RASFs.[7] However, a comprehensive picture of SFs' contribution to RA pathogenesis has largely remained elusive, perhaps due to their complex features that mutate in response to the proinflammatory milieu.[8] To date, a number of single cytokines that induce the inflammatory behaviour of SFs have been reported (eg, interferon (IFN)-γ and IL-17 from T cells and TNF-α, IL-1β, IFN-α, IL-18 and transforming growth factor-β1 (TGF-β1) from monocytes).[9] To make matters more complicated, in *in vivo*, SFs are expected to be exposed to a more complex environment. Data show that some cytokine combinations (eg, TNF-α and IL-17) synergistically enhance the expression of inflammatory molecules.[10] Those findings emphasise the need to simultaneously analyse the mechanisms underlying the accelerated inflammatory behaviour of SFs in the presence of single cytokines and cytokine combinations.

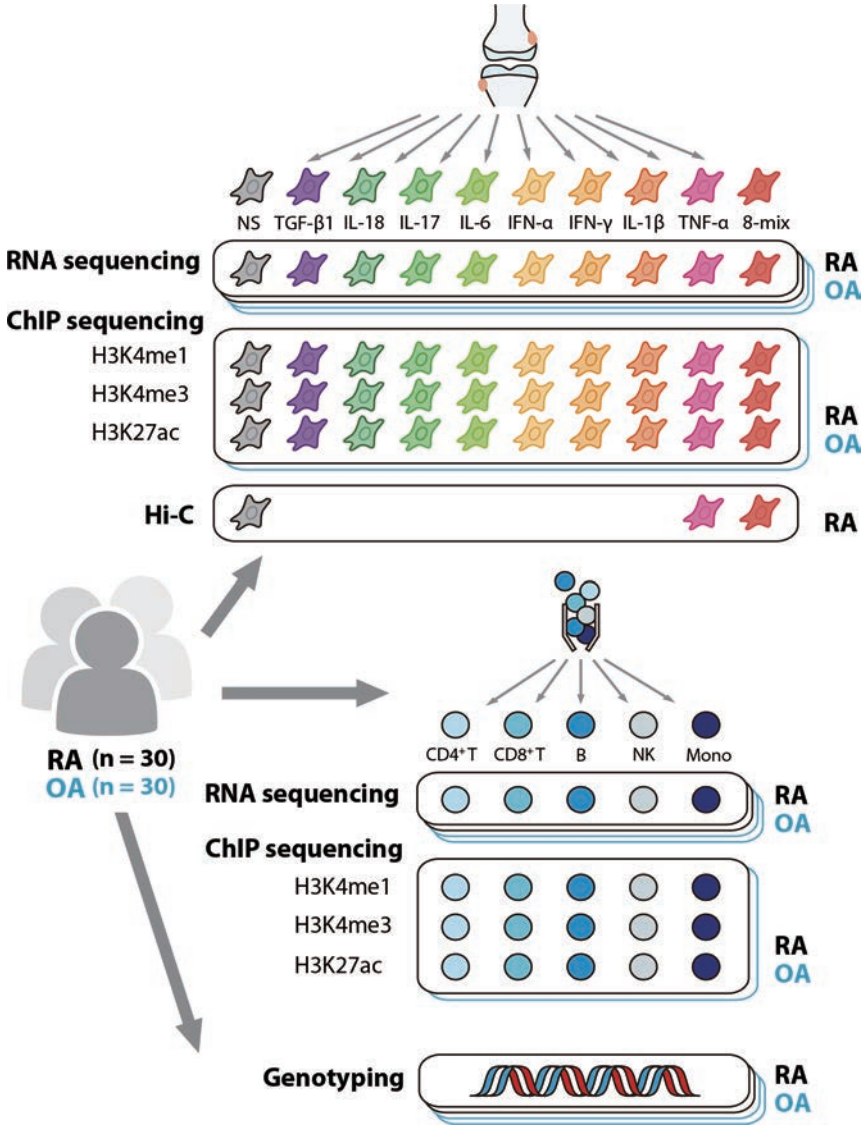

Figure 1 Experimental design for integrative analysis of activated SFs from RA and OA patients. Our study design included SFs stimulated by eight different factors plus a combination of all the factors. Specifically, cells were treated for 24 hours with one of the following: IFN-α 100 U/mL, IFN-γ 200 U/mL, TNF-α 10 ng/mL, IL-1β 10 ng/mL, IL-6/sIL-6R 200 ng/mL, IL-17 10 ng/mL, TGF-β1 10 ng/mL or IL-18 100 ng/mL or 8-mix, a mixture of the above eight cytokines. In addition, we used five freshly isolated PBMC populations ($CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, monocytes) from the same patient cohort. RNA sequencing of individual samples from RA and OA patients (n=30 per each) was carried out, and ChIP sequencing and Hi-C analysis were conducted with pooled samples. SNP genotyping array was performed in all patients. IFN, interferon; IL, interleukin; NK cells, natural killer cells; NS, non-stimulated; OA, osteoarthritis; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SFs, synovial fibroblasts; SNP, single nucleotide polymorphism; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α.

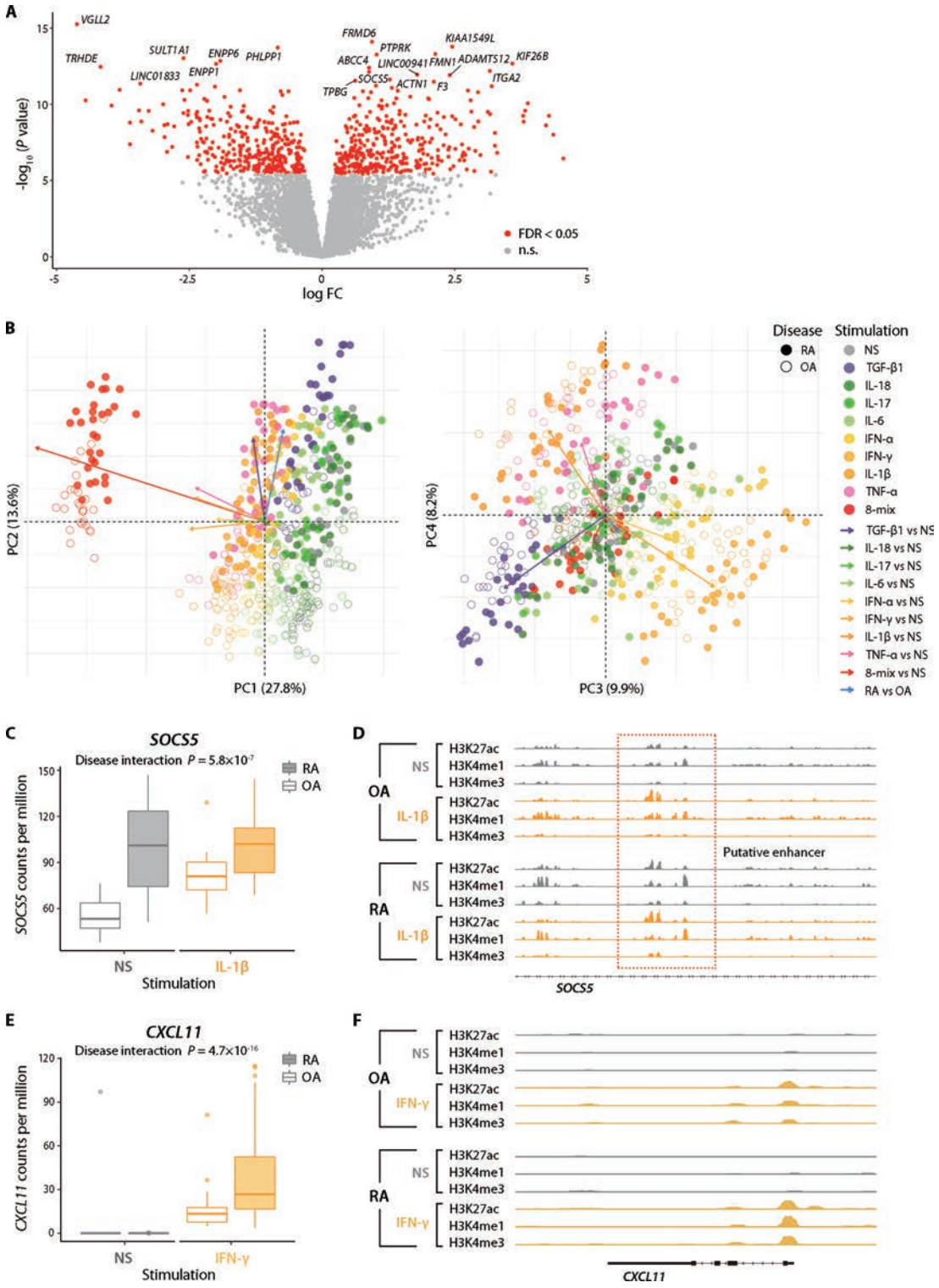

Figure 2 Distinctive transcriptomic and epigenomic signatures in RASFs. (A) A volcano plot comparing RASFs and OASFs under non-stimulated condition. Red points mark the genes with significantly increased or decreased expression in RASFs (FDR<0.01). (B) Principal component analysis (PCA) of gene expression levels for the top 1000 variable genes. Samples projected onto PC1/PC2 (left) or PC3/PC4 (right). Numbers in parentheses indicate contribution ratio (percentage of variation) of the first 4 PCs. Arrows link the centroid of indicated groups and adjusted to start from the origin. (C) Transcript abundances of *SOCS5* from RNA sequencing data in SFs under non-stimulation and stimulation by IL-1β. boxes, IQR; whiskers, distribution; dots, outliers. (D) Organisation of transcriptional regulatory regions around *SOCS5* gene in SFs under non-stimulation and stimulation by IL-1β. Boxed area indicate a putative enhancer. data were visualised using the Integrative Genomics Viewer (IGV). (E) Transcript abundances of *CXCL11* from RNA sequencing data in SFs under non-stimulation and stimulation by IFN-γ. boxes, IQR; whiskers, distribution; dots, outliers. (F) Organisation of transcriptional regulatory regions around *CXCL11* gene in SFs under non-stimulation and stimulation by IFN-γ. Boxed area indicates a putative enhancer. Data were visualised using the IGV. IFN, interferon; IL, interleukin; NS, non-stimulated; n.s., not significant; OA, osteoarthritis; PCA, principal component analysis; RA, rheumatoid arthritis; SFs, synovial fibroblasts; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α.

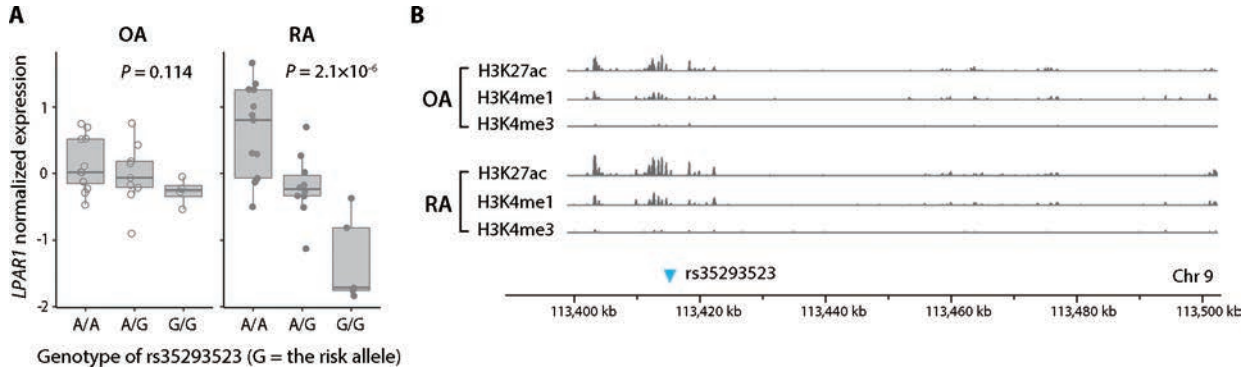

Figure 3 Disease-specific function of RA genetic risk loci. (A) Expression of *LPAR1* in OASFs (left) and RASFs (right) under non-stimulated condition. Individuals are stratified according to the rs35293523 genotype. Nominal p values in eQTL mapping are shown. (B) Organisation of transcriptional regulatory regions around *LPAR1* gene and positional relationship of RA risk loci (blue triangle, rs35293523) in OASFs and RASFs under non-stimulated condition. Data were visualised using the Integrative Genomics Viewer. eQTL, expression quantitative trait locus; OA, osteoarthritis; RA, rheumatoid arthritis; SFs, synovial fibroblasts.

Here, we used integrative methods to analyse genomic, transcriptomic and epigenomic features of RASFs in the presence of various proinflammatory cytokines in RA joints.[11] Analyses of activated SFs revealed an overview of relationships between pathogenic gene expressions, epigenomic modulations and RA susceptibility loci. Chromatin remodelling induced by synergistic proinflammatory cytokines were associated with RA heritability, and some transcription factors (metal-regulatory transcription factor-1 (MTF1), runt-related transcription factor 1 (RUNX1)) could be crucial for the structural rearrangement and the formation of inflammatory arthritis.

MATERIALS AND METHODS

See online supplementary materials and methods.

RESULTS

RASFs display distinctive transcriptomic and epigenomic signatures

We stimulated cultured SFs from RA and osteoarthritis (OA) patients (n=30 each) with eight different cytokines (IFN-α, IFN-γ, TNF-α, IL-1β, IL-6/sIL-6R, IL-17, TGF-β1, IL-18) or a combination of all 8 (8-mix). We also fractionated peripheral blood mononuclear cells (PBMCs) from the same patients into five major immune cell subsets ($CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer (NK) cells, monocytes) (figure 1).

Under non-stimulatory condition, RASFs and OASFs showed profound differences at the level of transcriptome (figure 2A) and epigenome (online supplemental figure 1A). Genes highly expressed in RASFs were enriched in cell adhesion associated pathways (online supplemental figure 1B), which is consistent with previous reports,[12 13] and the differences were largely preserved after stimulation (figure 2B, (online supplemental figure 1C)). Although most of the genes induced by each cytokine stimulation were shared between the diseases, some genes showed different behaviours (online supplemental figure 1D). For instance, *SOCS5*, coding a cytokine signalling regulator, was induced by IL-1β in OASFs, though it was highly expressed even under non-stimulatory condition in RASFs (figure 2C). Epigenomic data supported the activity of an enhancer located in *SOCS5* intron region in non-stimulated RASFs (figure 2D), which might be due to long-time cytokine exposure. Moreover, *CXCL11*, coding a chemokine associated with T cell chemotaxis, was highly induced by IFN-γ in RASFs compared with OASFs (figure 2E), and the corresponding difference in epigenome modification were seen (figure 2F). Genes differentially expressed between RASFs and OASFs, as well as those induced by each cytokine stimulation, are listed in online supplemental table 1). In addition, the relationship between the inflammatory environment and the subpopulation of SFs, which has been attracting attention in recent years, is discussed in online supplementary note 1.[14]

Gene regulatory effects of RA risk loci are modulated by an environmental perturbation

We next performed cis-eQTL (expression quantitative trait locus) analysis to evaluate the effect of genetic variants on gene expressions. Together with tissue-by-tissue analysis, we also used Meta-Tissue software (online supplementary materials) for a meta-analysis across SFs under 10 stimulations and 5 PBMC subsets. To improve analytical ability, we jointly analysed the RA and OA samples, and afterwards we compared the effect sizes between the diseases for significant eQTLs. We considered variants with FDR <0.1 in single tissue analysis or m value >0.9 in meta-analysis as significant eQTLs.[15 16]

As a result, 3245–4118 genes in SFs and 2557–2828 genes in PBMCs had significant eQTLs (online supplemental figure 2A). In total, 2368 genes showed eQTL effects only in SFs (online supplemental figure 2B). Although similar eQTL effect sizes were observed in RASFs and OASFs, when the analyses were limited to differential peak regions between the diseases, some loci showed genome-wide significant difference (online supplemental figure 2C). In total, 12 loci showed significant interaction with the disease term (FDR <0.1, (online supplemental table 2). One example is rs35293523-*LPAR1* in non-stimulated SFs (figure 3A, RA: $p=2.1 \times 10^{-6}$, OA: p=0.114, interaction FDR=0.0078). This locus is located in H3K27ac peak which is significantly greater in RASFs (figure 3B, $p=2.93\times10^{-8}$), indicating its enhancer activity in RA. As this single nucleotide polymorphism (SNP) is in tight LD with bone mineral density associated GWAS variant (rs12338959, $r^2=0.99$ in East Asian (EAS), $r^2=0.79$ in European (EUR) population),[17] epigenomic modulation of this locus could be associated with osteoporosis, a common comorbidity of RA.[18]

On the other hand, some of the RA GWAS loci had eQTL effects in SFs in a stimulation dependent manner (online supplemental table 3), (online supplemental figure 3). One example is rs6074022, which is in tight LD ($r^2=0.95$ in EUR, $r^2=0.9$ in EAS population) with an established RA risk SNP rs4810485.[19]

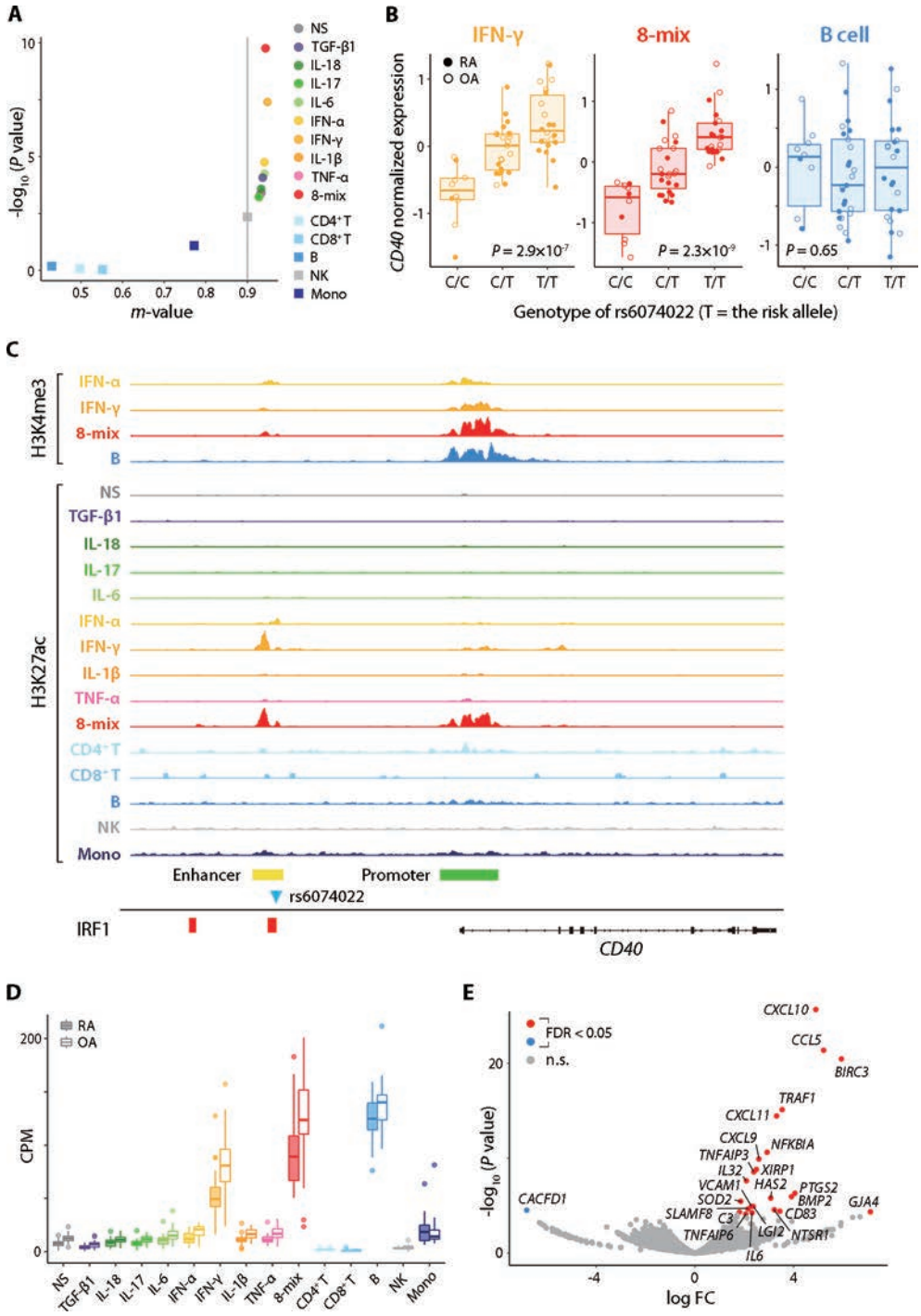

Figure 4 Stimulation-specific function of RA genetic risk loci. (A) A dot plot of rs6074022-*CD40* Cis-eQTL meta-analysis posterior probability m values versus tissue-by-tissue analysis -$\log_{10}$ p value. The grey solid line (*m*-value=0.9) corresponds to the significance threshold in this study. (B) Expression of *CD40* in RASFs (filled circles) and OASFs (open circles) stimulated by IFN-γ (left), 8-mix (middle) and B cells (right) from each individual plotted according to the rs6074022 genotype. Nominal p values in eQTL mapping are shown. (C) Transcriptional regulatory regions around the *CD40* gene and positional relationship of rs6074022 (blue triangle) in stimulated SFs and PBMCs. IRF1 biding sites were obtained from the public epigenome browser ChIP-Atras. Data were visualised using the Integrative Genomics Viewer. (D) Transcript abundance of *CD40* from RNA sequencing data in stimulated SFs and PBMCs. (E) A volcano plot of differential gene expression analysis comparing the presence or absence of CD40 ligand (CD40L) for IFN-γ-stimulated SFs. Orange and blue points mark the genes with significantly increased or decreased expression, respectively, for the addition of CD40L (FDR<0.01). Boxes, IQR; whiskers, distribution; dots, outliers in (B) and (D). CPM, counts per million; eQTL, expression quantitative trait locus; IFN, interferon; IL, interleukin; NK cells. natural killer cells; NS, non-stimulated; n.s., not significant; OA, osteoarthritis; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SFs, synovial fibroblasts; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α.

rs6074022 had robust eQTL effects on *CD40* in SFs, especially under IFN-γ or 8-mix stimulations (figure 4A,B). Importantly, the presence of an active regulatory region at rs6074022 was inferred only under these conditions (figure 4C). Although *CD40* is also expressed by B cells (figure 4D), the eQTL effect was not observed at this locus (figure 4B).

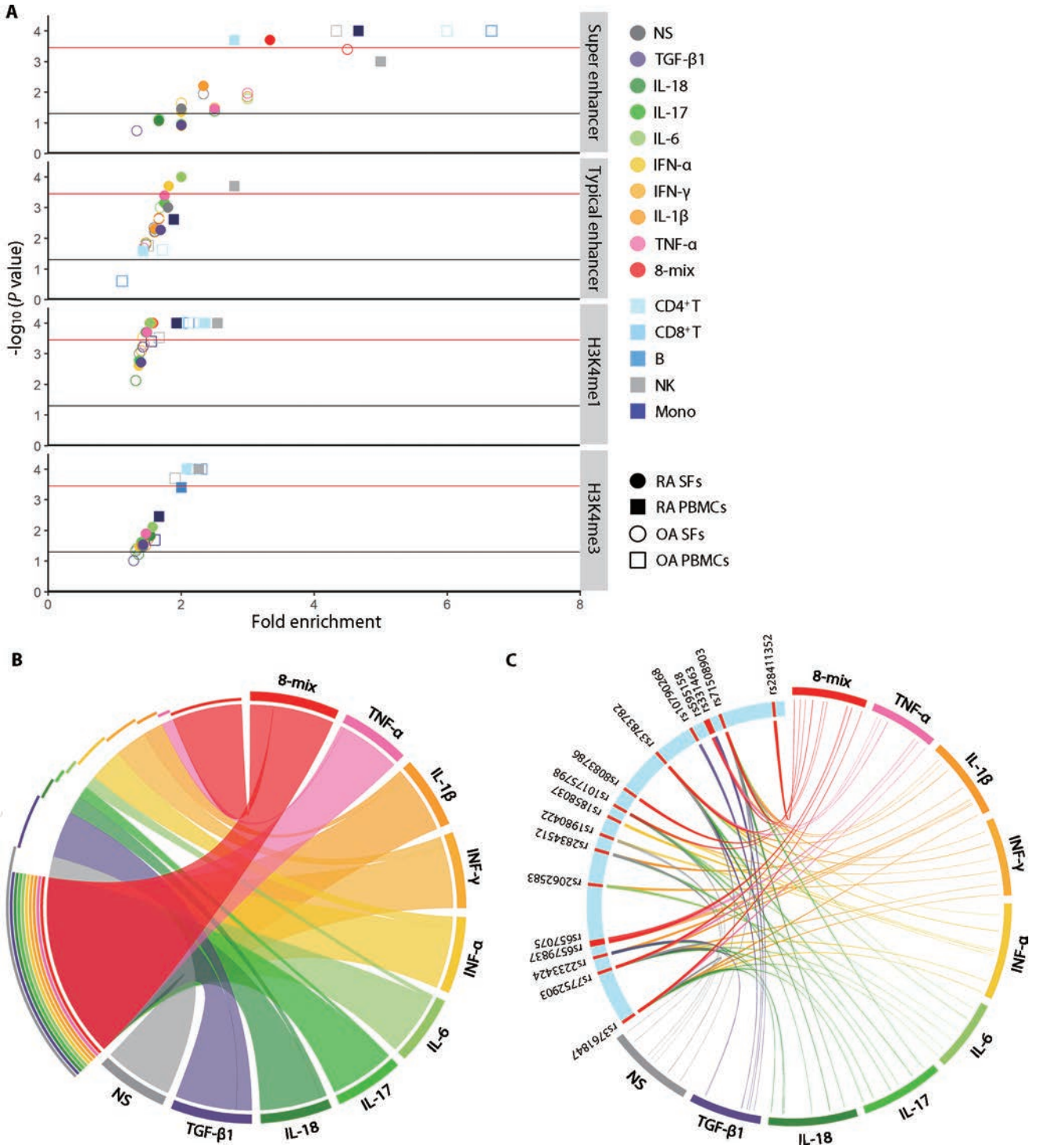

Figure 5 Enrichment of RA genetic risk in SFs SEs under eight cytokine stimulation. (A) Enrichment of RA risk loci in transcriptional regulatory regions of stimulated SFs and PBMCs. Active enhancers were classified into SEs and TEs following standard rose algorithms. The red solid lines and the black solid lines are the cutoffs for Bonferroni significance and p=0.05, respectively. (B) A Circus plot showing the overlap of SEs in SFs under different stimulatory conditions. only the regions unique to each condition or common to all of the conditions are depicted. (C) A Circus plot showing the overlap of RA risk loci and SEs in SFs under different stimulatory conditions. IFN, interferon; IL, interleukin; NK cells, natural killer cells; NS, non-stimulated; OA, osteoarthritis; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SE, super-enhancer; SFs, synovial fibroblasts; TE, typical enhancer; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α.

The biological role of the CD40-CD40L pathway in SFs has been discussed.[20 21] We performed transcriptomic analysis of RASFs stimulated with a 2-trimer form of the CD40 ligand and IFN-γ. As a result, some cytokines (eg, *IL6*) and chemokines (eg, *CCL5*, *CXCL10*) were significantly upregulated by the ligation of CD40L (figure 4E). Taken together, we conjecture that CD40 expression in SFs is influenced by genetic and environmental predisposition, and the CD40-CD40L pathway might have a pathogenic role in RASFs.

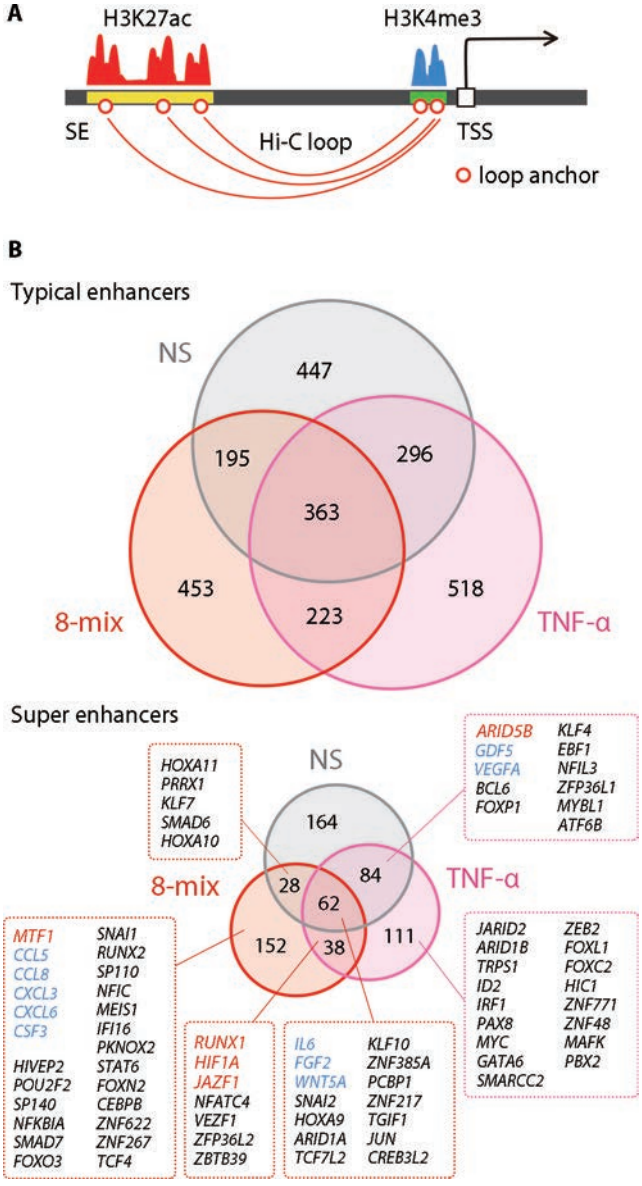

Figure 6 RA pathogenic factors regulated by SEs in SFs treated with eight cytokines. (A) A schematic image of 'SE-contacted genes'. (B) A Venn diagram representing the overlap of TE-contacted (top) or SE-contacted (bottom) genes in SFs under different stimulatory conditions. Red, blue and black text highlight genes whose contacted SEs overlap with RA risk loci, cytokines and chemokines and transcription factors, respectively. NS, non-stimulated; RA, rheumatoid arthritis; SE, super-enhancer; SFs, synovial fibroblasts; TE, typical enhancer; TNF-α, tumour necrosis factor-α; TSS, transcriptional start site.

Genetic risk of RA accumulate in the transcriptomic and epigenomic perturbations induced by synergistic proinflammatory cytokines

As acquired epigenomic differences between RA and OA are not necessarily associated with inherited genome, we next sought to reveal the condition most closely associated with RA susceptibility.

First, to elucidate the transcriptomic changes that is associated with RA genetic risk, we performed gene-set enrichment analysis using MAGMA software (online supplementary materials). This analysis indicated that perturbed gene sets subject to IFN-α, IFN-γ and 8-mix stimulation significantly overlapped with RA risk loci in both EUR and EAS populations (online supplemental figure 4AB). Those data contrasted with the non-significant association of transcriptome differences between the diseases with RA genetic risk. These findings indicate that there is an accumulation of RA genetic risk in the pathways that are perturbed under specific stimulatory conditions in cultured SFs.

Next, to elucidate the epigenomic changes which is associated with genetic risk, we assessed the enrichment of GWAS top-associated loci in regulatory regions including super-enhancers (SEs). SEs are large clusters of enhancers collectively bound by an array of transcription factors (TFs) to define cell identity, and they are hotspots for disease susceptibility.[22–24] Although the significant overlap of SEs in Th cells and B cells with RA risk loci has been reported,[22] SFs have not been examined. Here we compared the enrichment of RA risk loci to SEs and typical-enhancers (TEs), and risk loci showed significant enrichment with SEs in $CD4^+$ T cells and B cells, as well as with SEs in 8-mix stimulated SFs (figure 5A). Some risk loci showed overlap with SEs which appear uniquely under 8-mix treatment (figure 5B,C). When we performed a similar analysis using risk loci for type 1 diabetes mellitus (a representative non-articular autoimmune disease), only SEs in $CD4^+$ T cells and B cells showed significant enrichment (online supplemental figure 4C). The number or width of 8-mix SEs were comparable to those in other stimulations (online supplemental figure 5). Consequently, SFs might behave as key players in RA pathogenesis especially under combination of cytokines.

SEs induced by cytokine mixtures regulate genes crucial for RA pathogenesis

While the 8-mix is an artificial stimulatory condition, its unique impact on epigenomes (figure 5B), synergistic enhancement of arthritic gene expressions (online supplemental figure 6), and significant association with RA genetic risk (online supplemental figure 4A,B, figure 5A) induced us to interpret that it reflects some aspect of SF behaviour which is relevant to RA pathogenesis. The contribution of each cytokine to 8-mix condition is discussed in online supplementary note 2.

In order to characterise the genes regulated by 8-mix SEs, we combined the three-dimensional (3D) genome architectures (chromatin loops detected by Hi-C analysis), the position of SEs, promoter regions (defined with H3K4me3 ChIP sequencing analysis). We annotated 'SE-contacted genes' such that one side of Hi-C loop anchors overlapped an SE, the other side coincided with the transcriptional start site (TSS) and coexisted with the H3K4me3 peak (figure 6A). SEs were highly overlapped with Hi-C loop anchors than were TEs or H3K4me1 peaks (online supplemental figure 7A). When the TSS and H3K27ac peak was connected by a Hi-C loop, the variation of mRNA expression showed a significant correlation with the H3K27ac peak variation (online supplemental figure 7B). These results underscore the validity of connecting active enhancer marks and TSS by Hi-C loops as previous reports.[25 26] When we compared the expression of SE-contacted genes and TE-contacted genes, the former showed significantly higher expression than the latter (online supplemental figure 7C).[24]

Next, we compared genes contacted by either SEs or TEs of SFs under three different conditions: non-stimulated, TNF-α or the 8-mix. The proportions of overlap between these conditions were smaller in SE-contacted genes (9.7%) than TE-contacted genes (14.5%) (figure 6B), indicating that the stimulation-specific expression profile and SEs formation are associated. SE-contacted genes included a number of TFs (eg, *MTF1*,

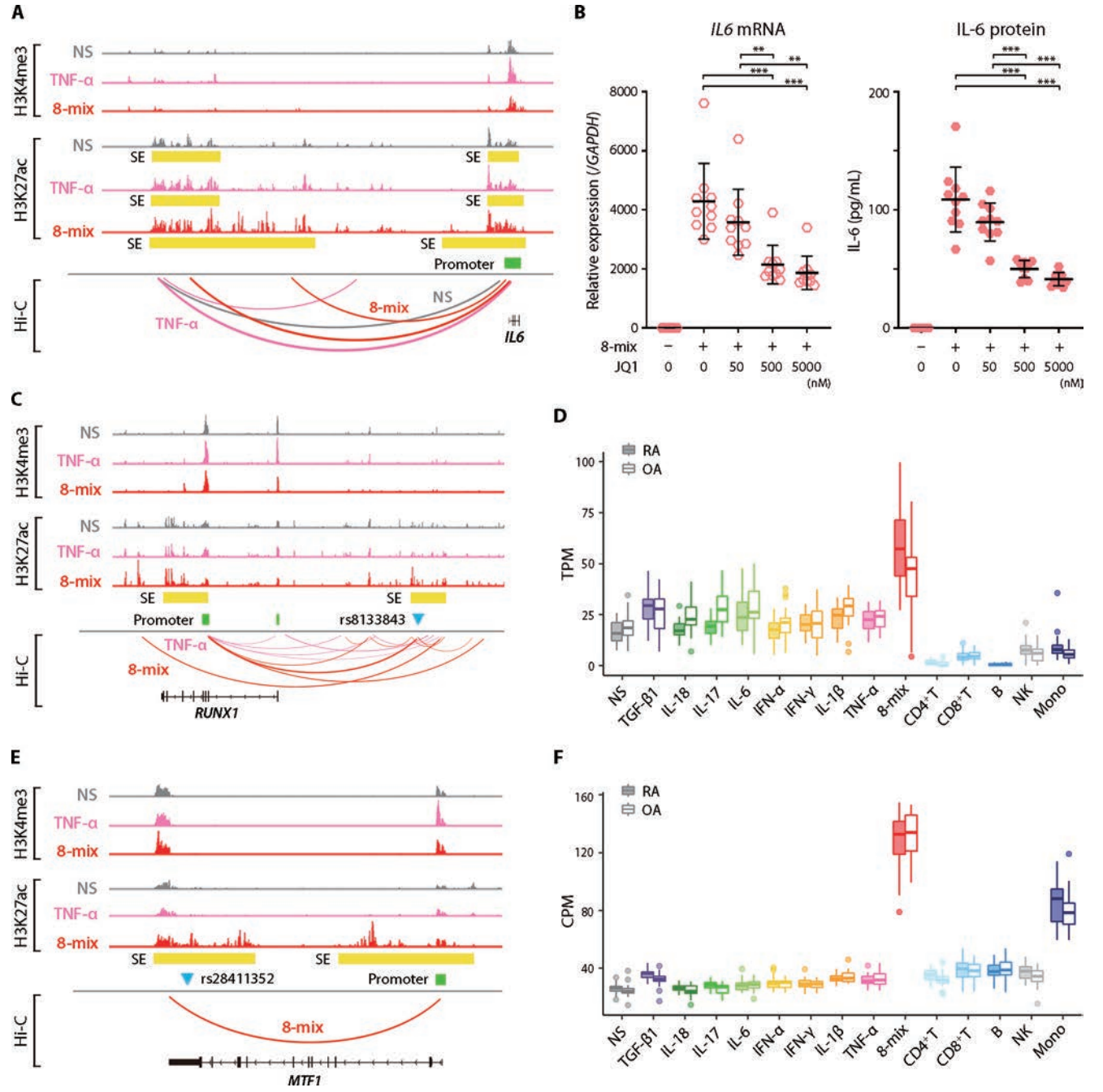

Figure 7 Representative SE-contacted genes in SFs treated with eight cytokines. (A, C, E) Organisation of transcriptional regulatory regions around *IL6* (A), *RUNX1* (C) and *MTF1* (E) genes and positional relationship of RA risk loci (blue triangle, rs8133848 for *RUNX1* and rs28411352 for *MTF1*) and chromatin conformation in stimulated SFs. Data were visualised using the Integrative Genomics Viewer. (B) Expression of IL-6 in SFs (n=10) treated with JQ1. The mRNA and protein expression were quantified by qRT-PCR (left) and ELISA (right), respectively. Horizontal crossbars, mean; error bars, SD. P values were determined using one-way ANOVA followed by Tukey's multiple comparison test (**p<0.01, ***p<0.001). (D, F) Transcript abundances of *RUNX1* isoform (RUNX1b) (D) and *MTF1* (F) from RNA sequencing data for stimulated SFs and PBMCs. Boxes, IQR; whiskers, distribution; dots, outliers. ANOVA, analysis of variance; CPM, counts per million; IFN, interferon; IL, interleukin; *MTF1*, metal-regulatory transcription factor-1; NK cells, natural killer cells; NS, non-stimulated; OA, osteoarthritis; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; *RUNX1*, runt-related transcription factor 1; SE, super-enhancer; SFs, synovial fibroblasts; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α; TPM, transcripts per million.

RUNX1), cytokines (eg, *IL6*) and chemokines (eg, *CCL5*, *CCL8*) (figure 6B, (online supplemental table 4)).

IL6 is a representative example of an 8-mix SE-contacted gene. Although this gene is regulated by an SE (almost 30 kb long) that exists upstream of the TSS in non-stimulated or TNF-α stimulated SFs, this SE elongates to 70 kb long under the 8-mix and an additional Hi-C loop emerges (figure 7A). When we inhibited SE formation with JQ1, a BRD4 inhibitor, the increased IL-6 expression under the 8-mix was disturbed (figure 7B). The necessity of SEs for elevated IL-6 production under synergistic inflammation was inferred.

Another example is *RUNX1*, a master-regulator involved in hematopoiesis.[27–29] In our study, RA risk locus rs8133843 overlapped with an 8-mix SE that exists upstream of *RUNX1*

A

TF	Motif	SEs enrichment *P* value: 8-mix SEs-connection	8-mix vs TEs	8-mix vs NS SEs	TNF-α SEs-connection	TNF-α vs TEs	TNF-α vs NS SEs	NS SEs-connection	NS vs TEs
SNAI1		yes	1.0×10^{-59}	1.0×10^{-14}	no	1.0×10^{-27}	> 0.05	no	1.0×10^{-45}
TCF4		yes	1.0×10^{-50}	1.0×10^{-13}	no	1.0×10^{-22}	> 0.05	no	1.0×10^{-40}
SNAI2		yes	1.0×10^{-48}	1.0×10^{-19}	yes	1.0×10^{-22}	> 0.05	yes	1.0×10^{-34}
MTF1		yes	1.0×10^{-18}	1.0×10^{-03}	no	1.0×10^{-05}	> 0.05	no	1.0×10^{-08}
SREBF1		no	1.0×10^{-09}	1.0×10^{-09}	yes	1.0×10^{-03}	> 0.05	no	1.0×10^{-04}
RARA		no	1.0×10^{-09}	1.0×10^{-08}	no	1.0×10^{-03}	1.0×10^{-06}	yes	1.0×10^{-05}
JUN		yes	1.0×10^{-03}	1.0×10^{-14}	yes	> 0.05	> 0.05	yes	1.0×10^{-03}
FOXL1		no	1.0×10^{-08}	1.0×10^{-04}	yes	1.0×10^{-04}	> 0.05	no	1.0×10^{-05}
ZNF740		no	1.0×10^{-07}	> 0.05	no	1.0×10^{-09}	1.0×10^{-02}	yes	1.0×10^{-17}
IRF1		no	> 0.05	1.0×10^{-33}	yes	> 0.05	> 0.05	no	> 0.05
CEBPB		yes	> 0.05	1.0×10^{-30}	no	> 0.05	1.0×10^{-06}	no	> 0.05

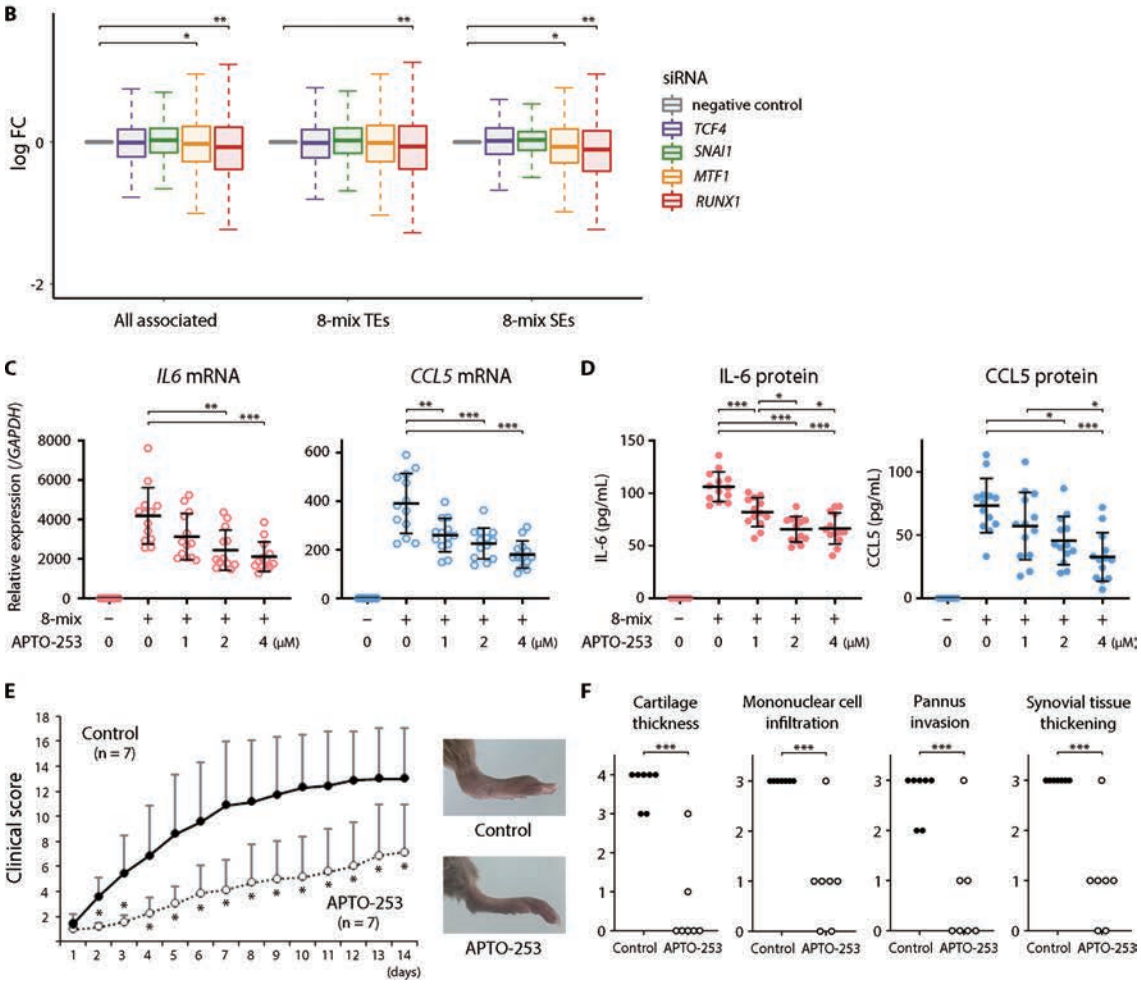

Figure 8 Transcription factors associated with stimulation-induced SE formation and arthritis progression. (A) Table depicts transcription factor binding motifs enriched at SEs in stimulated SFs. Following are summarised: attribution to SE-contacted genes, relative enrichment p values to TEs in each stimulatory condition or to SEs of non-stimulated condition. (B) Expression of SE- or TE-contacted genes in SFs stimulated by eight cytokines in cells depleted of specified transcription factors (*TCF4*, *SNAI1*, *MTF1* and *RUNX1*) relative to control SFs. Boxes, IQR; whiskers, distribution. P values, paired t-test (*p<0.05, ***p<0.001). (C, D) Expression of IL-6 and CCL5 in SFs (n=13) treated with APTO-253. The mRNA and protein level were quantified by qRT-PCR (C) and ELISA (D), respectively. Horizontal crossbars, mean; error bars, SD. P values, one-way ANOVA followed by Tukey's multiple comparison test (*p<0.05, **p<0.01, ***p<0.001). (E, F) Therapeutic effect of APTO-253 on CIA model. Following the onset of arthritis, mice were intravenously injected with either control or 15 mg/kg APTO-253 for twice per day for two consecutive days per week. Clinical (E) and pathological scores (F). Dots, mean; error bars, SD. P values, Mann-Whitney U test (*p<0.05, ***p<0.001). AVOVA, analysis of variance; CIA, collagen-induced arthritis; IL, interleukin; *MTF1*, metal-regulatory transcription factor-1; NS, non-stimulated; *RUNX1*, runt-related transcription factor 1; SE, super-enhancer; SFs, synovial fibroblasts; TE, typical enhance; TNF-α, tumour necrosis factor-α.

(figure 7C). A Hi-C loop was formed with the promoter immediately above the second exon of *RUNX1* only in the 8-mix, and the *RUNX1* expression was higher in the 8-mix compared with others (figure 7D).

MTF1, a zinc finger TFs, is another example. RA risk locus rs28411352 overlapped with an 8-mix unique SE that exists upstream of the *MTF1* (figure 7E). The Hi-C loop was detected with the promoter only under the 8-mix. *MTF1* expression was upregulated in the 8-mix (figure 7F).

TFs associated with stimulation-induced SE formation control arthritis progression

Finally, we searched for candidate modulators that were crucial for SE formation, especially in the 8-mix. In the previous study, key TFs for SE formation were reported to be controlled by SEs themselves, forming a self-regulatory network.[24] From this perspective, we used motif analysis to focus on SE-contacted TFs that were also enriched in 8-mix SEs (figure 8A). Among SE-contacted genes, TFs such as SNAI1, TCF4 and MTF1 showed significant motif enrichment in 8-mix SEs. MTF1 was the only example that also showed the overlap of 8-mix SE and RA risk variant (figure 7E). Although the RUNX1 motif was not enriched in 8-mix SEs compared with non-stimulated SEs, its motif was significantly enriched in SEs compared with the background sequence, both in 8-mix and without stimulation ($p=1.0\times10^{-16}$ and 1.0×10^{-20}, respectively). *In vitro* validation analysis showed that the expression of 8-mix SE-contacted genes was significantly suppressed by *MTF1* and *RUNX1* knockdown ($p=2.0\times10^{-3}$ and 4.3×10^{-4}, respectively) (figure 8B, (online supplemental figure 8). The effect of *MTF1* knockdown was more pronounced in 8-mix SE-contacted genes than TE-contacted genes, as anticipated from its motif enrichment in SEs. In *in vitro* assay, the increased 8-mix SE-contacted gene expressions (IL-6, CCL5) from stimulated RASFs was reduced by treatment with APTO-253, a MTF1 inhibitor (figure 8C,D).[30] Furthermore, in a collagen induced arthritis (CIA) model, APTO-253 demonstrated significant preventive (online supplemental figure 9) and therapeutic activity (figure 8E,F) on arthritis formation. Collectively, these results indicated that certain TFs play critical roles in the formation of epigenomic structures induced by synergistic proinflammatory cytokines and support MTF1 inhibition as a promising therapy candidate for RA (online supplemental figure 10).

DISCUSSION

In this study, we were able to describe the dynamic landscape of activated SFs and their contribution to RA pathogenesis. RASFs demonstrated distinct epigenomic and transcriptomic features from OASFs, which might be due to acquired epigenomic modifications from long-standing inflammation. Importantly, heritable RA risk was enriched in stimulation dependent epigenomic structures in SFs, a topic that has not been addressed deeply in previous reports.

Recent large-scale eQTL studies enhanced our understanding of complex diseases.[31 32] Considering the importance of studying disease-relevant tissues for functional understanding of GWAS variants,[15 33] we conducted cis-eQTL analysis of SFs, which are major local effector cells in arthritic joints. One example of eQTL-eGene pairs in SFs was the association of an RA risk SNP (rs4810485) with *CD40* expression. Although no significant eQTL effects of this locus in B cells was observed in our data, a previous report showed the protein-level QTL association of CD40 in $CD19^+$ B cells and this locus.[34] On the contrary, a larger eQTL study by the GTEx consortium that showed genome-wide significant eQTL effects of rs6074022 on *CD40* in lung (mainly fibroblasts) ($p=3.1\times10^{-26}$) and cultured fibroblasts ($p=8.4\times10^{-16}$), but not in EBV-transformed lymphocytes ($p=0.04$).[35] Naturally, although the possible importance of CD40 signals in B cells for RA pathogenesis cannot be neglected, our results shed light on the role of CD40-CD40L signals in a genetic network of synovitis.

The GWAS-SEs enrichment analysis indicated the importance of SFs under synergistic stimulations in the development of RA (analogous to $CD4^+$ T cells and B cells). During cytokine synergy, Hi-C analysis suggested that there were dynamic conformational changes in 3D structures involving SEs and the promoter of pathological molecules. We found marked expression of 8-mix stimulated SE-contacted genes (ie, *IL6*, *RUNX1*, *MTF1*).

SEs can collapse when their cofactors (eg, BET family) are perturbed.[36] On the other hand, selective modulation of disease-associated SEs in a cell type-specific manner may have better safety profiles than pan-SEs inhibitors (ie, JQ1). In the present study, we analysed TFs that have the potential to be selective SE modulators in activated SFs. Our results suggested that MTF1 participates in SE formation, putatively making a feedback loop to maintain the epigenomic machinery. MTF1 regulates gene expression in response to zinc and various stresses.[37] In the setting of disease, MTF1 could contribute to tumour metastasis and chemoresistance.[38] In the present study, APTO-253, a MTF1 inhibitor,[30] demonstrated inhibitory activity on the expression of pathogenic molecules (IL-6, CCL5) from RASFs and antiarthritic activity in a CIA model. Previously, the zinc-ZIP8-MTF1 axis was identified as a catabolic regulator of cartilage destruction.[39] Furthermore, intra-articular injection of adenovirus expressing-MTF1 in an OA mouse model promoted the expression of various inflammatory molecules in SFs. This evidence supports the essential role of MTF1 as a genetic hub for joint destruction and inflammation.

There are some limitations of this study. First, the number of patients included in the cis-eQTL analysis was limited owing to sample accessibility, resulting in putatively large false negatives. Second, previous reports have shown that culture procedure could affect the phenotype of SFs.[39–42] It should be noted that the results presented were obtained from the early passage SFs, and using directly isolated cells may have led to less biased analysis. Third, the stimulation endpoint was only 24 hours, and more detailed effects of each cytokine could have been analysed if expression information at different stimulation endpoints had been available. Finally, isolated PBMCs were not artificially stimulated with cytokines ex vivo. Thus, it is not clear whether the eQTL difference between SFs and PBMCs is attributable solely to cell type difference.

Overall, our multiomics approach using activated local cells in joints shed light on the concept of SF-targeted therapy from the perspective of epigenome remodelling related to genetic risk and would be beneficial in searching for novel drug targets.

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Contributors HT, SS, KI, YKo, KY and KF designed the research project. MO conducted bioinformatics analysis on the advice of KI, AS performed RNA sequencing. HT performed ChIP sequencing and other *in vitro* experiments. TS and KS performed Hi-C. HT, YT, HI, JH, YKa and ST contributed human samples. SS performed figure editing. HT and MO wrote the manuscript with critical inputs from YKo, KY and KF.

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CLINICAL SCIENCE

Regulatory eosinophils induce the resolution of experimental arthritis and appear in remission state of human rheumatoid arthritis

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ABSTRACT

Objectives Eosinophils possess pro-inflammatory functions in asthma. However, our recent studies have suggested that innate lymphoid cells type 2 (ILC2s) and eosinophils have proresolving properties in rheumatoid arthritis (RA). Nothing is known yet about the mechanisms determining the double-edged role of eosinophils. Therefore, we investigated whether asthma, a paradigm eosinophilic disease, can elicit resolution of chronic arthritis.

Methods Ovalbumin-triggered eosinophilic asthma was combined with K/BxN serum-induced arthritis, where lung and synovial eosinophil subsets were compared by single-cell RNA sequencing (scRNA-seq). To investigate the involvement of the ILC2–interleukin-5 (IL-5) axis, hydrodynamic injection (HDI) of IL-25 and IL-33 plasmids, IL-5 reporter mice and anti-IL-5 antibody treatment were used. In patients with RA, the presence of distinct eosinophil subsets was examined in peripheral blood and synovial tissue. Disease activity of patients with RA with concomitant asthma was monitored before and after mepolizumab (anti-IL-5 antibody) therapy.

Results The induction of eosinophilic asthma caused resolution of murine arthritis and joint tissue protection. ScRNA-seq revealed a specific subset of regulatory eosinophils (rEos) in the joints, distinct from inflammatory eosinophils in the lungs. Mechanistically, synovial rEos expanded on systemic upregulation of IL-5 released by lung ILC2s. Eosinophil depletion abolished the beneficial effect of asthma on arthritis. rEos were consistently present in the synovium of patients with RA in remission, but not in active stage. Remarkably, in patients with RA with concomitant asthma, mepolizumab treatment induced relapse of arthritis.

Conclusion These findings point to a hitherto undiscovered proresolving signature in an eosinophil subset that stimulates arthritis resolution.

Key messages

What is already known about this subject?

► Our recent studies have suggested that innate lymphoid cells type 2 (ILC2s) and eosinophils have proresolving, but not proinflammatory, properties in rheumatoid arthritis. However, nothing is known about the mechanisms determining the proresolving role of eosinophils.

What does this study add?

► Synovial regulatory eosinophils (rEos) triggered by allergic asthma initiate resolution of chronic arthritis and tissue regeneration.

► Mechanistically, joint-resident rEos expand on systemic distribution of interleukin-5 produced by ILC2s in the lungs.

How might this impact on clinical practice or future developments?

► These findings demonstrate the existence of a proresolving subset of eosinophils that act as effector cells for the resolution of chronic joint inflammation.

INTRODUCTION

Eosinophils have multiple functions in health and disease. Circulating eosinophils range from 0 to 500 cells/µL of human blood. However, their number can increase up to 20-fold in certain pathological conditions.[1] Eosinophils are formed from multipotent haematopoietic stem cells in the bone marrow (BM) microenvironment and once matured, escape to peripheral sites. In the BM, granulocyte progenitors differentiate into eosinophil progenitors, if the transcription factor GATA-binding factor 1 becomes activated. On further maturation, they start to express Siglec-F and respond to interleukin-5 (IL-5).[2]

Eosinophils express a wide range of molecules that are stored in granules and lipid bodies throughout their cytoplasm. Their capacity to promptly release large amounts of mediators places eosinophils as critical regulators of a broad variety of immunological processes. Eosinophils are traditionally associated with the immune defence against parasites[3 4] and the development of type 2 immune disorders such as allergy and asthma.[5] The proinflammatory effector function of eosinophils is well known in asthma, characterised by inflammation of the airways and structural remodelling of the lungs. Asthma has different causal pathways, dividing this disease into several endotypes.[6] Its most common form is type 2 allergic asthma, identified by the secretion of type 2 cytokines (IL-4, IL-5 and IL-13), IgE production and eosinophilia.[7] In asthma,

eosinophils are primarily activated by innate lymphoid cells type 2 (ILC2s).[8] ILC2s expand on release of the cytokines thymic stromal lymphopoietin, IL-33 and IL-25[9] and have been shown to orchestrate lung inflammation.[10]

However, the restricted view of eosinophils as primarily inflammatory cells has been recently challenged. Several studies emphasise on the homeostatic role of eosinophils, including their involvement in immune maintenance,[11] organ development[12] and tissue regeneration.[13 14] For instance, eosinophils are responsible for the modulation of classical macrophages into alternatively activated macrophages (AAMs).[15] Unexpectedly, recent studies have suggested that ILC2s and eosinophils have proresolving, but not proinflammatory, properties in rheumatoid arthritis (RA).[16–18] In RA, the inflammatory process in the joints is triggered by cytokines such as IL-6, TNFα and IL-1β that are secreted by proinflammatory bone marrow-derived monocytes (BMDMs).[19] RA has a high level of chronicity and potential mechanisms forcing resolution of inflammation have been incompletely defined,[20] but likely depend on the establishment of an anti-inflammatory effector cell population. Stunningly, in the very early phases of RA with higher probability of resolution, a type 2 cytokine signature has been reported,[21 22] suggesting that eosinophils may act in a context dependent manner, either supporting or ceasing certain forms of inflammatory processes.

Little is known about the mechanisms that allow eosinophils to enhance inflammatory processes on the one hand, while acting as homeostatic proresolving cells on the other hand. It can be hypothesised that the proinflammatory action of eosinophils and their homeostatic functions depend on different eosinophil subsets. Indeed, in asthmatic lungs, two distinct eosinophil subsets have been discovered, classified by a different expression of Siglec-F.[23] Herein, we show that the induction of asthma indeed causes resolution of arthritis and is associated with the emergence of a specific subset of regulatory eosinophils (rEos) in the joints. In contrast to lung eosinophils, these cells display proresolving characteristics. They expand on exposure to IL-5, released by ILC2s in the lungs. Depletion of eosinophils by genetic approach or by anti-IL-5 antibody treatment reversed asthma-induced resolution of arthritis. In human, rEos were consistently present in the blood and synovium of patients with RA in remission. Furthermore, the treatment with the monoclonal anti-IL-5 antibody mepolizumab led to arthritis relapse in patients with asthma. Altogether, these findings demonstrate the existence of a proresolving subset of eosinophils that act as effector cells for the resolution of chronic joint inflammation.

METHODS

Mice

Wildtype (WT) BALB/cJRj were purchased from Janvier Labs. ΔdblGATA[24] mice and IL-5tg/4Get mice[25 26] were on Balb/c background. B6(C)-Il5$^{tm1.1(icre)Lky}$/J mice were purchased from The Jackson Laboratory and were on C57BL/6J background. All mice were housed in a temperature-controlled and humidity-controlled facility with free access to food and water.

Ovalbumin (OVA)-induced asthma model

Mice at the age of 6 weeks were sensitised two times with intraperitoneal injection of 100 µg OVA (InvivoGen) complexed to adjuvant alum (10%, Thermo Scientific) at day −20 and −13. Thereafter, mice were challenged with 50 µg of OVA (dissolved into 1 x phosphate-buffered saline (PBS)) intranasally (anaesthesia with isoflurane) at day –2, –1 and 0 as previously described.[27] Mice were challenged a second time with 50 µg OVA intranasally at day 4, 5 and 6.

K/BxN serum-induced arthritis (SIA)

Nine weeks old mice were injected intraperitoneally with 150–200 µL pooled serum from arthritic adult K/BxN mice as described.[28] Development of arthritis was evaluated for each paw using a semiquantitative scoring system (0–4 per paw; maximum score of 16) as previously described.[18]

r/inflammatory eosinophils (iEos) synovial cavity transfer

r/iEos were sorted from the blood and BM of IL-5tg/4Get mice and dissolved into 25 µL 1 x PBS. The cells or mock 25 µL 1 x PBS were injected into the synovial cavity of ΔdblGATA mice after anaesthesia with isoflurane at day 3, 5 and 7 post K/BxN serum transfer.

IL-25/33 plasmid DNA purification and hydrodynamic administration

IL-25 and IL-33 plasmid DNAs[17] were purified using EndoFree Plasmid Kits (QIAGEN) and freed from remaining endotoxin using the MiraCLEAN Endotoxin Removal Kit (Mirus) according to the manufacturer's instructions. Overall, 10 µg IL-25 plasmid DNA and 10 µg IL-33 plasmid DNA in 2.1 mL 1 x PBS were hydrodynamically administered into WT and ΔdblGATA mice at day 5 after K/BxN serum transfer.

IL-5 in vivo neutralisation

Overall, 20 µg LEAF-purified anti-mouse/human IL-5 antibody (BioLegend) or 20 µg Ultra-LEAF-purified rat IgG1, κ isotype control antibody (BioLegend) in 150 µL 1 x PBS was injected into the tail vein of SIA and OVA/SIA mice from day 4 until day 8 after K/BxN serum transfer.

Patient characteristics

Peripheral blood and synovial samples (ultrasound-guided needle biopsy) were taken from patients with RA fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA.[29] Disease activity was defined by Disease Activity Score 28 (DAS28) based on erythrocyte sedimentation rate (ESR).[30] For flow cytometry analyses of eosinophils, blood of healthy donors (n=10, age 31.1±12, 80% female), active RA (n=10, DAS28–ESR 2.7–5.8 units, age 65.7±8.7, 80% female), and non-active patients with RA (n=10, DAS28–ESR<2.6 units, age 59.8±9.3, 50% female) was collected in ethylenediaminetetraacetic acid (EDTA) tubes. Synovial biopsy samples were obtained from patients with active RA (n=6, DAS28–ESR 3.6–6.1 units, age 66.3±9.8, 83% female) and patients with RA in remission (n=10, DAS28 <2.6 units, age 52±9.5, 70% female). In addition, we identified eight patients with RA and concomitant asthma receiving the anti-IL-5 antibody mepolizumab in our institution. Patients were treated in routine care for RA and received mepolizumab at a dose of 100 mg via subcutaneous injection for treatment of their asthma, which failed to respond to inhaled glucocorticoids and bronchodilatators according to the approved indication. DAS28–ESR Score was documented before and after mepolizumab treatment. Flares in the same patients with RA were treated with antitumour necrosis factor alpha antibody adalimumab at a dose of 40 mg per subcutaneous injection.

Bronchoalveolar lavage fluid (BALF) collection and staining

BALF was collected via flushing lungs four times with 0.75 mL saline through the trachea. The BALF was centrifuged at 22.86 g for 5 min to generate cytospin slides. May-Grünwald-Giemsa (Carl Roth) staining of cytospin slides was performed according to the manufacturer's instructions and representative pictures were taken with the BZ-X710 all-in-one fluorescence microscope (Keyence).

Histological analysis

Lungs of mice were fixed in 4% paraformaldehyde (PFA) at 4°C overnight. Serial paraffin sections (2 µm) were stained with H&E staining for semiquantification of lung inflammation. Hind paws of mice were fixed overnight in 4% PFA at 4°C and afterwards decalcified in 14% EDTA for 14 days until bones were pliable. Serial paraffin sections (2 µm) were stained with H&E staining for quantifying inflammation and with tartrate-resistant acid phosphatase (TRAP) staining using the acid phosphatase, leucocyte (TRAP) Kit (MilliporeSigma) for quantifying bone erosion and number of osteoclasts. The quantification of lung inflammation score, paw inflammation area, bone erosion area and number of osteoclasts per paw was performed on an Axio Lab. A1 microscope (Carl Zeiss), equipped with a digital camera and image analysis system (OsteoMeasure, Osteometrics).

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of IgE were measured by ELISA using purified rat anti-mouse IgE (BD), purified mouse IgE, κ isotype standard (BD) and biotin rat anti-mouse IgE (BD) according to the manufacturer's instructions. Serum levels of IL-4, IL-5, IL-13 and IL-17 were measured by ELISA using mouse IL-4/IL-5/IL-13/IL-17 DuoSet ELISA Kit (all from R&D) according to the manufacturer's instructions.

Flow cytometry

For single-cell generation, lungs were chopped with a scalpel into small pieces and digested with 1 mg/mL Collagenase A and 0.1 mg/mL DNaseI (both from MilliporeSigma) in Roswell Park Memorial Institute (RPMI) medium (+10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS)) at 37°C for 1 hour. Digested lungs were put through 40 µm cell strainers and lysed with 10 mL red cell lysis buffer (RCL buffer: 0.15 M NH_4Cl, 0.01 M $KHCO_3$, 0.1 mM EDTA in ddH_2O, 0.2 µm filtered). Ankles were cut into small pieces with scissors and digested with 1 mg/mL Collagenase A and 0.1 mg/mL DNaseI in RPMI medium (+10% FBS and 1% PS) at 37°C for 1 hour. Digested ankles were put through 40 µm cell strainers. Mesenteric lymph nodes were minced and put through 40 µm cell strainers. Blood was lysed two times with 5 mL RCL buffer and put through 40 µm cell strainers. For cell isolation from the BM, the left femur was flushed 3x with 1 x PBS. Then, the BM cells were lysed with 3 mL RCL buffer and put through 40 µm cell strainers. For surface marker staining, isolated single cells were first incubated with anti-mouse CD16/32 antibody (1:1000, BioLegend, clone 93) in 1 x PBS for 5 min in the dark at 4°C, and then stained with the surface markers: CD11b-FITC (BD, M1/70), CD11b-APC/Cy7 (BioLegend, M1/70), CD11c-PE (BioLegend, N418), CD19-APC (BD, 1D3), CD278-BV421 (BD, 7E.17G9), CD3ε-PE/Cy5 (BioLegend, 145–2 C11), CD4-FITC (BD, H129.19), CD45-APC efluor780 (eBioscience, 30-F11), CD49b-PE/Cy7 (BioLegend, DX5), FcεRIα-PE/Cy7 (BioLegend, MAR-1), F4/80-PE (BioLegend, BM8), I-A/I-E-pacific blue (BioLegend, M5/114.15.2), KLRG1-BV510 (BioLegend, 2F1/KLRG1), Ly-6G-PerCP/Cy5.5 (BioLegend, 1A8), Siglec-F-BV510 (BD, E50-2440), Siglec-F-PE (BD, E50-2440), Siglec-F-BV421 (BD, E50-2440) in 1 x PBS in the dark at 4°C for 20 min. After washing, cells were resuspended in fluorescence-activated cell sorting (FACS) buffer (1 x PBS with 2% FBS and 5 mM EDTA) for flow cytometric analyses.

For Ki67 staining, cells were fixed after surface staining with fixation buffer at 4°C in the dark for 30 min and then stained with intracellular marker Ki67-PE/Cy7 (BioLegend, 16A8) in 1 x permeabilisation buffer in the dark at 4°C for 30 min (Foxp3/Transcription Factor Staining Buffer Set from eBioscience). After washing, cells were resuspended in FACS buffer for analyses.

For T-helper cell staining, isolated lung cells (1×10^6) were stimulated with 50 ng/mL phorbol12-myristate-13-acetate (PMA), 1 µg/mL lonomycin (both from MilliporeSigma) and 1 x monensin (eBioscience) in 1 mL RPMI medium (+10% FBS and 1% PS) in a cell incubator for 4–6 hours. After stimulation, cells were harvested and washed for surface maker staining with CD4-FITC (BD, H129.19) at 4°C in the dark for 20 min. After washing, cells were fixed with fixation buffer, and then stained with the intracellular markers IFNγ-PerCP-Cy5.5 (eBioscience, XMG1.2), IL-4-APC (BD, 11B11), and IL-17A-PE (eBioscience,eBio17B7) in 1 x permeabilisation buffer in the dark at 4°C for 30 min. Then, cells were washed and resuspended in FACS buffer for analyses.

Whole human blood was stained with CD125 (IL-5Ra)-PE (Miltenyi Biotec, REA705), CD45-FITC (BioLegend, H130), Siglec-8-APC (BioLegend, 7C9), CD62L-BV421 (BioLegend, DREG-56), CD63-PE/Cy7 (BioLegend, H5C6), CD69-PerCP (BioLegend, IV A91), and CD123-BV510 (BioLegend, 6H6) at 4°C for 20 min. The cells were measured after TQ-Prep (Beckman Coulter) sample preparation according to the manufacturer's instructions.

Flow cytometry was performed on the Navios, Gallios or Cytoflex S flow cytometer (all from Beckman Coulter). Flow cytometry data were analysed by Kaluza V.2.1, CytExpert V.2 (both from Beckman Coulter) or FlowJo (BD Biosciences).

Light sheet fluorescence microscopy (LSFM)

For eosinophil visualisation in ankle joints, mice were injected intravenously with 5 µg/mouse of Alexa Fluor 647 anti-mouse Siglec-F antibody (BD, E50-2440) in 100 µL 1 x PBS, 9 days after K/BxN serum transfer and 1 hour before sacrifice. After sacrifice, the mice were perfused with 15 mL 1 x PBS with 5 mM EDTA through the left ventricle and afterwards with 15 mL 4% PFA to rinse erythrocytes and fix the bone tissues from inside, respectively. Thereupon, the hind paws were fixed in 4% PFA at 4°C for 4 hours, dehydrated with increasing alcohol concentrations and cleared with ethyl cinnamate (MilliporeSigma) as described.[31] The LSFM imaging was performed with a LaVision BioTec Ultramicroscope (LaVision BioTec) with an Olympus MVX10 zoom microscope body (Olympus), a LaVision BioTec Laser Module, an Andor Neo sCMOS Camera with a pixel size of 6.5 µm, and detection optics with an optical magnification range 1.263–12.63 and a numerical aperture (NA) of 0.5. To show the location of eosinophils, a 488 or 561 nm optically pumped semiconductor laser was used for generation of autofluorescent signals. For Siglec-F-AF647 excitation, a 647 nm diode laser was used. Emitted wavelengths were detected with specific detection filters: 525/50 nm and 620/60 nm for autofluorescence and 680/30 nm for Siglec-F-AF647. An optical zoom factor of 1.25, a thickness of 3 µm and a sheet NA of 4 µm were

used. Evaluations were done with ImageJ and Imaris software (Oxford Instruments).

Eosinophil sorting for single-cell RNA sequencing (scRNA-seq)

Eosinophils for scRNA-seq were sorted from the lungs and ankle joints of SIA and OVA/SIA mice at day 7 post K/BxN serum transfer. For single-cell isolation, lungs were chopped with a scalpel into small pieces and digested (4 mL/lung) with 1 mg/mL Collagenase A and 0.1 mg/mL DNaseI in RPMI medium (+10% FBS and 1% PS) at 37°C, 700 rpm for 45 min. Digested lungs were put through 70 µm cell strainers. After lysing once with 10 mL RCL buffer, lungs were removed of fat tissue using 10 mL serological pipettes (SARSTEDT AG&Co). Cells from three lungs (three mice) were pooled for staining and sorting. Ankles were cut into small pieces with scissors and digested (2 mL/ankle) with 2 mg/mL Collagenase A and 0.03 mg/mL DNaseI in RPMI medium (+10% FBS and 1% PS) at 37°C, 230 rpm for 45 min. Digested ankles were put through 70 µm cell strainers. Cells from 16 ankles (8 mice) of OVA/SIA mice and 24 ankles (12 mice) of SIA mice were pooled for staining and sorting. Lung/ankle cells were first incubated with anti-mouse CD16/32 antibody (1:1000, BioLegend, clone 93) in 1 x PBS for 5 min in the dark at 4°C, and then stained with CD45-APC efluor780 (eBioscience, clone 30-F11), CD11b-FITC (BD, clone M1/70), and Siglec-F-PE (BD, clone E50-2440) in 1 x PBS for 20 min in the dark at 4°C. After washing, cells were resuspended in FACS buffer for sorting on MoFlo Astrios EQ (Beckman Coulter). CD45+ CD11b+ Siglec-F+ granulocytes were sorted into 1 x PBS with 4% FBS for scRNA-seq.

ScRNA-seq of sorted eosinophils and data analysis

Sorted CD45+ CD11b+ Siglec-F+ granulocytes were subjected to Chromium Single Cell 3′ Solution V.3 (10 x Genomics) library preparation according to the manufacturer's instructions. Libraries were sequenced on a HiSeq 2500 (Illumina) platform to a depth of at least 200 million reads each. Reads were converted to FASTQ format using the mkfastq command from Cell Ranger (V.3.0.1, 10 x Genomics) and mapped to the mouse reference genome (mm10, V.3.0.0, 10 x Genomics) using the count command from Cell Ranger with standard parameters. Low quality cells expressing less than 300 genes or more than 20% mitochondrial genes were removed. Clustering of cells, identification of cluster markers, differential expression analysis and visualisations were performed using the Seurat (V.3.1.0) package for R (V.3.6.1).[32] To compare gene expression between tissues or treatment conditions, individual data sets were combined using the Seurat integration approach for SCT-normalised data. Gene ontology (GO)-term enrichment analysis was performed with the clusterProfiler (V.3.12.0) package.[33] Genes with an adjusted p value<0.05 were assigned as differentially expressed. Genes with an adjusted p value <0.05 were considered for further cellular pathway analysis with ingenuity pathway analysis (IPA, QIAGEN) software.[34]

r/iEos sorting for in vitro analysis

r/iEos were sorted from the blood and BM of IL-5tg/4Get mice. The blood was lysed two times with 10 mL RCL buffer. BM cells were flushed out from the femur and tibia with 1 x PBS using 1 mL syringe and 27 G needle. Cells from blood and BM were pooled and put through 40 µm cell strainers. Cells were stained with CD45-APC efluor780 (eBioscience, clone 30-F11), CD125-APC (Miltenyi, clone REA343), and Siglec-F-PE (BD, clone E50-2440) in 1 x PBS for 20 min in the dark at 4°C. After washing, cells were resuspended in FACS buffer for sorting on MoFlo Astrios EQ (Beckman Coulter). CD45+ CD125 int Siglec-F int granulocytes were sorted as rEos, CD45+ CD125 int Siglec-F high granulocytes were sorted as iEos into 1 x PBS with 4% FBS for further analysis.

Transmission electron microscopy (TEM)

Sorted r/iEos were washed with 1 x PBS and fixed with 2.5% glutaraldehyde (Carl Roth) in 0.1 M phosphate buffer for at least 48 hours. Thereupon, cells were postfixed in 2% buffered osmium tetroxide (Carl Roth) for 2 hours, and then dehydrated in graded alcohol concentrations and embedded in epoxy resin according to the standard protocol. For orientation, 1 µm semithin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a TEM 906E (Carl Zeiss). Quantification of granules, vesicles and mitochondria was performed with imageJ.

Extracellular flux assay using the Seahorse platform (Agilent)

For metabolic analyses of r/iEos, the distinct eosinophil subtypes were sorted from the blood and BM of IL-5tg/4Get mice. For the Glycolysis Stress Test, Seahorse XF RPMI Medium (Agilent Technologies) was supplemented with 2 mM L-glutamine and for the Mito Stress Test with 10 mM glucose, 1 mM pyruvate and 2 mM L-glutamine (all from Agilent Technologies). iEos and rEos were resuspended in Glyco Assay Medium or Mito Assay Medium and were plated in a concentration of 2×10^5 cells/ 180 μL on Seahorse XF96 Cell Culture Microplates (Agilent Technologies) precoated with adhesive solution (Agilent Technologies) according to the manufacturer's instructions. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) using final concentrations of 10 mM glucose, 2 µM oligomycin and 50 mM 2-deoxy-D-glucose (ECAR) or 2 µM oligomycin, 2 µM carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, 1 µM rotenone and 1 µM antimycin A (OCR) (all from MilliporeSigma) were measured in a 96-well XF Extracellular Flux Analyzer (Agilent Technologies) according to the manufacturer's instructions. Data were obtained with the Seahorse Wave Desktop Software (Agilent Technologies) and changes in ECAR and in OCR, in response to the mentioned compounds, were used to calculate glycolytic function and mitochondrial respiration parameters using Microsoft Excel.

Coculture of BMDMs with r/iEos or r/iEos supernatant

BMDMs were generated from Balb/c BM cells by incubating them for 7 days in macrophage differentiation medium composed of dulbecco's modified Eagle's medium (DMEM) medium (Gibco) with 10% FBS (Gibco), 1% PS (Gibco), 1 x MEM non-essential amino acid solution (MilliporeSigma), 50 µM 2-mercaptoethanol (Gibco) and 15% L929 conditioned medium. After differentiation, the cells were plated in 96-well plates at a concentration of 1×10^6 cells/mL in macrophage culture medium, composed of RPMI medium (Gibco) with 10% FBS, 2 mM L-glutamine (Gibco) and 1% PS, overnight at 37°C, 5.5% CO_2. Adherent cells were stimulated for 4, 8, 12 or 24 hours with 100 ng/mL lipopolysaccharides (LPS)/MilliporeSigma), 10 ng/mL IL-4 (BioLegend), or were cocultured with sorted r/iEos (2×10^6 cells/mL), or 35% (v/v) supernatant from r/iEos, generated after culturing r/iEos for 48 hours in RPMI medium with 10% FBS, 1% PS and 10 ng/mL IL-5 (BD).

Confocal imaging with human synovial slides

Epitopes were retrieved from deparaffinised sections using a heat-induced method. Briefly, sections were alternatively bathed

in boiling Citrate buffer (10 mM citric acid monohydrate, pH 6.0) and Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA, 0.05% Tween-20, pH 9.0). Each bathing step was repeated five times for 2 min each. After washing with 1 x PBS, sections were blocked first for endogenous biotin with the Endogenous Biotin-Blocking Kit (Thermo Fisher) according to the manufacturer's instructions, and then with 2.5% goat serum in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonicacid (HEPES) at room temperature (RT) for 1 hour. Sections were incubated with primary antibody for eosinophil–peroxidase (EPX) (mouse/Abcam/AHE-1/1:200), EAR1/NR1D1 (rabbit/MilliporeSigma/1:20) and CD68 (biotin/GeneTex/FA-11/1:100) in 2.5% goat serum in 10 mM HEPES overnight at 4°C. After washing in 1 x PBS, sections were incubated with the secondary antibodies Alexa Fluor 555 goat anti-mouse IgG (Abcam/1:200), DyLight 650 goat anti-rabbit IgG (Abcam/1:50) and Alexa Fluor 488 rat anti-streptavidin (BioLegend/1:50) in 10 mM HEPES at RT for 3 hours. After washing with 1 x PBS, sections were mounted with Fluoroshield with 4′,6-diamidino-2-phenylindole (DAPI)/MilliporeSigma) and covered with coverslips. Images were acquired with a Leica TCS SP 5 II confocal microscope with acousto-optic tunable filter and acousto-optical beam splitter, and equipped with photomultiplier tubes (PMTs) and hybrid detectors (HyD) on a DMI6000 CS frame. Representative images were generated with an HCX PL APO CS 63.0×1.30 GLYC 21°C UV objective. For quantification, two random fields within the synovial tissue were visualised by tile scan (4×4 ~ 0.96 mm^2) with an HCX PL APO 40×1.25–0.75 Oil objective. Fluorescence signals were generated via three sequential scans. In the first imaging sequence DAPI and DyLight 650 were simultaneously excited with a 405 nm diode laser and a 633 nm helium–neon laser, respectively. DAPI was detected with PMT at 413–480 nm and DyLight 650 signals were detected with HyD at 650–750 nm. The second sequence for detecting Alexa Fluor 488 used an argon laser at 488 nm for excitation and an HyD detector at 496–560 nm. A third imaging sequence involved an excitation of Alexa Fluor 555 with a 514 nm argon laser and its detection with PMT at 560–650 nm. Generated images were deconvoluted with Huygens Professional and processed with Imaris software. Quantification of EPX+ EAR1− and EPX+ EAR1+ cells was performed with ImageJ.

RNA isolation and real-time polymerase chain reaction (PCR)

Mouse ankle joints were first homogenised using the Precellys Lysing Kit (Bertin corp.) on Precellys 24 (VWR Peqlab). Then, the total ankle joint RNA was extracted using RNAPure peqGOLD (VWR Peqlab) according to the manufacturer's instructions. RNA from stimulated or cocultured macrophages was extracted by using TRIfast peqGOLD (VWR Peqlab) according to the manufacturer's instructions. Extracted RNA was freed from genomic DNA using the DNase I Kit (Thermo Scientific) and reversely transcribed into cDNA using the high capacity cDNA Reverse Transcription Kit (AppliedBiosystems). Real-time PCR was performed using Takyon ROX SYBR 2X MasterMix dTTP blue (Eurogentec) on CFX96TM Real-Time System (Bio-Rad) with the primers listed in the online supplemental table 1. Gene expression was normalised with Actb.

Statistical analysis

All statistical analyses were performed using GraphPad Prism Software V.8. Data were presented as mean±SEM. Statistical significance was calculated by two-tailed Student's t-test (Gaussian distribution) or Mann-Whitney test (not normally distributed) for two-group comparison and one-way analysis of variance (ANOVA) (Gaussian distribution), Kruskal-Wallis test (not normally distributed) or two-way ANOVA for multiple comparisons. Statistical details (eg, number of samples/group, number of independent experiments) can be found in the figure legends. P values less than 0.05 were considered statistically significant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

RESULTS

Asthmatic responses trigger resolution of inflammatory arthritis

To evaluate whether an asthmatic attack can elicit resolution of arthritis, we challenged OVA-triggered asthmatic mice[27] with K/BxN SIA.[28] Therefore, 6 weeks old Balb/c mice were sensitised with an OVA/Alum (100 µg) mixture by intraperitoneal route, 20 and 13 days before induction of arthritis by K/BxN serum transfer (day 0). Then, mice were challenged with OVA allergen (50 µg) intranasally at day –2, –1 and 0 and again at day 4, 5 and 6 to trigger an asthmatic attack (figure 1A). As shown in figure 1B–D, the lungs of OVA-treated mice presented the full pattern of allergic asthma with and without induction of SIA. Both OVA and OVA/SIA-treated animals displayed strong eosinophilia, characterised by high numbers of eosin-red-stained eosinophils in the BALF (figure 1B). Moreover, the lungs showed strong inflammation accompanied by infiltration of ILC2s (Lineage− ICOS+ KLRG1+) and eosinophils (CD45+ Ly6G− CD11b+ Siglec-F+) (figure 1B–D and online supplemental figure S1A,B). As expected, the OVA and OVA/SIA-challenged mice showed increased IgE serum levels (figure 1E). While IL-4 and IL-13 serum levels were not increased, systemic IL-5 levels were specifically upregulated in the two OVA-treated groups (figure 1F). These observations show that eosinophilic asthma developed in both OVA and OVA/SIA-treated animals exhibiting characteristic features of lung inflammation.

Interestingly, asthma led to a rapid resolution of arthritis after the second OVA challenge (figure 1G). Following the whole course of SIA, both groups were able to fully resolve the disease 20 days post serum transfer (online supplemental figure S1C). However, asthmatic response led to an earlier initiation of the resolution phase, associated with less tissue destruction (online supplemental figure S1C,D). Furthermore, asthma was able to reduce inflammation, bone erosion and the number of osteoclasts in the affected joints (figure 1H,I). This effect was accompanied by decreased expression of inflammatory cytokine genes such as Il1b and Il6, and osteoclast-related genes such as Fos and Mmp9 in the synovium of the asthmatic mice (online supplemental figure S1E,F).

To delineate the cellular composition in the synovium, proinflammatory and anti-inflammatory myeloid cells were quantified by flow cytometry in OVA, SIA and OVA/SIA mice. Indeed, neutrophil (CD45+ CD11b+ Ly6G+) infiltration was decreased in the OVA/SIA group compared with SIA mice (figure 1J and online supplemental figure S2A,B). Moreover, the asthmatic response favoured the switch from classical proinflammatory macrophages (MΦ/CD45+ Ly6G− Siglec-F− CD11b+ F4/80+ MHCII+) to proresolving AAMs (CD45+ Ly6G− Siglec-F− CD11b+ F4/80+ MHCII−) in the affected joints (figure 1K and online supplemental figure S2A,C). In accordance, lower expression of the classically activated MΦ gene Nos2 and higher expression of the AAM genes Cd163 and Cd206 could be observed in the synovium of OVA-challenged mice (figure 1L). Since the hallmark of allergic asthma is the expansion of eosinophils, we also investigated the presence of these cells in the

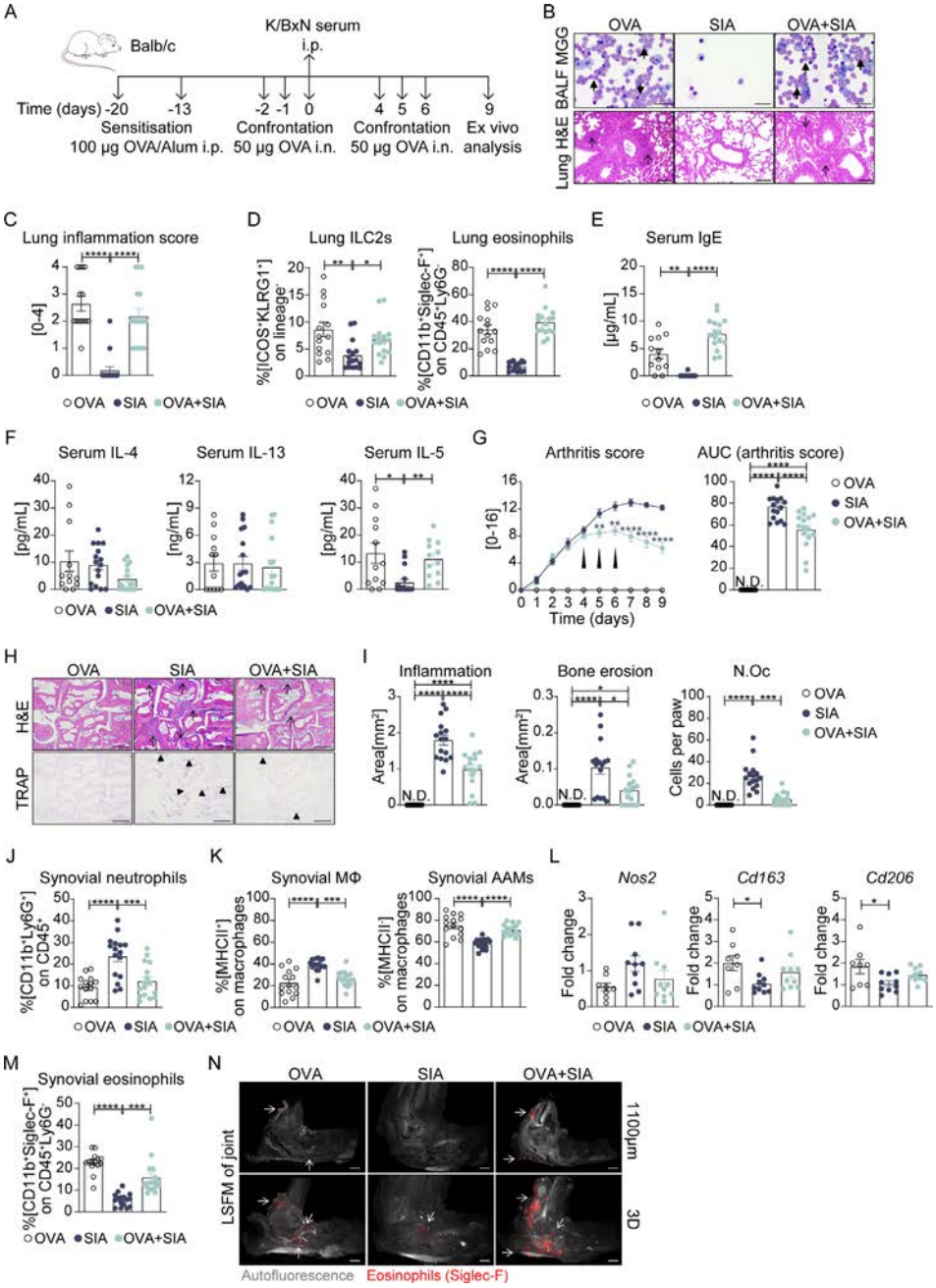

Figure 1 Ovalbumin (OVA)-induced asthma initiates resolution of inflammatory arthritis. (A) Experimental outline of OVA-induced asthma with K/BxN serum-induced arthritis (SIA) in Balb/c wildtype mice. i.n., intranasal; i.p., intraperitoneal. (B,C) Representative bronchoalveolar lavage fluid (BALF) May-Grünwald-Giemsa (MGG) staining and lung H&E staining (B), semiquantification of lung inflammation (C) of OVA, SIA and OVA+SIA groups (n=14–16/group) at day 9 post SIA. Bold arrows indicate eosinophils; thin arrows illustrate inflammation. Scale bar 40 and 100 µm, respectively. (D) Frequency of lung ILC2s (ICOS+ KLRG1+ gated on lineage– cells) and lung eosinophils (CD11b+ Siglec-F+ gated on CD45+ Ly6G– cells) analysed by flow cytometry in the afore-mentioned three groups (n=14–16/group) at day 9 post SIA. See also online supplemental figure S1A,B. (E,F) Serum IgE levels (E) and serum interleukin (IL)-4, IL-13 and IL-5 levels (F) at day 9 post SIA in OVA, SIA and OVA+SIA groups (n=12–16/group). (G) Arthritis score and area under the curve (AUC) of arthritis score of OVA, SIA and OVA+SIA groups (n=14–16/group) during the course of SIA. (H,I) Representative hind paw H&E and tartrate-resistant acid phosphatase (TRAP) staining (H) and quantification of hind paw inflammation area, bone erosion area and number of osteoclasts per paw (N. Oc) (I) at day 9 post SIA in OVA, SIA and OVA+SIA groups (n=14–16/group). Arrows indicate inflammation; triangles illustrate bone erosion. Scale bar 500 µm. (J,K) Frequency of synovial neutrophils (CD11b+ Ly6G+ gated on CD45+ cells) (J), synovial classical macrophages (MΦ/ MHCII+ gated on CD45+ Ly6G– Siglec-F– CD11b+ F4/80+ cells), and alternatively activated macrophages (AMMs/ MHCII– gated on CD45+ Ly6G– Siglec-F– CD11b+ F4/80+ cells) (K) at day 9 post SIA in OVA, SIA and OVA+SIA groups (n=14–16/group) analysed by flow cytometry. See also online supplemental figure S2A–C. (L) mRNA expression of Nos2, Cd163 and Cd206 in the synovial tissue of OVA, SIA and OVA+SIA groups (n=8–10/group), 9 days post SIA. (M) Frequency of synovial eosinophils (CD11b+ Siglec-F+ gated on CD45+ Ly6G– cells) at day 9 post SIA in OVA, SIA and OVA+SIA groups (n=14–16/group) analysed by flow cytometry. See also online supplemental figure 2A,D. (N) Light sheet fluorescence microscopy (LSFM) of eosinophils (Siglec-F/red) in the ankle joints (autofluorescence/grey) of OVA, SIA and OVA+SIA groups, 9 days post SIA. White arrows illustrate eosinophils. Scale bar 500 µm. Data are shown as mean±SEM. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

synovium. Flow cytometry analyses revealed a strong increase of eosinophils (CD45+ Ly6G− CD11b+ Siglec-F+) in the joints of OVA/SIA mice (figure 1M and online supplemental figure S2A,D). Interestingly, even in non-arthritic mice, OVA challenge induced eosinophils in the joints (figure 1M and online supplemental figure S2A,D). Besides, LSFM clearly demonstrated an accumulation of eosinophils (Siglec-F+/red) in the joints on asthmatic response (figure 1N and online supplemental videos 1–3). Taking together, these results show that the proresolving impact of asthma on arthritis is associated with expansion of eosinophils in the affected joints.

Eosinophils are essential for asthma-induced resolution of arthritis

To determine the importance of eosinophils in asthma-induced resolution of arthritis, the combined asthma/arthritis model was applied to eosinophil-deficient (ΔdblGATA) mice.[24] According to the literature,[35] eosinophil-deficient mice were not protected from asthma manifestation, as shown by preserved lung inflammation and unchanged infiltration of ILC2s into the lungs (figure 2A–D). Moreover, ΔdblGATA mice presented similar IgE serum levels as WT mice on OVA challenge (figure 2E). Strikingly, mice without eosinophils developed more severe arthritis that did not resolve on asthma induction (figure 2F). As expected, OVA challenge only reduced inflammation and bone erosion in WT mice, but not in the eosinophil-deficient animals (figure 2G,H). A partial decrease of osteoclast numbers could be observed in ΔdblGATA mice after OVA exposure, but not to the same extent as in WT mice (figure 2G,H). As supposed, eosinophils were absent in the joints of ΔdblGATA mice (figure 2I). Furthermore, neutrophils, classical macrophages and AAMs were analysed in the synovium by flow cytometry (figure 2J,K). While no difference could be observed in the frequency of neutrophils, OVA/SIA-treated ΔdblGATA mice no longer presented a shift of classical macrophages to AAMs as it was seen in WT OVA/SIA mice (figure 2J,K). Altogether, these data demonstrate that eosinophils are the main effector cells driving asthma-induced resolution of arthritis.

Lungs and joints possess distinct eosinophil subsets explaining their opposed function

To delineate why OVA-induced eosinophils on the one hand favour asthma in the lungs and on the other hand resolve arthritis in the joints, we performed scRNA-seq of eosinophils isolated from lungs and joints of OVA/SIA-treated animals. Tissues were prepared from OVA/SIA mice, 7 days after induction of arthritis and 1 day after the last OVA challenge. Lungs and ankle joints were digested to generate single cells and viable eosinophils were sorted via CD45+, CD11b+ (to discriminate from alveolar macrophages), Siglec-F+, and were subjected to scRNA-seq analyses (figure 3A and online supplemental figure S3A). Interestingly, integration of joint-derived and lung-derived data followed by unbiased clustering revealed a distinct eosinophil composition in arthritic joints than in asthmatic lungs. In total, eight clusters within the eosinophil compartment could be identified (figure 3B). Among them, clusters 0 and 1 were mainly made up of asthmatic lung eosinophils and cluster 2 was primarily made up of eosinophils from the arthritic joint (figure 3C). Differential gene expression analyses identified that 2229 genes were upregulated and 4534 genes were downregulated in the joint-enriched cluster 2 compared with the lung-enriched clusters 0 and 1 (figure 3D).

GO analyses of the 100 most significantly altered genes showed that these genes were highly associated with inflammatory responses and cell migration (figure 3E). Furthermore, the lung-associated clusters 0 and 1 highly expressed proinflammatory molecules such as neurotoxin (Rnase2a), toll-like receptor 4 (Tlr4) and various chemokines (Ccl24, Ccl8, Cxcl16), while the joint cluster 2 strongly upregulated proresolving mediators such as 15-lypoxygenase (Alox15), 5-lipoxygenase (5-LOX) activating protein (Alox5ap), NFKB inhibitor alpha (Nfkbia) and aconitate decarboxylase 1 (Acod1) (figure 3F,G). In accordance, calculations of affected pathways with IPA software showed that the gene profile of the joint-enriched cluster 2 when compared with the lung-enriched clusters 0 and 1 was associated with inhibition of inflammatory responses, especially joint inflammation (online supplemental figure S3B–D). In summary, asthmatic response triggers a proinflammatory eosinophil subset in the lungs and a proresolving eosinophil subset in the joints.

Previously, it has been described that inflammatory and rEos can be separated by their expression of Siglec-F, whereby iEos are high and rEos are intermediate for Siglec-F.[23] Indeed, our scRNA-seq analyses also showed that the lung-enriched clusters 0 and 1 highly expressed several genes characteristic of iEos such as Acp5, Itgax, Mif, Grn, Slc3a2, Tlr4, Cd33, Il13ra1 and Retnla (figure 3F,H). In contrast, joint-enriched cluster 2 shared many genes with the rEos subset such as Sell, Runx3, Ear1, and to a lesser extend Pon2 and Ldlr (figure 3F,H). We could also show that allergic asthma strongly induced iEos (Siglec-F high) in the lungs, whereas only rEos (Siglec-F int) were upregulated in the arthritic joints (figure 3I,J).

Eosinophils generate and store proinflammatory and regulatory mediators in specific granules. The secretion of these molecules can be selectively regulated and is central for eosinophil function.[36] We performed proteome profile arrays with supernatants of eosinophils isolated from lungs and joints of OVA/SIA-treated animals to investigate their distinctive secretion profile (online supplemental figure S4A,B). In line with the scRNA-seq data, lung eosinophils were mainly characterised by enhanced release of chemokines such as CCL11, CCL17, CCL22 and CXCL16, associated with the stimulation and migration of proinflammatory immune cells.[37] In contrast, synovial eosinophils showed increased secretion of lipocalin-2, CXCL5, MMP-3, MMP-9, osteopontin and serpin E1, being related to tissue remodelling and repair.[38–42] Altogether, these findings reveal that the lung and joint microenvironment induces distinct eosinophil subsets with divergent functional profiles.

rEos and iEos present distinct metabolic profiles and immunological functions

Next, we evaluated the activation state and functionality of rEos (Siglec-F int) versus iEos (Siglec-F high). To isolate enough cells for in vitro studies, we took advantage of IL-5 transgenic, IL-4 reporter mice (IL-4/GFP-enhanced transcript, 4Get) that constantly express these two eosinophil subsets.[25 26] rEos and iEos were isolated by FACS from the BM and blood of IL-5tg/4Get mice, according to their Siglec-F expression (online supplemental figure S4C). May-Grünwald-Giemsa staining of rEos and iEos revealed that these two subtypes presented a distinct differentiation state (online supplemental figure S4D). The segmented nucleus of iEos compared with the donut shaped nucleus of rEos indicates that iEos are further maturated. While the granule number was similar within the cytoplasm of iEos and rEos, only iEos presented sombrero vesicles as shown by TEM, suggesting enhanced granule release (figure 4A,B). These

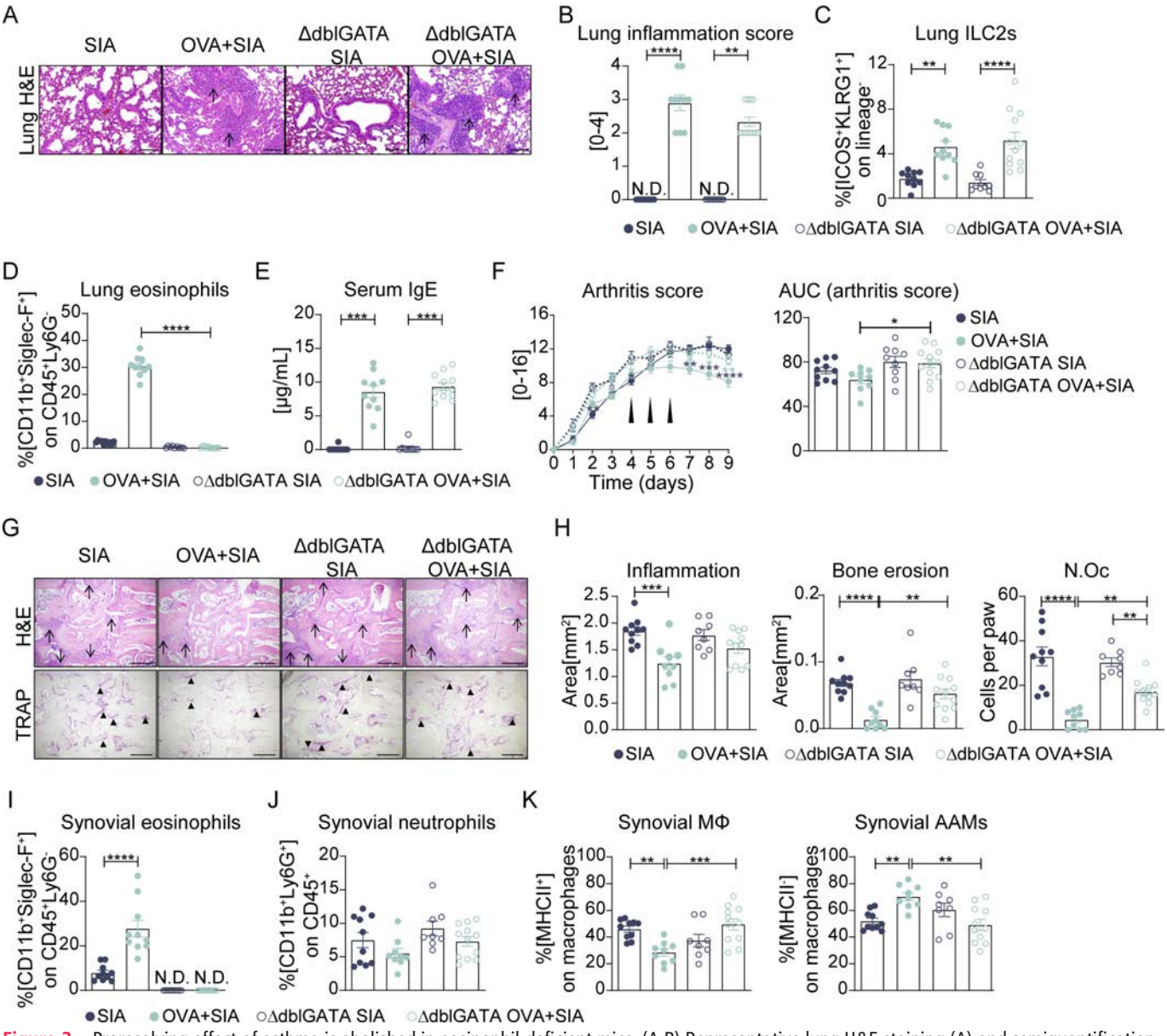

Figure 2 Proresolving effect of asthma is abolished in eosinophil-deficient mice. (A,B) Representative lung H&E staining (A) and semiquantification of lung inflammation (B) in serum-induced arthritis (SIA), ovalbumin (OVA)+SIA, ΔdblGATA SIA and ΔdblGATA OVA+SIA groups (n=9–12/group), 9 days post SIA. Thin arrows indicate inflammation. Scale bar 100 µm. (C,D) Frequency of lung ILC2s (ICOS+ KLRG1+ gated on lineage− cells) (C) and lung eosinophils (CD11b+ Siglec-F+ gated on CD45+ Ly6G− cells) (D) at day 9 post SIA in the afore-mentioned four groups (n=9–12/group) analysed by flow cytometry. (E) Serum IgE levels in SIA, OVA+SIA, ΔdblGATA SIA and ΔdblGATA OVA+SIA groups (n=9–12/group), 9 days post SIA. (F) Arthritis score and area under the curve (AUC) of arthritis score of SIA, OVA+SIA, ΔdblGATA SIA and ΔdblGATA OVA+SIA groups (n=9–12/group) during the course of arthritis. (G,H) Representative hind paw H&E and tartrate-resistant acid phosphatase (TRAP) staining (G) and quantification of hind paw inflammation area, bone erosion area and number of osteoclasts per paw (N. Oc) (H) at day 9 post SIA in SIA, OVA+SIA, ΔdblGATA SIA and ΔdblGATA OVA+SIA groups (n=9–12/group). Arrows indicate inflammation; triangles illustrate bone erosion. Scale bar 500 µm. (I–K) Frequency of synovial eosinophils (CD11b+ Siglec-F+ gated on CD45+ Ly6G− cells) (I), synovial neutrophils (CD11b+ Ly6G+ gated on CD45+ cells) (J), synovial MΦ and synovial alternatively activated macrophages (AAMs) (MHCII+/MHCII− gated on CD45+ Ly6G− Siglec-F− CD11b+ F4/80+ cells) (K) at day 9 post SIA in SIA, OVA+SIA, ΔdblGATA SIA and ΔdblGATA OVA+SIA groups (n=9–12/group) analysed by flow cytometry. Data are shown as mean±SEM. Asterisks mark statistically significant difference (*p<0.05, **p<0.01; ***p<0.001; ****p<0.0001).

findings are in line with the previous characterisation of rEos and iEos.[23]

TEM analysis revealed that rEos possessed enhanced numbers of mitochondria compared with iEos, assuming that the metabolic pathway of these cells could be differentially regulated (figure 4A,B). The metabolic profile of the two Eos subsets was analysed by extracellular flux assays, determining the glycolysis and mitochondrial respiration/oxidative phosphorylation in the cells. The measurement of the ECAR revealed a higher glycolytic activity in iEos than in rEos (figure 4C). In contrast, the energetic supply of rEos relied more on oxidative phosphorylation as shown by the assessment of the OCR (figure 4D), which is in line with the high amount of mitochondria in these cells.

Based on the distinct gene expression signature, morphology and metabolic profile of rEos and iEos, we assumed that these cells also implement different immunological functions. Eosinophils

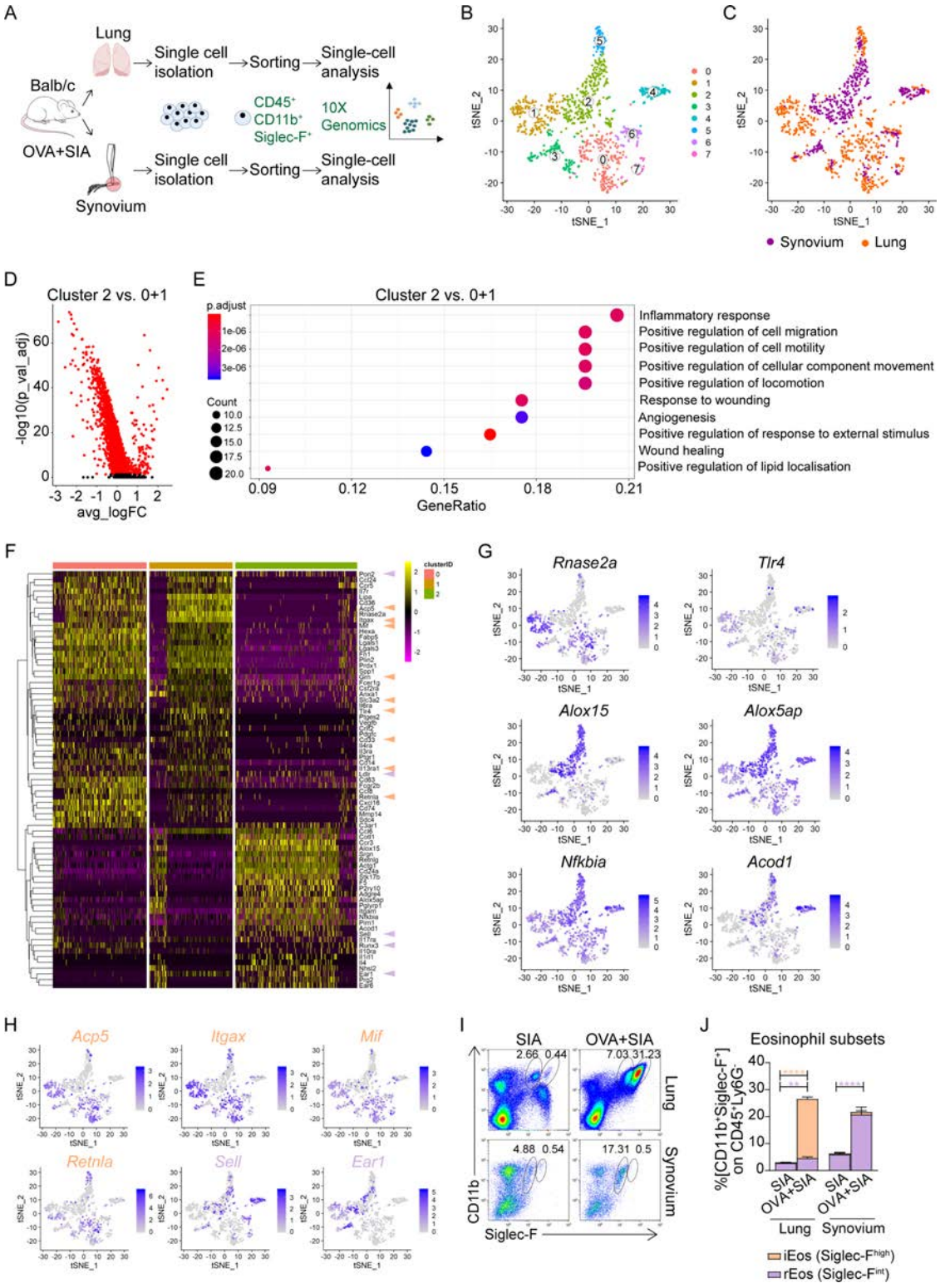

Figure 3 Single-cell RNA sequencing (scRNA-seq) reveals distinct eosinophil subsets in lungs and joint tissue. (A) Scheme of eosinophil isolation for scRNA-seq from the lungs and ankle joints of the ovalbumin (OVA)+serum-induced arthritis (SIA) group (n=3–8/tissue), 7 days post SIA. See also online supplemental figure 3A. (B,C) t-distributed stochastic neighbour embedding (tSNE) of the integrated scRNA-seq data sets from the two tissues identifying eight cell clusters (B) that delineate distinct subpopulations of lung and synovial eosinophils (C). (D,E) Volcano plot (D) and gene ontology enrichment analysis (E) of differentially expressed genes between cluster 2 and clusters 0+1. (F) Heatmap of differentially expressed genes of clusters 0, 1 and 2. (G) tSNE plots showing the expressional distribution of Rnase2a, Tlr4, Alox15, Alox5ap, Nfkbia and Acod1. (H) tSNE plots showing the expressional distribution of Acp5, Itgax, Mif, Retnla, Sell and Ear1. (I,J) Representative density plots (I) and percentage (J) of inflammatory eosinophils (iEos/ CD11b+ Siglec-F high gated on CD45+ Ly6G– cells) and regulatory eosinophils (rEos/CD11b+ Siglec-F int gated on CD45+ Ly6G– cells) analysed at day 9 post SIA in the lungs and synovium of SIA and OVA+SIA groups (n=9–10/group). Genes with an adjusted p value<0.05 were assigned as differentially expressed. Adjusted p value<0.05 were considered significantly enriched. Data are shown as mean±SEM. Asterisks mark statistically significant difference (**p<0.01; ****p<0.0001).

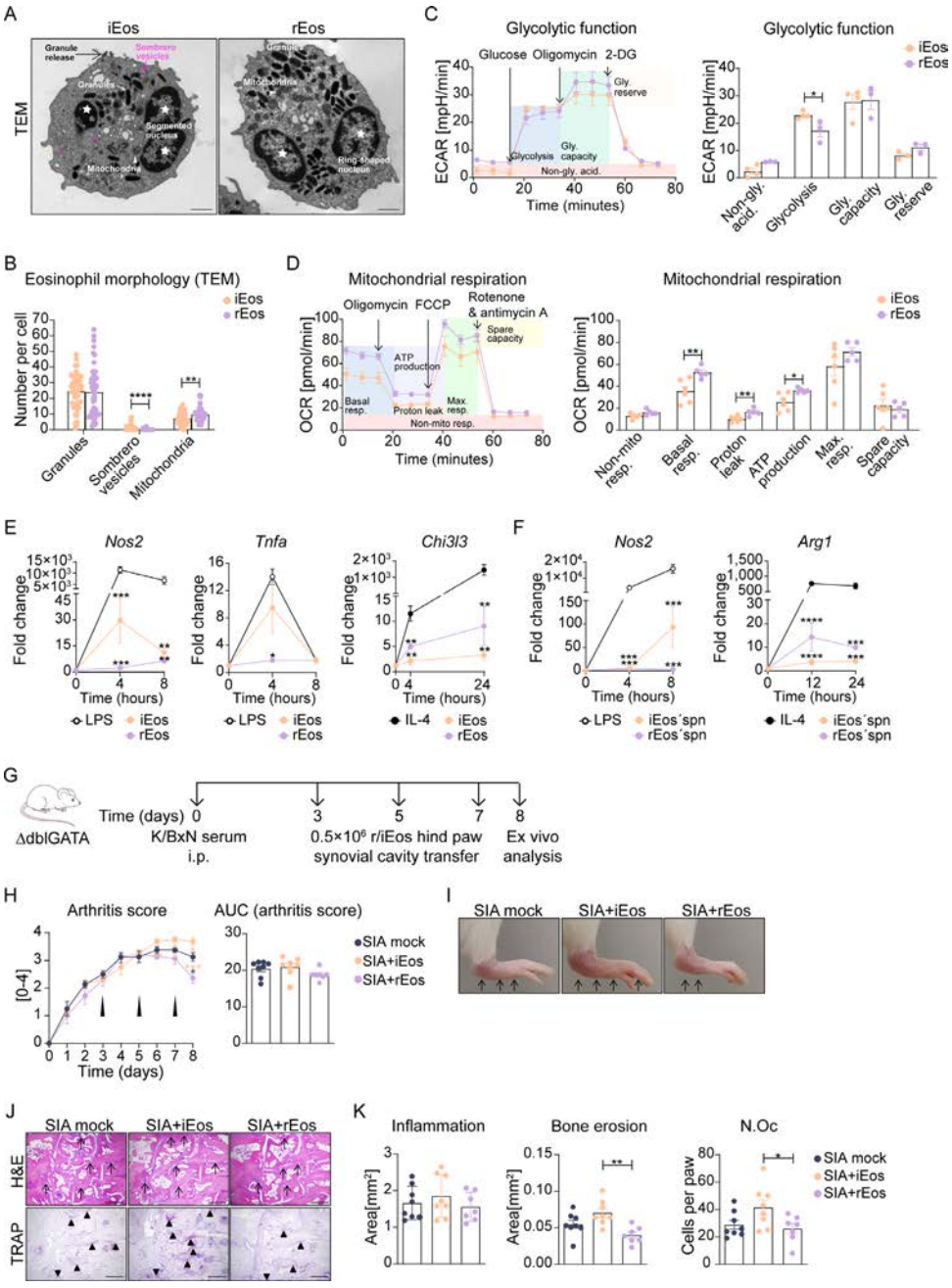

Figure 4 rEos and iEos possess distinct metabolic profiles and immunological functions. (A,B) Representative transmission electron microscopy (TEM) images (A) and quantification of granules, sombrero vesicles and mitochondria (B) of rEos (Siglec-F int) and iEos (Siglec-F high) (n=51/sample) sorted from the blood and bone marrow (BM) of IL-5tg/4Get mice. White arrows indicate granules; magenta arrows show sombrero vesicles; white triangles illustrate mitochondria; stars mark the nucleus. Scale bar 1 µm. See also online supplemental figure 4C. (C) Extracellular acidification rate (ECAR) profile plot in cells injected with glucose, oligomycin, and 2-deoxy-D-glucose (2-DG) and glycolytic function parameters, analysed by extracellular flux assay in rEos and iEos sorted from the blood and BM of IL-5tg/4Get mice (n=3–4/sample, representative of three independent experiments). (D) Oxygen consumption rate (OCR) profile plot in cells injected with oligomycin, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and rotenone and antimycin A and mitochondrial respiration parameters, analysed by extracellular flux assay in rEos and iEos sorted from the blood and BM of IL-5tg/4Get mice (n=5–6/sample, representative of three independent experiments). (E) mRNA expression of Nos2, Tnfa, and Chi3l3 in macrophages cocultured for 0, 4, 8 and 24 hours with iEos (2:1) or rEos (2:1) compared with 100 ng/mL lipopolysaccharides (LPS) or 10 ng/mL IL-4 stimulations (n=3/sample, representative of three independent experiments). (F) mRNA expression of Nos2 and Arg1 in macrophages stimulated for 0, 4, 8, 12 and 24 hours with supernatant from iEos (1:3) or rEos (1:3) compared with 100 ng/mL LPS or 10 ng/mL IL-4 stimulations (n=3/sample, representative of three independent experiments). (G) Experimental design of rEos and iEos injection into the synovial cavity of ΔdblGATA mice during the course of serum-induced arthritis (SIA). (H) Arthritis score and area under the curve (AUC) of arthritis score of ΔdblGATA SIA mock, ΔdblGATA SIA+iEos and ΔdblGATA SIA+rEos groups (n=7–8/group). (I–K) Representative images of the hind paws (I), representative hind paw H&E and tartrate-resistant acid phosphatase (TRAP) staining (J) and quantification of hind paw inflammation area, bone erosion area and number of osteoclasts per paw (N. Oc) (K), 9 days post SIA of ΔdblGATA SIA mock, ΔdblGATA SIA+iEos and ΔdblGATA SIA+rEos groups (n=7–8/group). Arrows indicate inflammation; triangles show bone erosion. Scale bar 500 µm. Data are shown as mean±SEM. Asterisks mark statistically significant difference (*p<0.05; **p<0.01, ***p<0.001, ****p<0.0001).

have been reported to play a fundamental role in the priming of proresolving AAMs.[15] On asthmatic response, we observed a clear shift of proinflammatory classical macrophages to AAMs in the affected joints (figure 1K). To compare the potential of rEos with iEos to guide macrophage polarisation, we performed in vitro coculture experiments of macrophages with rEos and iEos. In addition, macrophages were stimulated with rEos and iEos supernatant. IL-4 and LPS stimulations were used as positive controls for the formation of AAMs and classical MΦ, respectively. Interestingly, these analyses revealed that rEos as well as their supernatant were able to trigger the generation of AAMs, measured by upregulated gene expression of the AAM genes, chitinase-like 3 (Chi3l3) and arginase1 (Arg1) (figure 4E,F). By contrast, iEos and their supernatant led to the formation of classical MΦ, analysed by an increased mRNA expression of nitric oxide synthase 2 (Nos2) and TNFα (Tnfa) in stimulated macrophages (figure 4E,F). These data suggest that the switch into a regulatory macrophage population in the joints during the asthmatic response is mediated by rEos.

To further analyse the functionality of the two Eos subsets, rEos and iEos were injected into the synovial cavity of eosinophil-deficient (ΔdblGATA) mice during the course of arthritis at day 3, 5 and 7 post serum transfer. The mice were analysed at day 8 post SIA induction (figure 4G). As shown in figure 4H,I, only rEos were able to trigger an early resolution of SIA. Conversely, iEos even seem to enhance SIA inflammation as demonstrated in the arthritis score and the representative images of the hind paws (figure 4H,I). Histological analyses showed that rEos had the tendency to reduce inflammation, bone erosion and the number of osteoclasts in the affected joints (figure 4J,K). These results indicate that the rEos subset is able to induce resolution of arthritis, while iEos show the opposite effect by exacerbating inflammatory responses.

ILC2s trigger arthritis resolution through the induction of rEos in the joints

During an asthmatic response, eosinophils are induced primarily by ILC2s.[10] We could already show a strong upregulation of ILC2s in the lungs during allergic asthma (figure 1D). Thus, we tested whether ILC2s, known to have proresolving action in arthritis,[17] were responsible for the activation of rEos in the arthritic joints. To do so, ILC2s were induced in WT and eosinophil-deficient (ΔdblGATA) mice by HDI of IL-25 and IL-33 plasmids, 5 days post serum transfer. At day 9 post serum transfer, we performed the ex vivo analyses (figure 5A). We could confirm an increased abundance of ILC2s on overexpression of the cytokines IL-25 and IL-33 in WT and eosinophil-deficient mice (figure 5B). ILC2 increase was accompanied by enhanced SIA resolution in WT mice (figure 5C). Remarkably, the induction of ILC2s could not alter the course of arthritis in eosinophil-deficient animals (figure 5C). Histological analysis further confirmed that eosinophils were necessary for ILC2-mediated resolution of SIA (figure 5D,E). As expected, ILC2 expansion in WT mice was linked to a strong upregulation of rEos in the inflamed joints as shown by flow cytometry and LSFM (figure 5F,G). Interestingly, serum IL-5 levels were strongly upregulated on ILC2 boost, showing even higher concentrations in ΔdblGATA mice compared with WT mice (figure 5H). These data show that ILC2s induce resolution of arthritis in an eosinophil-dependent manner probably by the secretion of IL-5.

Eosinophil maturation, activation and survival are dependent on IL-5.[43] We could already demonstrate that allergic asthma was leading to a systemic upregulation of IL-5, while the other type 2-related cytokines IL-4 and IL-13 remained unchanged (figure 1F). To investigate in which compartment IL-5 is predominantly expressed in the OVA/SIA-treated mice, we used IL-5 reporter mice, expressing tdTomato under the control of the IL-5 promotor. Flow cytometry analyses revealed that IL-5-producing cells expanded drastically in the lungs after the second OVA challenge at day 6, and stayed high also at day 9 post serum transfer (figure 5I,J). However, IL-5-expressing cells were almost undetectable in the blood as well as in the synovium of OVA/SIA-treated animals, suggesting that IL-5 is primary produced in the lungs, and then acts systemically through the circulation (figure 5I,J). The analyses of IL-5-expressing cell subsets in the lungs demonstrated that ILC2s are the main producers of IL-5 when compared with CD4+ T cells or all other IL-5-producing cells (figure 5K). These findings illustrate that during allergic asthma, lung ILC2s secrete IL-5, driving the activation and expansion of rEos in the arthritic joints.

Regulatory phenotype of joint-resident eosinophils is enhanced by IL-5-mediated priming

Next, we characterised whether rEos infiltrate into the synovium from other tissue compartments such as lungs, BM and blood, or proliferate within the synovium on activation of the ILC2–IL-5 axis. In fact, rEos were also upregulated in the lungs, BM and blood after OVA challenge, suggesting a possible infiltration of rEos in the joints from the circulation (figure 5L,M and online supplemental figure S5). Nonetheless, Ki67 staining revealed that rEos possessed the ability to directly proliferate within the arthritic joints, and the proliferating ability was enhanced on OVA treatment (figure 5N), indicating that the increase of rEos likely results from enhanced infiltration and proliferation.

Then, we delineated whether joint-resident eosinophils from the beginning possess regulatory functions or whether asthmatic challenge is necessary to prime the proresolving potential of these cells. Thus, synovial eosinophils from SIA and OVA/SIA mice were compared by scRNA-seq (sorting strategy in online supplemental figure S6A). Integration of the data from the two treatments followed by unbiased clustering identified six individual subclusters within the synovial eosinophil compartment (online supplemental figure S6B). However, OVA-challenged and unchallenged synovial eosinophils were distributed equally among these six clusters (online supplemental figure S6C). Nonetheless, differential gene expression analyses revealed that 32 genes were upregulated and 200 genes were downregulated in OVA/SIA-challenged eosinophils (online supplemental figure S6D). Interestingly, several proinflammatory genes were decreased in synovial eosinophils on asthmatic response such as Cd33, Il13ra1 and Il6ra, whereas a number of regulatory genes such as Alox15 and Retnlg were increased (online supplemental figure 6E,F). Yet, synovial eosinophils from SIA and OVA/SIA mice largely displayed an overlapping expression pattern in contrast to eosinophils from lungs and joints in OVA/SIA-challenged animals. Accordingly, rEos were specifically increased in the synovium during SIA without additional trigger (online supplemental figure S6G). These data suggest that joint-resident eosinophils initially possess a regulatory phenotype, which is further enhanced by IL-5-mediated priming.

Neutralisation of IL-5 blocks OVA-induced resolution of arthritis

To clarify the importance of systemic IL-5 for the rEos-triggered resolution of RA, we blocked IL-5 with a monoclonal antibody (anti-IL-5) during the course of arthritis in SIA and OVA/

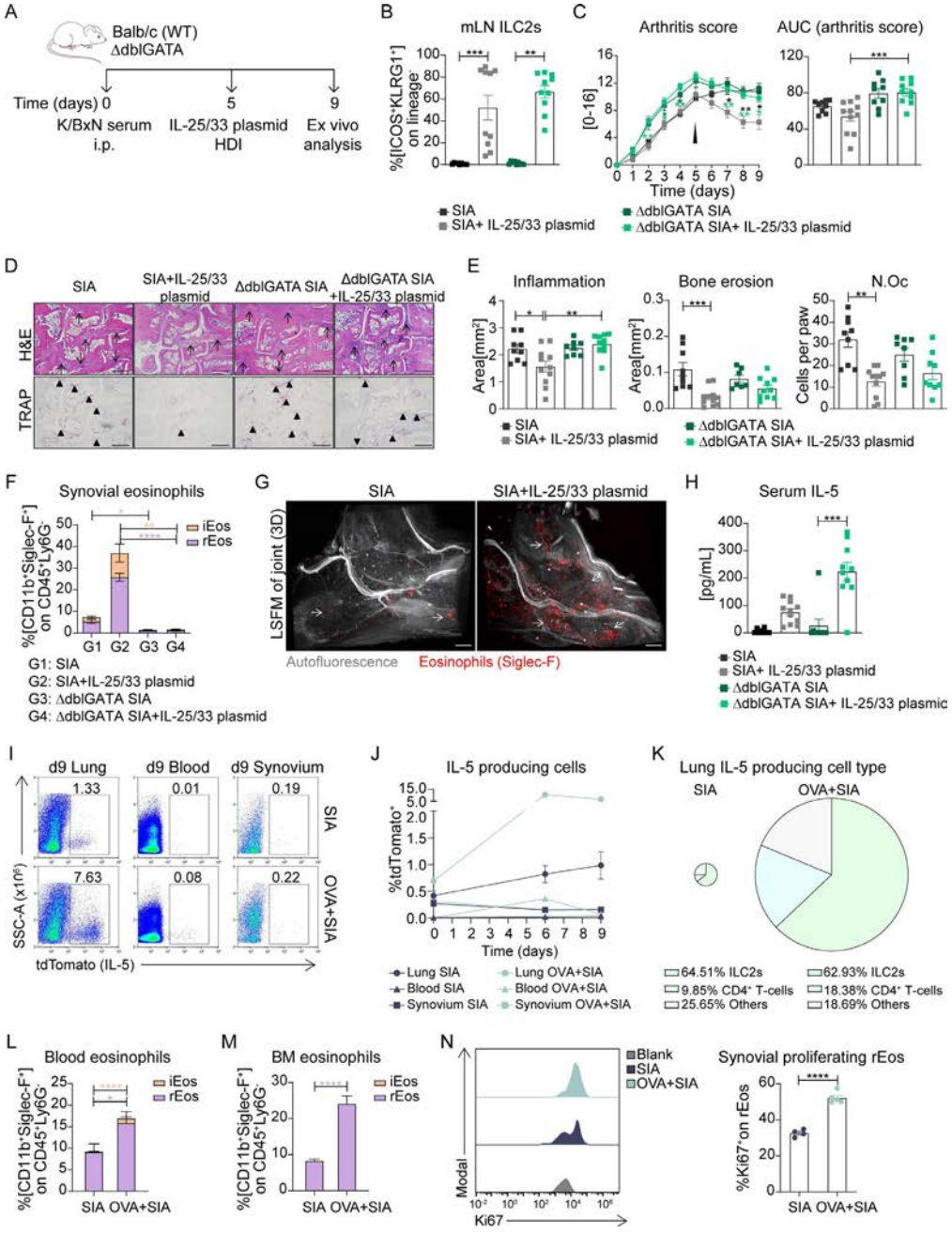

Figure 5 ILC2–interleukin (IL)-5 axis mediates resolution of arthritis through the induction of rEos in the joints. (A) Experimental outline of hydrodynamic injection (HDI) of IL-25/IL-33 plasmids at day 5 of serum-induced arthritis (SIA) in wildtype (WT) and ΔdblGATA mice. i.p., intraperitoneal. (B) Frequency of ILC2s (ICOS+ KLRG1+ gated on lineage− cells) in the mesenteric lymph nodes, 9 days post SIA in SIA, SIA+IL-25/33 plasmid, ΔdblGATA SIA and ΔdblGATA SIA+IL-25/33 plasmid groups (n=9–10/group) analysed by flow cytometry. (C) Arthritis score and area under the curve (AUC) of arthritis score of the afore-mentioned four groups (n=9–11/group) during the course of arthritis. (D,E) Representative hind paw H&E and tartrate-resistant acid phosphatase (TRAP) staining (D) and quantification of hind paw inflammation area, bone erosion area and number of osteoclasts per paw (N. Oc) (E), 9 days post SIA of SIA, SIA+IL-25/33 plasmid, ΔdblGATA SIA and ΔdblGATA SIA+IL-25/33 plasmid groups (n=7–11/group). Arrows indicate inflammation; triangles show bone erosion. Scale bar 500 µm. (F) Frequency of synovial iEos (CD11b+ Siglec-F high gated on CD45+ Ly6G− cells) and rEos (CD11b+ Siglec-F int gated on CD45+ Ly6G− cells) in the afore-mentioned four groups (n=9–10/group), 9 days post SIA, analysed by flow cytometry. (G) Light sheet fluorescence microscopy (LSFM) of eosinophils (Siglec-F/red) in the ankle joints (autofluorescence/grey) in SIA and SIA+IL-25/33 plasmid groups, 9 days post SIA. White arrows illustrate eosinophils. Scale bar 500 µm. (H) Serum IL-5 levels in SIA, SIA+IL-25/33 plasmid, ΔdblGATA SIA and ΔdblGATA SIA+IL-25/33 plasmid groups (n=8–11/group), 9 days post SIA. (I,J) Representative pseudo colour plots (I) and percentage (J) of tdTomato (IL-5)+ cells in the lungs, blood and synovium of IL-5 reporter mice induced with SIA compared with ovalbumin (OVA)+SIA (n=3–6/group) at day 0, 6 and 9 of SIA, analysed by flow cytometry. (K) Percentage distribution of tdTomato (IL-5)+ cells among ILC2s, CD4+ T cells and others in SIA and SIA+OVA groups (n=3/group, representative of three independent experiments), 9 days post SIA, analysed by flow cytometry. (L,M) Frequency of iEos and rEos in the blood (L) and bone marrow (M) of SIA and OVA+SIA groups (n=14–15/group), 9 days post SIA, analysed by flow cytometry. (N) Representative histogram and percentage of Ki67-stained rEos in the synovium of SIA and OVA+SIA groups (n=4–5/group), 9 days post SIA, analysed by flow cytometry. Data are shown as mean±SEM. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

SIA-treated mice (figure 6A). Lung inflammation, lung ILC2 infiltration and serum IgE levels were not altered in OVA/SIA mice after IL-5 inhibition compared with the treatment with an isotype control antibody, while the induction of iEos in the lungs was strongly diminished (figure 6B–E). Interestingly, IL-5 inhibition blocked resolution of arthritis elicited by allergic asthma (figure 6F). Histological analyses demonstrated that OVA/SIA mice injected with anti-IL-5 fully developed inflammation, bone erosion and osteoclasts in the joints (figure 6G,H). This failure of resolution correlated with suppressed expansion of rEos in the arthritic joints after IL-5 inhibition (figure 6I). These findings support that asthma-induced upregulation of rEos in the inflamed synovium is dependent of IL-5.

Resolution of arthritis by asthma depends on the asthma endotype

Asthma is a very heterogeneous disease and is divided into several endotypes. The major distinction is between neutrophilic and eosinophilic asthma, which possess independent causal pathways and cytokine profiles (Th1/Th17 and Th2, respectively).[44] Therefore, we investigated the impact of a Th17-driven asthmatic disease on the course of inflammatory arthritis. To do so, a house dust mite (HDM)-induced lung inflammation model[45] was combined with K/BxN SIA. Six weeks old Balb/c mice were repeatedly sensitised intranasally with HDM (25 µg) before induction of arthritis (day −20 to −16, day −13 to −9 and day −6 to −2). At day 0, mice were induced for SIA by K/BxN serum and then rechallenged with HDM (25 µg) during the course of arthritis from day 1 to 5 after serum transfer (online supplemental figure S7A). Comparable to the OVA-triggered asthmatic reaction, HDM exposure led to a robust inflammation in the lungs (online supplemental figure S7B,C). However, ILC2s and IL-5-producing cells were not increased in the lungs on HDM challenge (online supplemental figure S7D,E). Although significantly upregulated numbers of lung eosinophils could be detected on HDM administration, the percentage was much lower than in OVA-triggered asthmatic response (5% and 40%, respectively) (online supplemental figure S7F). Successful induction of a Th17-mediated asthmatic disease was confirmed by elevated numbers of lung Th17 cells (CD4+ IL-17A+) in HDM and HDM/SIA-treated groups compared with SIA mice, while lung Th1 cells (CD4+ IFNγ+) were unchanged and lung Th2 cells (CD4+ IL-4+) were even reduced after HDM challenge (online supplemental figure S7G). In sharp contrast to the OVA model, HDM treatment had no impact on systemic IL-5 levels (online supplemental figure S7H). However, systemic IL-17 levels were enhanced in HDM groups together with serum IgE (online supplemental figure S7H). Arthritis evaluation revealed that HDM exposure had an opposite effect on the disease severity than OVA treatment. It significantly amplified arthritis (online supplemental figure S7I). Histological analysis of the joints showed the same level of inflammation, bone erosion and number of osteoclasts in HDM/SIA mice as in the SIA group (online supplemental figure S7J,K). Similarly, the numbers of neutrophils, classical MΦ and AAMs were unchanged in the synovium of HDM/SIA mice compared with SIA alone (online supplemental figure S7L). In line with these observations, the HDM-induced asthma model was unable to recruit rEos into the inflamed joints, explaining the absent resolution (online supplemental figure S7M). The HDM-triggered exacerbation of arthritis is probably due to the systemic increase of the arthritogenic cytokine IL-17. Taken together, the ability of asthma to resolve inflammatory arthritis strongly depends on the disease endotype, whereby only type 2-driven eosinophilic asthma is able to guide rEos into the synovium, leading to the resolution of arthritis.

In human, rEos are associated with the resolution of RA

The morphology, tissue distribution, half-life and biological function of mouse and human eosinophils are very similar.[46] We, therefore, verified whether distinct eosinophil subtypes can also be found in human RA. First, we investigated the number and activation status of eosinophils (CD45+ CD125 int Siglec-8+) in the peripheral blood of healthy donors, active patients with RA (DAS28–ESR 2.7–5.8 units) and patients with RA in remission (DAS28–ESR<2.6 units) (figure 7A–C). The eosinophil activation markers, CD62L (rEos), CD123 (iEos),[23] CD69[47] and CD63,[48] were previously shown to be differentially regulated in asthmatic conditions. While the total eosinophil number and the expression of the proinflammatory surface molecules, CD123, CD63, and CD69, were similar among the different groups, the expression of the rEos protein CD62L was only upregulated in eosinophils from remission patients with RA (figure 7A–C). Furthermore, using confocal microscopy, we determined the infiltration of monocytes/macrophages (CD68+ cells) and rEos (EPX+ EAR1+ cells) in the synovium of active patients with RA (DAS28–ESR 3.6–6.1 units) versus patients with RA in remission (DAS28–ESR<2.6 units) (figure 7D). The synovial tissue of active patients with RA was characterised by high infiltration of monocytes/macrophages. rEos were detectable in the synovium of all patients with RA in remission, while only one-third of active patients with RA showed them (figure 7D). These data suggest that also in human the presence of rEos in the peripheral blood and synovium is associated with resolution of RA.

Based on murine studies,[49] patients with severe eosinophilic asthma are treated with monoclonal antibodies against IL-5 such as mepolizumab.[50] The purpose is to reduce hyper-eosinophilia and thereby restore the lung functionality.[51] We were able to test the impact of mepolizumab in patients with concomitant presence of eosinophilic asthma and RA. Importantly, these patients were in remission or low disease activity for arthritis (DAS28–ESR<3.2) for at least 6 months when they received mepolizumab. Remarkably, 6 of these eight patients developed a flare of their RA after mepolizumab therapy (figure 7E). Altogether, these results demonstrate that blocking IL-5 can exacerbate human RA.

DISCUSSION

Eosinophils are typically considered as downstream effector cells of inflammatory processes based on their ability to secrete granule-derived proinflammatory mediators. Effector function of eosinophils is mainly reported in the context of allergic responses and helminth infections. Nonetheless, eosinophils also store growth factors, anti-inflammatory cytokines and resolvins, allowing them to contribute to resolution of inflammation and tissue regeneration.[43] Notably, eosinophils are abundantly present in various tissues such as the BM, the spleen, the uterus, the adipose tissue, the small intestine and the skin, where they likely exert homeostatic tasks.[52] These observations suggest that eosinophils might acquire certain tissue-dependent phenotypes that explain their heterogeneous functions.[53]

Our study reveals that an immune-rEos population exists in the joints that is able to resolve chronic arthritis (figure 7F). The gene expression signature of these rEos is fundamentally different from their counterparts in the lungs. In particular, rEos in joints highly express proresolving 5-LOX and 12/15-LOX

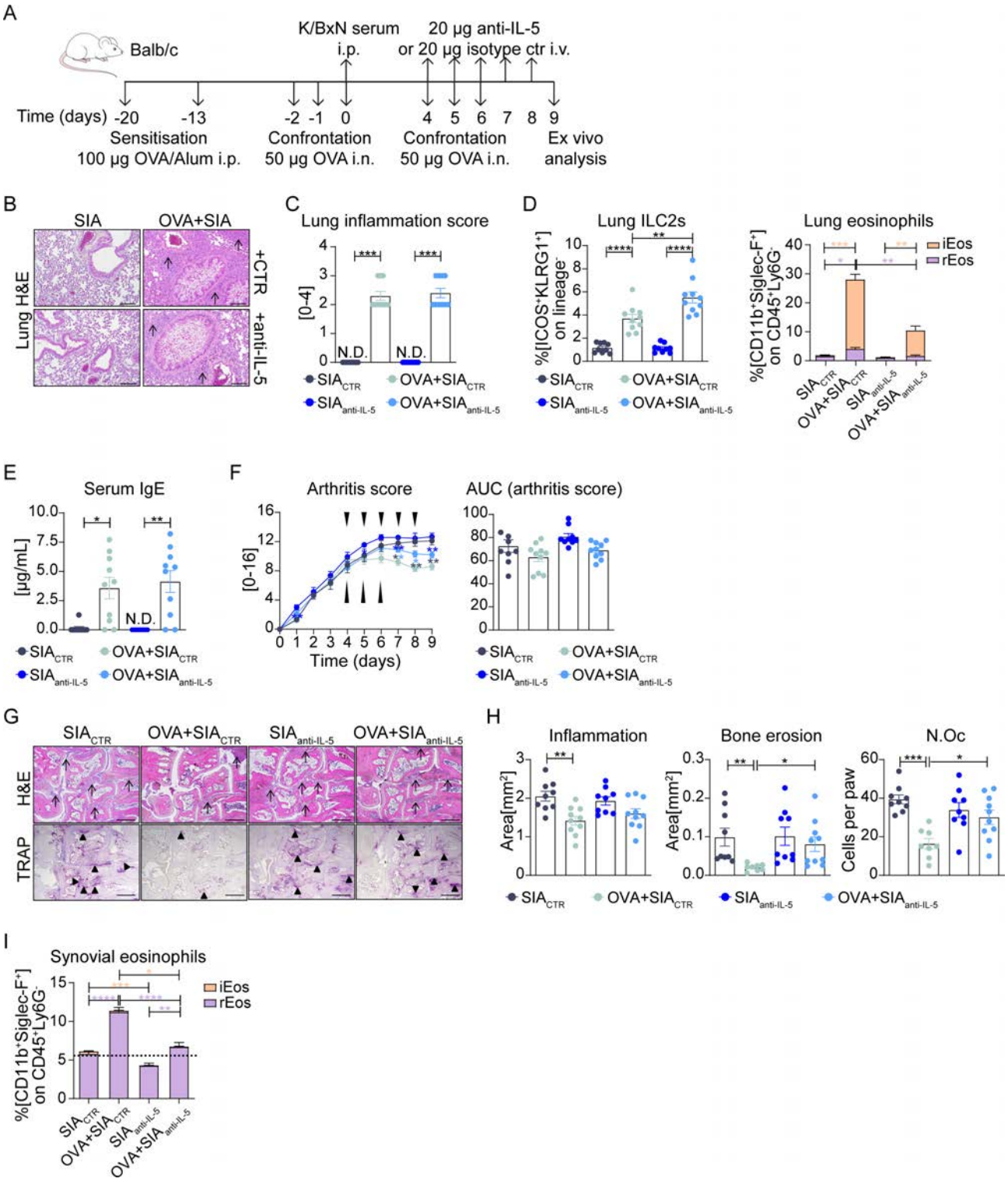

Figure 6 IL-5 inhibition abrogates ovalbumin (OVA)-induced resolution of arthritis. (A) Experimental outline of eosinophil depletion with 20 µg of monoclonal anti-interleukin (IL)-5 (anti-IL-5) antibody compared with isotype control in mice induced with serum-induced arthritis (SIA) and OVA+SIA. (B,C) Representative lung H&E staining (B) and semiquantification of lung inflammation (C) in SIA CTR, OVA+SIA CTR, SIA anti-IL-5, OVA+SIA anti-IL-5 groups (n=9–10/group) at day 9 post SIA. Thin arrows illustrate inflammation. Scale bar 100 µm. (D) Frequency of lung ILC2s (ICOS+ KLRG1+ gated on lineage– cells) and lung r/iEos (CD11b+ Siglec-F int/high gated on CD45+ Ly6G– cells) in the afore-mentioned four groups (n=9–10/group), 9 days post SIA, analysed by flow cytometry. (E) Serum IgE levels in SIA CTR, OVA+SIA CTR, SIA anti-IL-5, OVA+SIA anti-IL-5 groups (n=9–10/group) at day 9 post SIA. (F) Arthritis score and area under the curve (AUC) of arthritis score in the afore-mentioned four groups (n=9–10/group) during the course of arthritis. (G,H) Representative hind paw H&E and tartrate-resistant acid phosphatase (TRAP) staining (G) and quantification of hind paw inflammation area, bone erosion area and number of osteoclasts per paw (H) of SIA CTR, OVA+SIA CTR, SIA anti-IL-5, OVA+SIA anti-IL-5 groups (n=8–10/group) at day 9 post SIA. Arrows indicate inflammation; triangles show bone erosion. Scale bar 500 µm. (I) Frequency of synovial rEos and iEos in the afore-mentioned four groups (n=9–10/group), 9 days post SIA, analysed by flow cytometry. Data are shown as mean±SEM. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

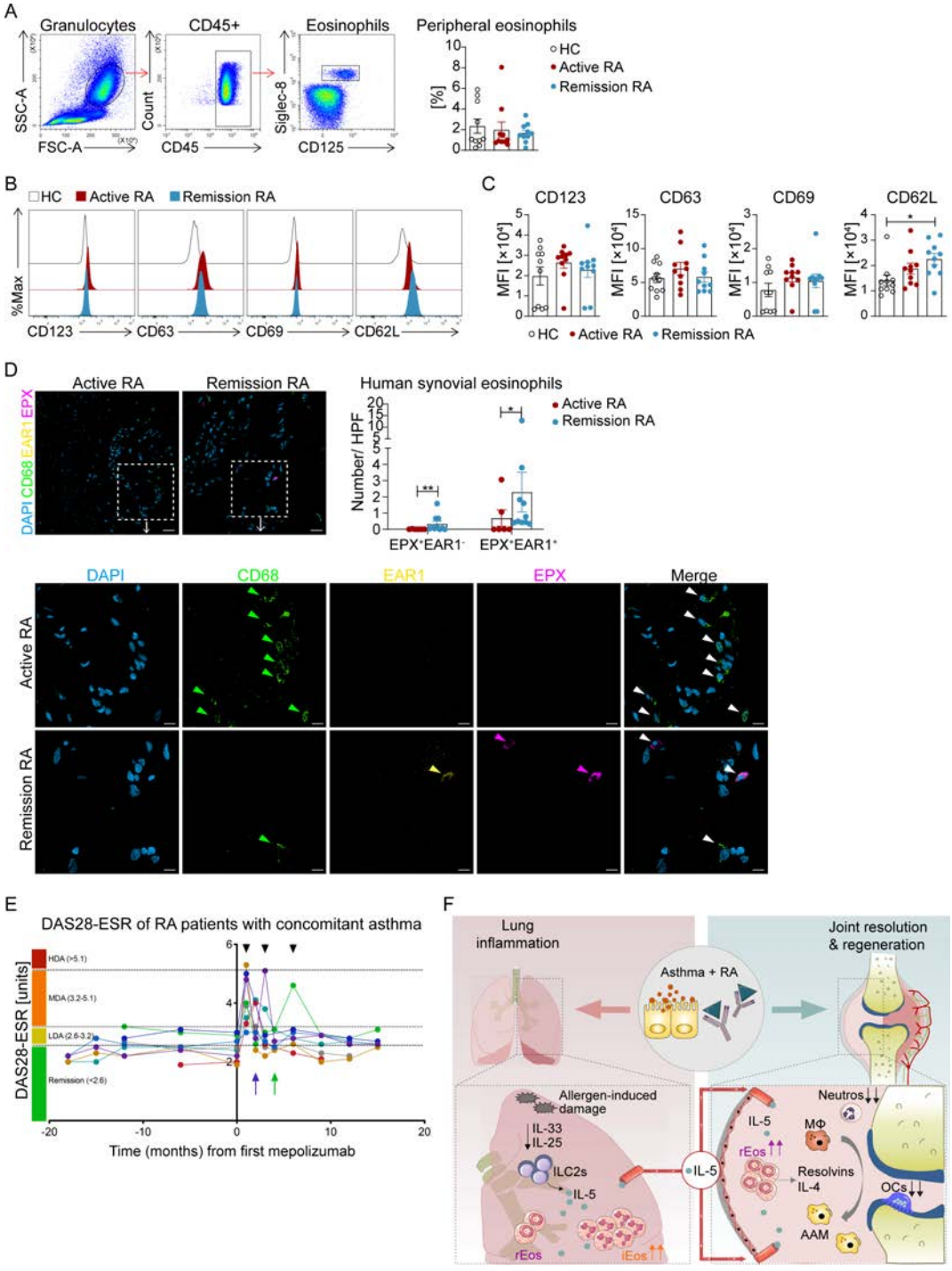

Figure 7 In human, resolution of rheumatoid arthritis (RA) is linked to an immune-regulatory eosinophil subset. (A) Gating strategy of eosinophils (CD45+ Siglec-8+ CD125 int granulocytes) in human peripheral blood. Frequency of blood eosinophils in healthy controls (HC) (n=10), active RA with a Disease Activity Score 28 (DAS28) based on erythrocyte sedimentation rate (ESR) between 2.7 and 5.8 units (n=10), and patients with RA in remission with a DAS28–ESR less than 2.6 units (n=10) measured by flow cytometry. (B,C) Representative histogram (B) and mean fluorescence intensity (MFI) (C) of CD123, CD63, CD69 and CD62L gated on peripheral eosinophils in HC, active RA and remission patients with RA (n=10/group). (D) Representative images of confocal microscopy in human synovial biopsies from active RA (DAS28–ESR 3.6–6.1 units, n=6) and RA remission (DAS28–ESR<2.6 units, n=10) patients stained for 4′,6-diamidino-2-phenylindole (DAPI), blue), CD68 (green), EAR1 (yellow) and eosinophil–peroxidase (EPX) (magenta) and quantification of EPX+ EAR1- and EPX+ EAR1+ cells per high-power field (HPF) by screening two random regions with an area of 0.96 mm^2 within the synovial tissue. Scale bar 20 µm (upper images) and 8 µm (lower images). (E) DAS28–ESR score in eight longitudinally followed patients with RA with concomitant asthma, from 20 months before and after mepolizumab treatment for eosinophilic asthma. Each line represents one patient; time point zero indicates the first exposure to mepolizumab; arrowheads indicate rescue treatment with adalimumab (a monoclonal antibody against tumour necrosis factor alpha); arrows indicate repeated exposure with mepolizumab. HDR, high-disease activity; LDA, low-disease activity; MDA, moderate-disease activity. (F) Schematic overview illustrating allergic asthma-mediated resolution of inflammatory arthritis via ILC2–interleukin (IL)-5–rEos axis. AAM, alternatively activated macrophage. Data are shown as mean±SEM. Asterisks mark statistically significant difference (*p<0.05; **p<0.01).

genes. The bioactive lipids generated by these enzymes are powerful mediators of resolution of inflammation.[54] The role of eosinophils in the resolution of arthritis likely extends beyond cell autonomous events and includes pathways resulting from interactions with macrophages. While classical macrophages amplify the inflammatory cascade through the release of proinflammatory cytokines such as TNFα and IL-1β, AAMs cease inflammation by taking up dead cells (efferocytosis) and secreting proresolving lipid mediators.[55] Eosinophils are known to foster AAM generation through the production of IL-4, IL-13 and 12/15-LOX-derived mediators.[56 57] ScRNA-seq confirmed high expression of Il4 and Alox15 in rEos in the joints. Our in vitro and in vivo experiments showed that rEos induce the polarisation of classical macrophages into AAMs in the synovium. Thus, synovial eosinophils may trigger the resolution process cell autonomously by secreting resolvins (12/15-LOX pathway) and indirectly by priming AAMs.

In addition to faster resolution of arthritis, accumulation of rEos in the joints also enabled better preservation of joint structure, indicating that rEos might contribute to tissue regeneration. Indeed, proteome profile arrays identified a specific secretion pattern of rEos different from iEos, associated with increased production of lipocalin-2, MMP-3, osteopontin and serpin E1, which are involved in tissue remodelling, wound repair and bone formation.[38–42] Therefore, synovial eosinophils may display dual function by ceasing inflammation and initiating synovial tissue recovery.

Interestingly, transcriptome analyses also identified the gene aconitate decarboxylase 1 (Acod 1) as primarily expressed in synovial rEos. The encoded enzyme synthesises the metabolite itaconate. Besides being an anti-inflammatory metabolite, itaconate is also responsible for the modulation of the energy metabolism towards mitochondrial respiration.[58] Recent data suggest that metabolic states directly influence the immunological function of cells. In this context, proinflammatory effector cells such as classical macrophages and neutrophils mainly use glycolysis as their energetic source, while anti-inflammatory, proresolving immune cells such as regulatory T cells (Tregs) and AAMs primarily rely on mitochondrial oxidative phosphorylation.[59–61] Strikingly, a similar metabolic separation is observed between the inflammatory and rEos subtypes, showing that iEos are high in glycolytic activity, whereas rEos possess increased mitochondria and use oxidative phosphorylation as energy source. The distinct modulation of the metabolic profile of iEos and rEos could accompany their functional differences. However, further studies on the function of itaconate in eosinophils are necessary.

Upstream of eosinophils, ILC2s produce IL-5, the most important cytokine for eosinophil development, activation and survival.[10 62] Herein, we show that ILC2s predominantly express IL-5 in the lungs on asthmatic response. This ILC2–IL-5 axis is essential for the expansion of rEos in the joints. These cells are already present in arthritic joints, but are not sufficient to initiate resolution. However, on IL-5-induced stimulation, rEos proliferate and migrate into the synovial tissue, allowing them to better fulfil their proresolving action. The anti-inflammatory function of ILC2s is likely dependent of rEos in the joints. However, bone erosion and osteoclast numbers were reduced by IL-25/IL-33 even in the absence of eosinophils. This is supported by previous studies, showing that the ILC2–IL-9–Treg axis[17] as well as the ILC2–IL-4/13 axis[16] is able to suppress inflammation-induced bone erosion. Moreover, Omata *et al* demonstrated that ILC2s inhibit osteoclast-mediated bone loss independently of inflammation.[63]

Human and mouse eosinophils exhibit many similarities, including their distribution in the body, expression markers and biological functions.[46] Numerous applications in humans, such as the treatment with the monoclonal anti-IL-5 antibody mepolizumab, are based on mouse studies.[50] Our human studies revealed that peripheral blood eosinophils from patients with RA in remission showed a regulatory phenotype. Moreover, rEos were primarily found in close proximity to macrophages in the synovium of patients with RA undergoing remission. In line with our murine data, remission of human RA is associated with the presence of rEos. Determining the priming of the immune-rEos subset from their progenitors, the eosinophil–basophil colony-forming units (Eo/B CFUs) would be of particular interest, since Eo/B CFUs have also been shown to rise in the peripheral blood and tissues of patients with inflammatory and hypersensitivity disorders.[64]

Interestingly, patients with RA with concomitant asthma, who were in remission for arthritis, showed a relapse of arthritis after mepolizumab treatment. Mepolizumab targets and neutralises human IL-5. Apart from eosinophils, the IL-5 receptor alpha chain is also expressed on basophils and mast cells in human.[65] While multiple studies have proven the efficacy of mepolizumab to reduce eosinophils, its effect on basophils and mast cells remains unclear.[66–68] Thus, aggravation of chronic arthritis in patients with asthma on mepolizumab therapy is most likely due to eosinophil inhibition. However, the role of other IL-5 receptor-expressing cells cannot be fully ruled out.

These results also suggest an inverse correlation between asthma manifestation and the risk of RA. Indeed, several human cohort studies confirm this assumption.[69] However, some studies show opposite results, where asthma is associated with higher incidence of arthritis.[70 71] These contradictory data are probably due to the high heterogeneity in the nature of asthma. Based on its causal pathway, asthmatic disease can be divided into neutrophilic and eosinophilic asthma that have completely divergent cytokine profiles.[6] In this context, a recent study of patients with asthma has identified three main patient clusters: Th2 high, Th17 high and Th2/Th17 low. The Th2-high and Th17-high clusters were inversely correlated and blocking one cytokine signature enhanced the other in a murine asthma model.[72] We could demonstrate that only eosinophilic asthma with a type 2 cytokine signature is leading to resolution of inflammatory arthritis, while an IL-17-related asthmatic response is in contrary exacerbating arthritis. Thus, correlation analyses addressing the relationship between asthma and RA should take into account the presence of divergent endotypes of asthma.

In summary, this study describes a regulatory phenotype of eosinophils that reside in the joints and foster resolution of arthritis. These cells expand in the synovium on activation of the ILC2–IL-5 axis. The proresolving effector function of these cells is mediated by releasing resolvins, modulating macrophage polarisation and triggering synovial tissue regeneration. Our data demonstrate that the immunological pathways causing asthma in the lungs are at the same time eliciting resolution of arthritis in the joints, supporting the hypothesis of a tissue-specific functional priming of eosinophils.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval All mouse experiments were performed according to the rules and regulations of the animal facility Franz-Penzoldt-Zentrum, Erlangen and approved by the animal ethical committee of the government of Unterfranken, Würzburg, Germany. All analyses of human material were performed in accordance with the institutional guidelines and with the approval of the ethics committee of the Universitätsklinikum Erlangen and the Hospital Clinic of Barcelona, and signed declaration of consent was obtained from each patient.

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CLINICAL SCIENCE

Which factors are associated with bone marrow oedema suspicious of axial spondyloarthritis as detected by MRI in the sacroiliac joints and the spine in the general population?

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ABSTRACT

Objective Identify factors associated with presence and extension of spinal and sacroiliac joints (SIJ)–MRI lesions suggestive of axial spondyloarthritis (axSpA) in a population-based cohort (Study of Health in Pomerania) aged <45 years.

Methods Spinal (sagittal T1/T2) and SIJ (semicoronal STIR sequences) MRIs were evaluated by two trained blinded readers. The presence (yes/no) and extension (Berlin MRI Score) of bone marrow oedema (BME) were captured. Degenerative spinal lesions were excluded and discrepancies resolved by consensus. Cross-sectional associations between clinical factors and presence/extension of BME were analysed by logistic/negative binomial regression. Record linkage of claims data was applied to identify participants with axSpA.

Results MRIs of 793 volunteers were evaluated. The presence of SIJ–BME (odds ratio) was strongly associated delivery during the last year (4.47, 1.49–13.41). For SIJ–BME extension, associations (incidence rate ratios, 95% CI) were found for delivery ((during last year) 4.52, 1.48–13.84), human leucocyte antigen (HLA)-B27+ (2.32, 1.30–4.14), body mass index (25–30 vs <25 kg/m²; 1.86 (1.19–2.89)) and back pain ((last 3 months) 1.55, 1.04–2.31), while for spinal BME, associations were found for age per decade (1.46, 1.13–1.90) and physically demanding work (1.46, 1.06–2.00). Record linkage was available for 694 (87.5%) participants and 9/694 (1.3%) had a record of axSpA (ICD M45.09).

Conclusion These population-based data support the hypothesis of mechanic strain contributing to BME in the general population aged <45 years and the role of HLA-B27+ as a severity rather than a susceptibility factor for SIJ–BME.

Key messages

What is already known about this subject?

- There is a relatively high frequency of inflammatory and fatty spinal/inflammatory sacroiliac joints (SIJ) and spinal MRI lesions suggestive of has been suspected.
- The reasons for these false-positive signals are still insufficiently investigated.

What does this study add?

- Human leucocyte antigen (HLA)-B27+, delivery during the last year in female adults and presence of back pain in the last 3 months seem to be the most important predictors for the extent of bone marrow oedema (BME) in the SIJ, while age and physically demanding work seem to be the most relevant predictors for the extent of BME in the spine.
- These data also support the role of HLA-B27 as a severity rather than a susceptibility factor for the occurrence of BME in the SIJ.

How might this impact on clinical practice or future developments?

- There is a different association between the occurrence and extension of BME in the SIJ in persons with high body mass index.
- The associations presented here are relevant for the interpretation of spinal MRIs of young people with back pain in blue collar jobs and their possible referral to rheumatologists for exclusion or confirmation of spondyloarthritis.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic rheumatic disease that is characterised by inflammatory back pain and several other musculoskeletal and extramusculoskeletal disease manifestations and comorbidities.[1] The classification criteria published in 2009 by the Assessment in Spondyloarthritis International Society (ASAS) have set the scene for a differentiation between the classical ankylosing spondylitis (AS), or radiographic SpA, and the non-radiographic form of SpA, based on the presence or absence of definite radiographic changes in the sacroiliac joints (SIJ).[2] Conventional radiography and MRI as imaging methods and human leucocyte antigen (HLA)-B27 as a laboratory test play an important role for diagnosis and classification of axSpA.[3] Recently, ASAS has published updates on the recommendations of how to interprete MRIs of the SIJ and the spine in patients with axSpA.[4 5] Yet, recent studies have shown that not only axSpA can be related to such changes in the axial skeleton and that differential diagnosis may sometimes be difficult.[6] Among others, we have recently demonstrated that there is a large proportion of false-positive MRIs suggestive of inflammatory activity as seen in axSpA, as well as frequent other MRI changes such as fat lesions, in the axial skeleton in

the normal population.[7 8] Furthermore, these changes may also occur in situations with physical demanding activities[9] or after pregnancy.[10]

The pathogenesis of axSpA is not fully understood to date but it seems to be largely genetically determined.[11] HLA-B27 is itself responsible for about 20% of the total genetic risk[11] and HLA-B27+ individuals carry a 10–20-fold increased risk of developing SpA.[12] In the last two decades, genetic studies have provided major insights into this topic, by identifying susceptibility alleles including >100 established loci which contribute roughly to 10% of the heritability of the disease, over and above the major effect of HLA-B27.[11] Although the association of AS with HLA-B27 was already reported in 1973,[12] it took more than 30 years to establish its role for the classification,[2] diagnosis[13] and the referral[14] of patients with axSpA. The clinical relevance of HLA-B27 has recently also been demonstrated in patients with psoriatic arthritis[15] and chronic inflammatory bowel disease.[16]

Using data from participants of a large population-based study <45 years,[17 18] we conducted a cross-sectional analysis to identify factors associated with MRI changes that are known to occur frequently in patients with axSpA in the SIJ and the spine.

METHODS

Study sample

The population-based project 'Study of Health in Pomerania' (SHIP) comprises the two separate cohorts SHIP and SHIP-TREND that are sampled in a north-eastern region of Germany and followed up every 4–6 years. SHIP is part of the Community Medicine Research net of the University Medicine of Greifswald, Germany, and conducts an extensive clinical examination programme in each follow-up of the cohorts[17]

Within the second follow-up of SHIP-2 and the baseline examination of SHIP-TREND-0, whole-body MRI was performed. For the present study, MRIs of the 793 volunteers being <45 years at the day of the MRI examination and who had complete MRI sets (both spine and SIJ) were included. The selection of volunteers from the SHIP project has been described in more detail elsewhere.[17]

MRI and reading of images

MRI was performed by the Department of Diagnostic Radiology and Neuroradiology in at one study site using one MRI device (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) and under the same standardised protocol.[18] For the entire spine T1 (TR 6.760 ms/TE 120 ms, flip angle 150°, slice thickness 4 mm, scan time 2:42 min) and T2 (TR 37.600 ms/TE 1.060 ms, flip angle 180°, slice thickness 4 mm, scan time 2:04 min) MRI sequences were available in a sagittal and for the SIJ respective STIR (TR 48.910 ms/TE 670 ms, flip angle 180°, slice thickness 5 mm) sequences in a semicoronal orientation were available. All images were blinded for additional participant information such as age and gender. Two readers, who first completed a training session of reference images including patients with axSpA, evaluated the MRI independently in a paired fashion to assess bone marrow oedema (BME) (defined as hyperintense signal on T2-weighted and hypointense signal on T1-weighted images in the spine or as hyperintense signal on STIR sequences in the SIJs). The ASAS definitions were used for defining lesions as 'positive'.[4 19] Spinal lesions with pathologic changes involving the vertebral endplate or being accompanied by abnormalities of the intervertebral disc (obvious dehydration, protrusion or prolapse) were considered as degenerative and were not counted. In case of disagreement for a lesion being present or not present, both readers adjudicated together in order to reach consensus.

In addition to a the binary approach of lesions being present or absent, quantification of the extension of BME lesions was performed based on the definitions of the Berlin SIJ and spine MRI Score.[20] Briefly, this score captures BME in a scale of 0–3. Both for the SIJ and the spine, a score of 0 means no BME, while a score of 1, 2 and 3 mean 0% to ≤33%, >33% to ≤66% and >66% of an SIJ quadrant or and 0% to ≤25%, >25% to ≤50% and >50% of a discovertebral unit, respectively. The final scores used for analysis were calculated based on consent of both readers in cases of disagreement.

Collection of clinical information

Clinical information was collected for age (in years), sex, smoking habit (current smoker, previous smoker, no smoker), mean spinal back pain level in the last 3 months prior to the MRI examination (on a numerical rating scale (NRS) rated 0–10), high-sensitivity C reactive protein (hsCRP, in mg/dL), HLA-B27 status (positive or negative), body mass index (BMI) (categorised according to the definitions of WHO, WHO to normal or underweight, overweight and obese), blue-collar or white-collar job, and whether the (female) patients had given birth within the last 12 months prior to the MRI examination.

Linkage of claims data

Claims data were available from the regional Association of Statutory Health Insurance including ICD-10 codes between 2002 and 2018. These data were used to obtain all ICD-10 codes of AS M45.xx including the quarterly period after the SHIP examination has been conducted.

As the claims data and those of the SHIP study have no common key for linkage, we applied record linkage as proposed in Vatsalan *et al*[21] based on: surname, name, date of birth and sex of participants with linkage consent. The names were normalised to upper-case letters and by removal of special characters, the indexing of candidate pairs was blocked via birth date. After comparison of candidate pairs, respective claims data were available for 694 of 769 (90.2%) participants who consented (769 of 793, 97.0%) in record linkage.

Statistical analysis

Descriptive measures (mean, median) are shown with SD, minimum and maximum. Frequencies and percentages are provided for categorical data (N). For all variables, the amount of missing values is reported.

The outcomes of affected SIJ quadrants or spinal segments are count data and were, therefore, modelled using negative binomial regression. The decision for a negative binomial regression model versus a Poisson model was based on likelihood ratio test in all outcome categories (SIJ, spine) and in the complete data. Effect estimates of the regression models were exponentiated resulting in incidence rate ratios (IRRs).[22] Associations for the presence of BME was modelled using multiple logistic regression (BME yes/no).

Missing values were accounted for by multiple imputations using the R-package mice.[23] Given the low number of missing values in each variable, m=10 imputations were considered sufficient. The imputation model comprised all variables of the analysis model. Imputations for categorical variables were conducted using logistic or generalised logistic regression; imputations of numerical variables were conducted using predictive means. All results from the regression models are pooled over all

imputations according to Rubins' rule.[24] The variance increase due to missing data was highest for HLA-B27 with 3.3%. We found no relevant difference between observations with complete data on those with incomplete data (data not shown).

In a sensitivity analysis we added information on known ICD-10 codes (M45.xx) to the regression models to examine changes and the robustness of results.

The interpretation of results is conducted in compliance with recommendations to avoid dichotomisation into statistically significant and insignificant results.[25] The use of p values is restricted to model diagnostics; all effects are described using effect estimates and 95% CIs.

RESULTS

A total of 793 MRIs of volunteers were evaluated by the two readers, with mean age 37.3±6.3 years, 49.4% male, 67 (8.4%) HLA-B27+, 53 (6.7%) with hsCRP >0.5 mg/dL, 451 (56.9%) with back pain in the last 3 months, (228 (28.8%) with back pain ≥4/10 on an NRS) were available. A physically demanding job was reported by 283 individuals (35.7%), 511 (64.3%) reported to work at a desktop. More than half (n=436) of the participants (55%) had a BMI >25 kg/m^2, and 247 were current smokers (31.1%). Delivery in the last year before the MRI examination was reported by 16 females (5%). Average physical activity >1 hour/day was reported by 653 subjects (82.4%). All characteristics including the numbers of missing values are shown in the table 1.

For the extension of BME on SIJ-MRIs, the largest IRR (95% CI) were found for delivery in the last year: 4.52 (1.48 to 13.84), HLA-B27+ : 2.32 (1.30 to 4.14) and BMI 25–30 versus <25 kg/m^2: 1.86 (1.19 to 2.89). For the extension of BME on spinal MRIs, IRRs were overall lower in size with the largest effects found for age per decade increase: 1.46 (1.13 to 1.90) and physically demanding work: 1.46 (1.06 to 2.00), see figure 1.

Regarding the presence of positive lesions on MRI only for the occurrence of delivery during the last year a strong association with SIJ–BME was found in females, with an OR of 4.47 (95% CI: 1.49 to 13.41) (figure 1).

Finally, in the comparison between SIJ and spinal MRIs, participants with back pain in the last 3 months (62.5% vs 56.9%) had more often SIJ BME than spinal BME, while spinal BME was more frequent than SIJ BME in participants working at a desktop (61.5% vs 54.4%), while smokers (66.9% vs 63.8%) and participants with back pain in the last 3 months (62.5% vs 56.9%) had more often SIJ BME than spinal BME, respectively, (data not shown).

In a subset of participants of the SHIP cohorts, information on ICD codes of all participants who consented to record linkage was available from the claims data: in 9/694 (1.3%) the ICD-10 code M45.09 (AS without specified location) was documented prior to the SHIP examination. However, an affirmation of the 'real' diagnoses of AS or axSpA by an expert was not obtained, since this was not part of the study protocol.

Results from sensitivity analyses did not reveal relevant differences to the results presented above. The largest change was found regarding the IRR for HLA-B27+ in SIJ–BME which decreased from 2.32 (1.30; 4.14) to 2.21 (1.24; 3.95).

DISCUSSION

In this population-based study, HLA-B27+, delivery during the last year in female participants and presence of back pain in the last 3 months were the most important predictors for the extent of BME in the SIJ, while age and physically demanding work

Table 1 Clinical, demographic and imaging characteristics of the 793 participants

Clinical, demographic and imaging characteristics	All (n=793)
Age (years)	
Mean (SD)	37.3 (6.3)
Median (min, max)	39.0 (21.0–45.0)
Sex	
Females	401 (50.6%)
Males	392 (49.4%)
HLA-B27	
Negative	689 (86.9%)
Positive	67 (8.4%)
Missing	37 (4.7%)
hsCRP >0.5 mg/dL	
No	708 (89.3%)
Yes	53 (6.7%)
Missing	32 (4.0%)
Back pain in last 3 month	
No	341 (43.0%)
Yes	451 (56.9%)
Missing	1 (0.1%)
Back pain in last 3 months ≥4 (NRS)	
No	228 (28.8%)
Yes	564 (71.1%)
Missing	1 (0.1%)
Physically demanding job	
No	507 (63.9%)
Yes	283 (35.7%)
Missing	3 (0.4%)
Work at desktop	
No	511 (64.4%)
Yes	270 (34.0%)
Missing	12 (1.5%)
BMI categories	
<25	357 (45.0%)
25 to <30	287 (36.2%)
≥30	149 (18.8%)
Smoking	
Never	295 (37.2%)
Previous	250 (31.5%)
Current	247 (31.1%)
Missing	1 (0.1%)
Birth within 1 year prior to the MRI examination	
No	385 (96 %)
Yes	16 (4.0%)
Average physical activity (annual)	
>2 hours	167 (21.1%)
1–2 hours	486 (61.3%)
<1 hour	140 (17.7%)
SIJ: number of affected quadrants	
Mean (SD)	0.236 (0.622)
Median (min, max)	0 (0–8.00)
SIJ: Berlin Score	
Mean (SD)	0.288 (1.08)
Median (min, max)	0 (0–24.0)
Spine: number of affected segments	
Mean (SD)	0.456 (0.911)
Median (min, max)	0 (0–6.00)
Spine: Berlin Score	
Mean (SD)	0.498 (1.04)
Median (min, max)	0 (0–8.00)

BMI, body mass index; HLA-B27, human leucocyte antigen B27; hsCRP, high-sensitivity C reactive protein; NRS, Numerical Rating Scale 0–10; SIJ, sacroiliac joints.

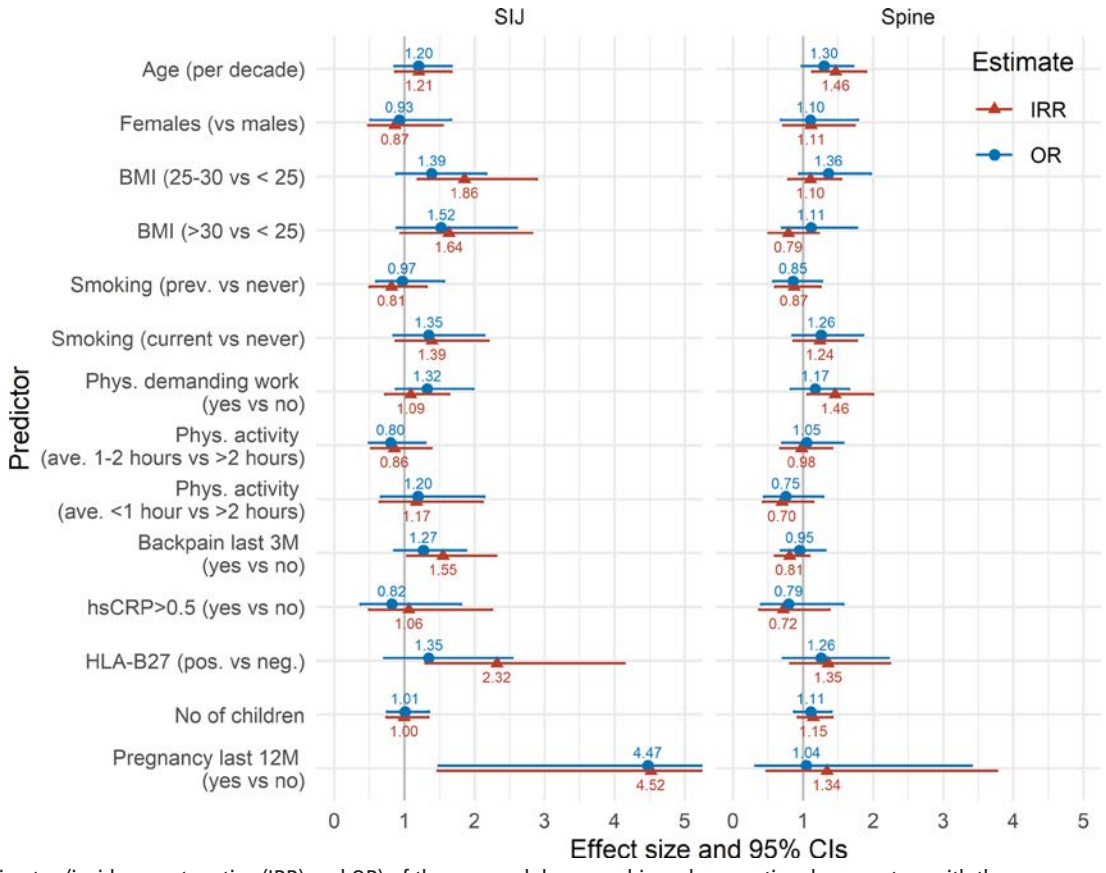

Figure 1 Esimates (incidence rate ratios (IRR) and OR) of the assessed demographic and occupational parameters with the occurrence of bone marrow oedema in the sacroiliac joints (SIJ) and the spine. BMI, bone mass index; hsCRP, high-sensitivity C reactive protein; HLA-B27, human leucocyte antigene B27.

were the most relevant predictors for the extent of BME in the spine.

These data support the hypothesis that mechanic strain contributes to BME found in the general population in subjects not diagnosed with SpA. In addition, HLA-B27+ was associated with the extent of BME but only contributes to a minor extent as a susceptibility factor for BME in the SIJ, while no association with spinal MRI changes was detected. Although the link between mechanically induced inflammation, genes and the immune system still needs to be elucidated, our data suggest, for the first time, that the recently supported[26–28] hypothesis coming from studies with patients also applies on a general population level.

Our finding of a clear association of BME in the SIJ after delivery within a relatively short period of time prior to the performance of the MRI is based on a small number of events (n=16). However, the result is clinically plausible, looking at the reported prevalence of MRI[29] and CT[30] findings of osteitis condensans ilii (OCI) in young patients with symptoms of low back pain. In a recent Belgian study,[10] 27 (77%) out of 35 women developed sacroiliac BME immediately postpartum, with 60% fulfilling the ASAS definition of a positive MRI in axSpA. Importantly, after 6 months, BME was still present in 46% of the participants. Whether and how OCI, a condition with a rather obvious mechanic pathogenesis, is associated with axSpA is unclear at present. In a recent study on OCI from Berlin, sacroiliac BME occurred frequently and equally often in both groups but only 7.4% of patients with OCI developed erosions in the SIJ even though about 35% of women with OCI were HLA-B27+.[31]

The association of back pain with the extension of BME in the SIJ but not the spine, even though the latter was more frequent, confirms its pathologic relevance related to the development of SpA, including the size of such lesions.[4] Nevertheless, it needs to be taken into account that the participants in this cohort study were not patients complaining about chronic or inflammatory back pain but were individuals from the general population. Thus, it may be expected that about 6% of the participants in this study had inflammatory back pain,[32] and based on a population prevalence of axSpA of 0.5%–1%, about four to eight patients may also have had axSpA.[33] Since it was not planned in this cohort study to directly diagnose patients, we cannot provide data on classification. Nevertheless, results obtained from record linkage of claims data showed that there were no established cases of axSpA in this study population, even though nine participants had been given the ICD code M45.09 somewhere in outpatient care at least once. This result is in line with the clinical experience that axSpA cases will be identified late or not at all.[34] These nine subjects were more likely to be HLA-B27+ and to have MRI changes in the SIJ (data not shown); this is consistent with this interpretation. It is also worth mentioning that the successful use of non-steroidal anti-rheumatic drugs (NSAIDs), which is not only part of the classification criteria for axSpA but sometimes also used as a diagnostic tool, was only present in a few patients and not associated with the presence of BME in this study (data not shown).

Furthermore, the prevalence of back pain, known to increase with age[35] during the first life decades,[36] was confirmed for subjects below the age of 45 in our study. The result that

participants working at a desktop had more often BME in the spine than in the SIJ may be methodologically explained since the differentiation of the severity of the physical workload (eg, blue or white collar) was not very sharp in this study. However, the finding that the extent of spinal BME was significantly associated with physically demanding work makes sense from the clinical point of view and is consistent with previous work.[37] Taken together, these results confirm that BME does occur in the younger general population with no established diagnosis of axSpA. Since physically demanding work is known to be associated with chronic back pain,[38] these associations are relevant for the interpretation of spinal MRIs of young people with back pain in blue collar jobs. Other assessments of physical activity showed smaller associations with the occurrence or extent of BME in our study. However, we did not assess the intensity of physical activity in more detail. In another study, physical inactivity over 14 years was shown to be associated with disc degeneration in the thoracic and the lumbar spine.[39] Similarly, physically demanding work may also have an effect of patients with axSpA. This is not necessarily related to inflammation.[40]

In addition, while the association of back pain with BMI and fat mass is well established,[41] our data suggest that there is also a different association between the occurrence and extension of BME in the SIJ in persons with high BMI (OR vs IRR, figure 1). This also seems to point in the direction of mechanical strain as a cause of more severe BME and is likely to stimulate research in this area.

Finally, only no or small associations between the occurrence of BME with any other of the investigated factors, such as smoking, hsCRP or female sex were found in our cohort. All of those factors are special interest in the overall topic of SpA-related MRI findings, since they represent established risk factors for low back pain[42] and also have been found to have an impact on the radiographic course of axSpA.[43]

In conclusion, in this cross-sectional analysis of MRIs of the SIJ and spine of participants of a population-based study, we identified predictors relevant to the presence of BME suggestive of axSpA. Our findings support the hypothesis of a mechanic origin of BME in the general population aged <45 years. On the other hand, HLA-B27 was identified to be a severity rather than a susceptibility factor especially for the presence of BME in the SIJ. Longitudinal analyses may be able to demonstrate causal relationships.

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TRANSLATIONAL SCIENCE

Immune response profiling of patients with spondyloarthritis reveals signalling networks mediating TNF-blocker function in vivo

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ABSTRACT

Objectives Antitumour necrosis factor (TNF) therapy has revolutionised treatment of several chronic inflammatory diseases, including spondyloarthritis (SpA). However, TNF inhibitors (TNFi) are not effective in all patients and the biological basis for treatment failure remains unknown. We have analysed induced immune responses to define the mechanism of action of TNF blockers in SpA and to identify immunological correlates of responsiveness to TNFi.

Methods Immune responses to microbial and pathway-specific stimuli were analysed in peripheral blood samples from 80 patients with axial SpA before and after TNFi treatment, using highly standardised whole-blood stimulation assays. Cytokines and chemokines were measured in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, and gene expression was monitored using nCounter assays.

Results Anti-TNF therapy induced profound changes in patients' innate immune responses. TNFi action was selective, and had only minor effects on Th1/Th17 immunity. Modular transcriptional repertoire analysis identified prostaglandin E_2 synthesis and signalling, leucocyte recirculation, macrophage polarisation, dectin and interleukin (IL)-1 signalling, as well as the nuclear factor kappa B (NF-kB) transcription factor family as key pathways targeted by TNF blockers in vivo. Analysis of induced immune responses before treatment initiation revealed that expression of molecules associated with leucocyte adhesion and invasion, chemotaxis and IL-1 signalling are correlated with therapeutic responses to anti-TNF.

Conclusions We show that TNFi target multiple immune cell pathways that cooperate to resolve inflammation. We propose that immune response profiling provides new insight into the biology of TNF-blocker action in patients and can identify signalling pathways associated with therapeutic responses to biological therapies.

Key messages

What is already known about this subject?

- Antitumour necrosis factor (TNF) therapy has revolutionised treatment of many chronic inflammatory diseases, including spondyloarthritis and rheumatoid arthritis. However, TNF inhibitors (TNFi) are not effective in 30%–40% of patients. The immunosuppressive effects of TNF blockers therefore expose a substantial fraction of patients to side-effects, in particular infections, without clinical benefit. Despite the extensive use of TNFi for many years, the biological basis for treatment failure remains unknown.

What did this study add?

- We demonstrate that anti-TNF therapy induces profound changes in patients' innate immune responses, but does not affect Th1/Th17 immunity.
- Modular transcriptional repertoire analysis showed that prostaglandin E_2 synthesis and signalling, leucocyte recirculation, macrophage polarisation, dectin and interleukin (IL)-1 signalling, as well as the NF-kB transcription factor family are key pathways targeted by TNF blockers in vivo.
- To investigate the concept that the immune status of patients before treatment initiation will define their response to TNFi treatment, we have searched for immunological transcripts that correlate with clinical efficacy of TNF blockers in stimulated immune cells. We found that high expression of molecules associated with leucocyte adhesion and invasion, chemotaxis and IL-1 signalling is correlated with favourable outcome of anti-TNF therapy.

INTRODUCTION

Chronic inflammatory diseases (CID) are challenging illnesses that often strike at a young age and cause lifelong morbidity, representing a considerable burden for the affected individuals and for society. Spondyloarthritis (SpA) is a family of related inflammatory disorders with common pathological

Key messages

How might this study impact on clinical practice or future developments?

- We have established a robust pipeline to monitor immune responses in patients that can be translated into a clinical setting. We show that immune response profiling can identify signalling pathways associated with therapeutic responses to TNFi. Further studies will assess whether this approach can be used to develop molecular biomarkers to help stratify patients to the most appropriate therapy.

and genetic features.[1–3] Clinical manifestations include spinal (axial) inflammation, peripheral arthritis, enthesitis and extra-articular features such as uveitis, psoriasis and inflammatory bowel disease.[4]

Antitumour necrosis factor (TNF) therapy has proven effective to reduce inflammation and clinical symptoms in SpA; however, little is known about how TNF inhibitors (TNFi) affect immune responses in patients, and TNFi have been associated with infectious complications,[5] including *Mycobacterium tuberculosis* reactivation.[6–8]

Furthermore, the high rate of non-responsiveness (30%–40%) to TNFi exposes a substantial fraction of patients to side effects without clinical benefit, and it is still not possible to determine which patients will respond to TNFi before treatment initiation.[9–11] The recent introduction of antibodies-blocking interleukin (IL)-17A has expanded the therapeutic options for axial SpA (axSpA), as well as psoriasis and psoriatic arthritis.[12 13] It is therefore important to develop tools to guide treatment decisions for patients affected by SpA and other CID, to optimise clinical care and contain healthcare costs.

Here, we investigated the global impact of TNFi on immune responses to microbial or pathway-specific stimuli, with the goal to enhance our understanding of the molecular mechanism of action of TNF blockers in patients with SpA and to identify immunological correlates of responsiveness to TNFi.

METHODS

Patients

Peripheral blood samples were obtained from 80 biologic-naïve patients fulfilling Assessment of SpondyloArthritis international Society (ASAS) criteria for axSpA,[14 15] attending the Rheumatology Departments of Cochin or Saint-Antoine Hospitals (Paris, France). A written informed consent has been obtained from each subject.

Patients' demographics, HLA-B27 status, information regarding symptoms, ongoing treatments, comorbidities and other main clinical features of SpA were recorded on a Case Record Form before and 3 months (D90) after initiation of anti-TNF therapy (see table 1 and online supplemental table 1).

Primary responsiveness to anti-TNF therapy was based on the Ankylosing Spondylitis Disease Activity Score (ASDAS).[16] The 'improvement score' was calculated as: ASDAS at baseline (D0)—ASDAS at D90. Patients achieving a delta ASDAS <1.1 were classified as non-responders.[16]

Whole-Blood TruCulture Stimulation was performed with TruCulture assays (Myriad RBM, Texas).[17] Multianalyte profiling of culture supernatants was performed with Luminex xMAP technology (Myriad-RBM, Austin, Texas, USA), gene expression analysis with nCounter Technology (NanoString), with the Human Immunology v2 Gene Expression CodeSet.[18 19]

Table 1 Clinical characteristics of the 80 patients with axial spondyloarthritis (axSpA) included in the study

Characteristic	SpA (n=80)
Female n (%)	25 (31%)
Median (IQR) age at sampling (years)	37 (19–64)
Median (IQR) disease duration (years)	2 (0–33)
HLA-B27 positive n (%)	63 (79%)
Current smokers n (%)	40 (50%)
Median (IQR) C reactive protein (CRP) (mg/L) at baseline	6.06 (0.09–62)
Median (IQR) BASDAI at baseline	49.80 (9.40–90)
Median (IQR) ASDAS at baseline	3.05 (1.13–4.79)
Axial involvement n (%)	80 (100%)
Axial and enthesial involvement n (%)	38 (47.5%)
Radiological sacroiliitis n (%)	48 (60%)
MRI sacroiliitis n (%)	63 (79%)
TNF blocker	
Soluble TNF receptor etanercept n (%)	53 (66.25%)
Monoclonal antibody adalimumab n (%)	13 (16.25%)
Monoclonal antibody golimumab n (%)	13 (16.25%)
Monoclonal antibody infliximab n (%)	1 (1.25%)
Extra-articular manifestations	
Psoriasis n (%)	16 (20%)
Uveitis n (%)	26 (33%)
IBD (%)	3 (4%)
Response at D90	
Median (IQR) CRP (mg/L) at D90	1.95 (0–51.80)
Median (IQR) BASDAI at D90	23.50 (0–78)
Median (IQR) ASDAS at D90	1.44 (0.64–3.45)
Patients with major ASDAS improvement n (%)	20 (25%)
Patients with clinically important improvement ASDAS n (%)	30 (37.5%)
Non-responder ASDAS n (%)	30 (37.5%)
Non-responder ASDAS treated with etanercept n (%)	22 (73.33%) (41.5%)†
Non-responder ASDAS treated with adalimumab n (%)	5 (16.67%) (38.5%)†
Non-responder ASDAS treated with golimumab n (%)	3 (10%) (23.1%)†
Non-responder ASDAS treated with infliximab n (%)	0 (0 %)
Non-responder BASDAI50 n (%)	52 (65%)

Median and IQR or percentages are shown.
*Percentage of total non-responders.
†Percentage of patients treated with the indicated drug.
ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; IBD, inflammatory bowel disease; TNF, tumour necrosis factor.

Purification of monocytes and in vitro cell stimulation

To generate in vitro derived macrophages, monocytes were isolated from healthy donors and cultured with macrophage colony-stimulating factor (M-CSF) in the presence or absence of TNFi. Cells were polarised towards M1 with LPS (20 ng/mL, Invivogen) and interferon (IFN)-γ (20 ng/mL, Milteny), or towards M2 with IL-4 and IL-13 (20 ng/mL, Miltenyi).

Data analysis

Quantitative set analysis of gene expression was performed using the R QuSage package.[20] Differential gene expression was analysed using the LIMMA package[21]; principal component analysis and hierarchical clustering were performed with Qlucore Omics Explorer (Qlucore).

Methods are described in detail in the online supplementary material.

RESULTS

TNFi affect immune responses to microbes and stimuli targeting specific immune receptors

We analysed immune responses in patients with axSpA with indications for TNFi treatment (table 1), using whole blood

('TruCulture') assays[17] (figure 1A). We stimulated blood samples from 12 patients with a range of microbial stimuli or signalling agonists, and we measured the levels of 31 secreted molecules (online supplemental tables 3 and 4, online supplemental figure 1A). Three months (D90) after TNFi initiation, the induction of many proinflammatory cytokines and chemokines (such as macrophage inflammatory protein-1beta (MIP-1β), IL-1Ra and IL-8) was reduced in response to various stimuli, indicating that TNFi target intracellular pathways shared by a broad range of immune activators (figure 1B). In contrast, TNFi had no major effects on IL-6, IFN-γ and IL-17 (online supplemental figure 1D), although the Th17 pathway is suggested to be of key importance in SpA pathophysiology.[22]

Only few secreted proteins increased after TNFi therapy. Among these was IL-10 following stimulation with gardiquimod (figure 1B), a selective ligand for TLR7.

These results show that TNFi induce selective changes in patients' immune responses, mostly detected in the challenged immune system, and not in the resting state (online supplemental figure 1D).

The effects of TNFi are detected after a single injection and remain stable over time

To determine the early effects of TNFi, we analysed 17 consecutive patients with axSpA 7 days after initiation of TNFi therapy (online supplemental figure 1B). Secretion of proinflammatory mediators was already affected after a single TNFi injection (figure 1C, D and G) and over a broad range of stimuli (online supplemental figure 2A). Production of IL-6, IL-17 and IFN-γ was largely unaffected (figure 1E,F).

The reduction in proinflammatory mediators was maintained at D90 (online supplemental figure 2B,C), demonstrating that the effects of TNFi on immune responses remain stable over time.

TNF blockers affect key transcriptional networks of innate immune responses

To gain insight into the mechanisms by which TNFi affect immune responses, we analysed the expression of immune-related genes before and at D7 and D90 after TNFi treatment. TNF blockade profoundly altered the transcription of a large number of genes (figure 2A).

The majority of genes differentially expressed after therapy were shared by different stimulation conditions, revealing a 'core immune response signature' targeted by TNFi (figure 2B), which included NF-kB genes, such as *NFKB1*, *RELA, NFKB2* and *RELB*, and NF-kB targets, such as *IL1A, IL1B* and *CCL20* (figure 2C and D, online supplemental figure 3A,B). In particular, TNFi strongly downmodulated expression of *PTGS2*, encoding cyclooxygenase (COX-2), the key enzyme in prostaglandin E_2 (PGE_2) biosynthesis and *PTGER4* encoding the PGE_2 receptor EP4 (figure 2D). TNFi-induced downmodulation of *PTGS2* and *PTGER4* did not depend on the NSAID index at baseline (online supplemental figure 4). Consistent with our analysis of secreted proteins (figure 1D), *IL17A*, *IFNG* and *IL6* were largely unaffected (online supplemental figure 3A).

The analysis of patients stratified into responders and non-responders showed that the majority of differentially expressed genes are common to both groups, although a number of genes are uniquely affected in each patient subset (online supplemental table 6 and online supplemental figures 5 and 6).

The effects of TNFi also on gene expression could be measured after a single injection and remained stable over time (online supplemental figure 7A).

To determine if changes in cell populations accounted for these effects, we analysed cell counts at D0 and D90. While leucocyte and monocyte counts remained stable, we observed a modest decrease of neutrophils and increase of lymphocyte counts after TNFi therapy (online supplemental figure 7B).

Modular transcriptional repertoire analysis reveals multiple mechanisms of TNFi action in vivo

The observation that TNFi affected several molecules in the same signalling pathway prompted us to further define the effects of TNFi on immune networks. We compared immune responses at D0 and D7 using Quantitative Set Analysis for Gene Expression (QuSAGE)[20] (online supplemental table 5). The modules 'NF-κB transcription factors' and 'NF-κB target genes' were among those most strongly downregulated by TNFi (figure 3A–C and online supplemental table 7), followed by the 'IL-1/IL-1R' module (figure 3A,B). Inspection of the individual genes in this module showed downregulation of *IL1A*, *IL1B*, *IRAK2*, *IL1R1* and *IL1RN*, as well as a substantial increase of *SIGIRR*, after TNF blockade (figure 3D).

TNFi therapy also reduced the activity of the 'dectin' module (figure 3A,B and online supplemental figure 8A), which groups C-type lectin receptors (CLRs) for *Candida albicans* and other fungi such as Dectin-2 (encoded by *CLEC6A*), or Mincle (encoded by *CLEC4E*) and associated signalling molecules, such as *CARD9*, a molecule involved in antifungal immunity that mediates signals from CLRs to the NF-κB pathway via BCL10.[23]

While gene set activities for most gene modules were reduced by TNFi, we observed increased activity at D7 of the 'cytotoxic molecules' module and of the 'M2-like monocytes' gene module, while the overall activity of the module 'M1-like monocytes' was reduced after TNFi, indicating that TNF blockers may affect monocyte/macrophage polarisation (figure 3).

In particular, we observed an upregulation of the genes encoding surface markers characteristic of regulatory macrophages, such as the mannose receptor *MRC1*, the scavenger receptors *MSR1* and *CD163*, the decoy receptor *IL1R2*, and of *IL10* (figure 3G and online supplemental figure 8B).

Analogous results were obtained at D90 after initiation of TNFi (online supplemental figure 8C), indicating the multiple immune pathways that mediate TNFi function in patients with SpA.

Many of the genes affected by TNFi are expressed in monocytes and macrophages, which prompted us to investigate the roles of these cells in the response to TNFi. We stimulated monocytes from patients with SpA with LPS in the presence or absence of etanercept (Eta), and measured transcript levels before and at different time points after stimulation (online supplemental figure 9). Several of the genes downregulated by etanercept were direct NF-κB target genes, such *NFKBIA*, *TNFAIP3*, *TNFAIP6* or *IL1A* (online supplemental figure 9).

TNFi skew macrophage polarisation towards an M2 phenotype in vitro

We then asked whether TNFi affect also macrophage gene expression. As the analysis of tissues is rarely performed in axSpA,[24] we investigated the effects of two TNFi, etanercept and adalimumab, on in vitro differentiated macrophages (figure 4A). Although the effects of adalimumab on gene expression were stronger in our system, a core of 56 genes was regulated by both TNFi (figure 4B–E).

We noted strong downregulation of M1-macrophages genes such as *IL18* (figure 4C, D and E), while expression of genes

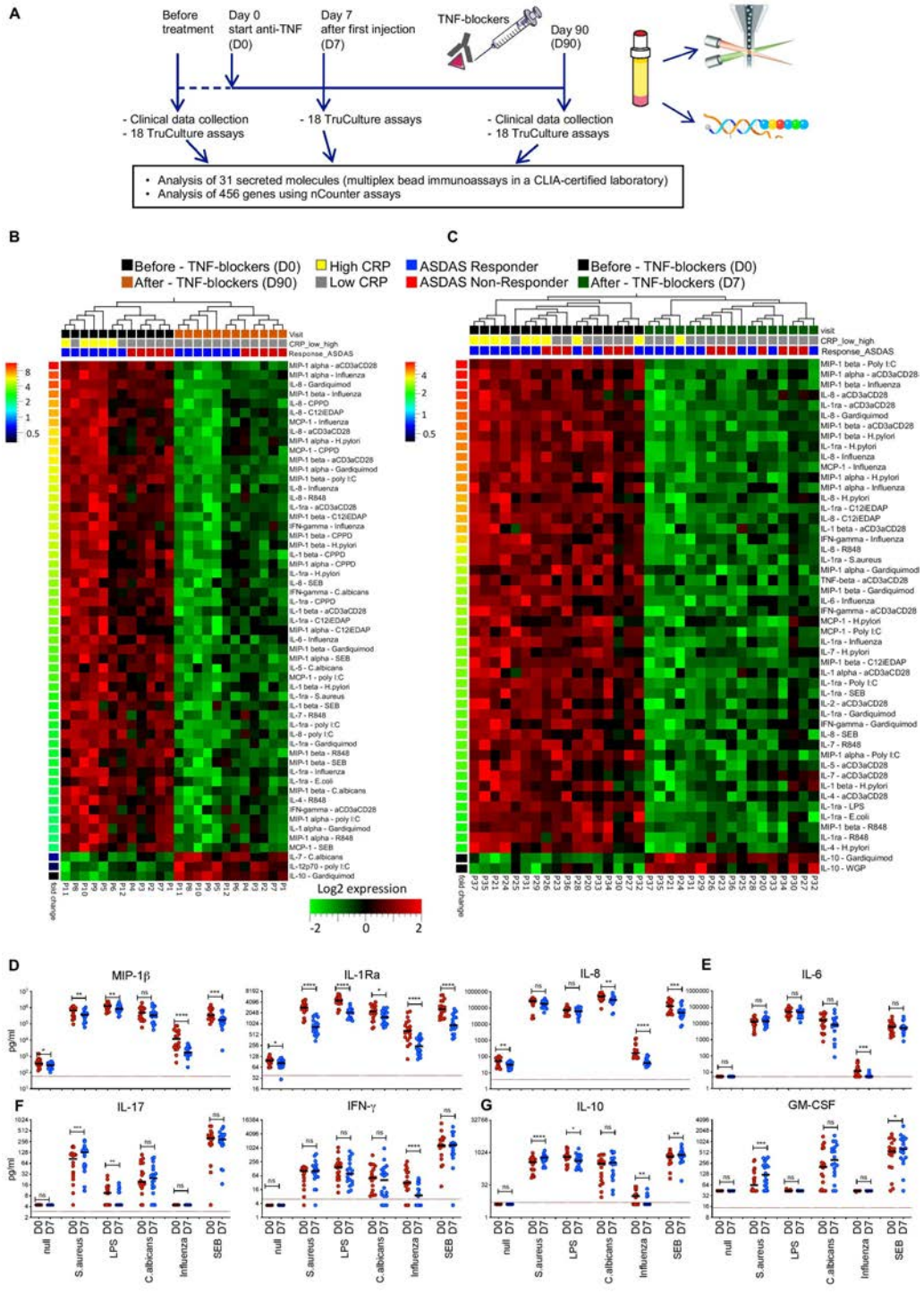

Figure 1 An immunological signature of antitumour necrosis factor (TNF) therapy. (A) Study design. Blood samples were collected from patients with axial spondyloarthritis (axSpA) prior to (D0), 7 days (D7, for a subset of patients), and 3 months (D90) after beginning TNF inhibitors (TNFi) treatment. Clinical efficacy was monitored at D90 according to the current standard of care. (B) The levels of 31 secreted molecules in response to 18 different immune stimuli were compared in samples from 12 patients at D0 (black rectangles) and D90 (orange rectangles). Patients with C reactive protein (CRP) levels >6 mg/L are marked with yellow rectangles, while CRP levels <6 mg/L are indicated with grey rectangles. Patients responding to anti-TNF therapy (delta ASDAS ≥1.1) are marked in blue and non-responders (delta ASDAS <1.1) are marked in red. The heatmap shows the levels of differentially secreted proteins (paired t-test, FDR≤0.05, fold-change ≥2, red indicates higher and green lower levels of protein secretion). Analyte-stimulus combinations were ranked by decreasing fold change (color-code bar, top left); patient IDs are indicated below the heatmaps. (C) The same analysis as in (B) was performed for additional 17 patients with axSpA, sampled at D0 (blue rectangles) and D7 (green rectangles). (D–G) Levels of proteins identified in (C), for 5 representative stimuli and the unstimulated (null) condition, in 17 patients with axSpA at D0 (red) and D7 (blue). Red lines indicate the least detectable dose (LDD) for each assay. P values were calculated using a Wilcoxon matched-pairs test (patients with SpA D0 vs D7) *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001; ns, not significant. Horizontal black bars indicate the median. Y-axes are log10 or log2 scales. ASDAS, Ankylosing Spondylitis Disease Activity Score; IFN, interferon; IL, interleukin.

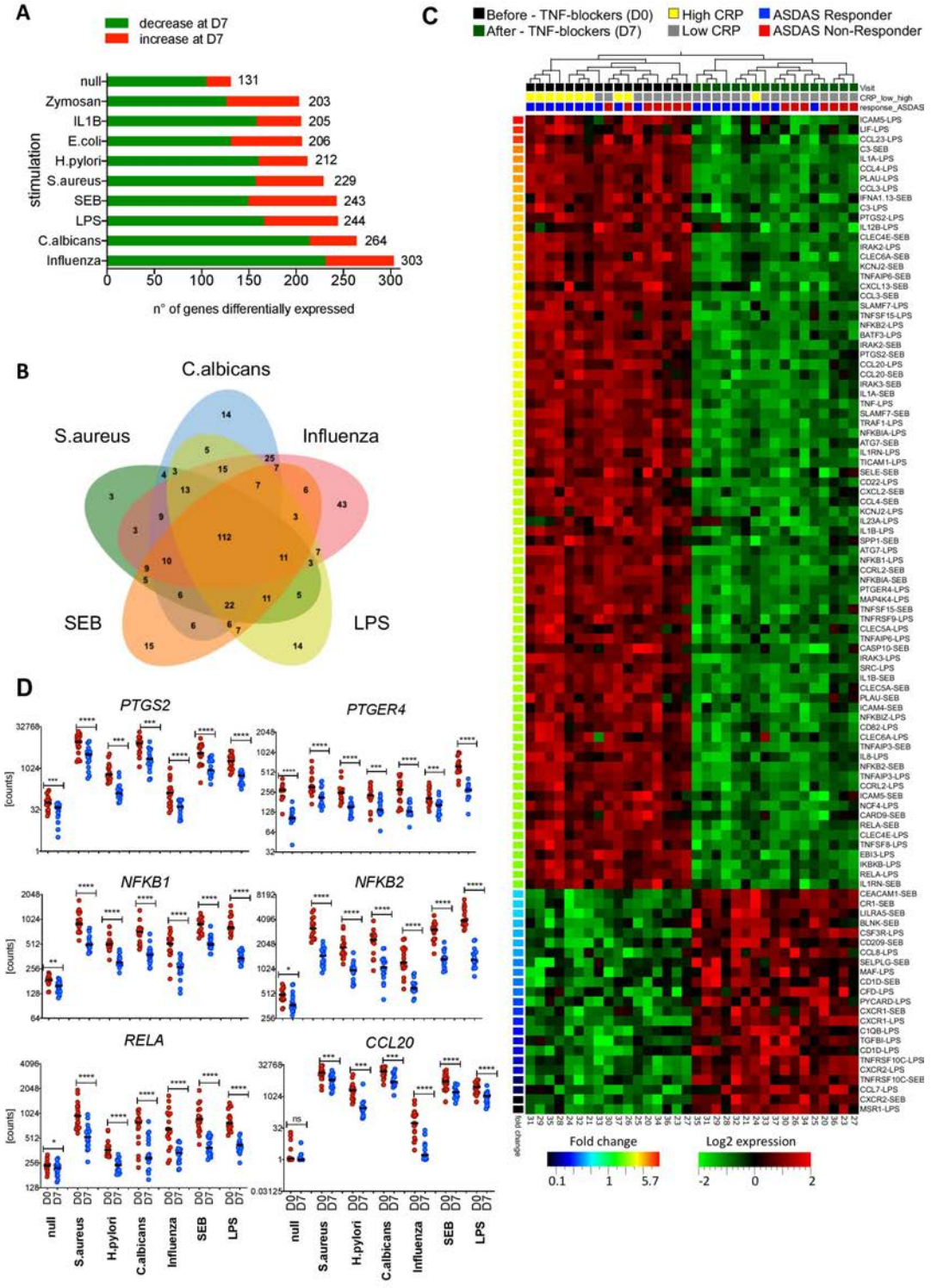

Figure 2 Tumour necrosis factor (TNF) blockers strongly affect key transcriptional networks of innate immune responses. (A) Number of genes differentially expressed in 10 different TruCulture stimulation assays performed at D0 and D7 (17 patients, paired t-test, false discovery rate (FDR)≤0.05). (B) Venn diagram of the genes differentially expressed as in (A), in five representative stimulation conditions. (C) Heatmap showing the genes most affected by TNF inhibitors (TNFi; D0, black rectangles vs D7, green) in lipopolysaccharides (LPS) and staphylococcal enterotoxin (SEB) stimulation conditions. Patients with C reactive protein (CRP) levels >6 mg/L are marked with yellow rectangles, while CRP levels <6 mg/L are indicated with grey rectangles. Patients responding to anti-TNF therapy (delta Ankylosing Spondylitis Disease Activity Score (ASDAS) ≥1.1) at M3 are marked in blue and non-responders (delta ASDAS <1.1) are marked in red. Paired t-test, FDR≤0.005 and fold-difference threshold of ≥2. Gene-stimulus combinations were ranked by decreasing fold change (colour code bottom left bar). (D) Expression levels of *PTGS2*, *PTGER4,* NF-κB family members, and *CCL20* for the unstimulated TruCulture assay and five representative stimuli at D0 (red) and D7 (blue) after initiation of TNFi therapy. P values were determined using a Wilcoxon matched-pairs test (D0 vs D7, *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001; ns, not significant, n=17). Horizontal black bars indicate the median.

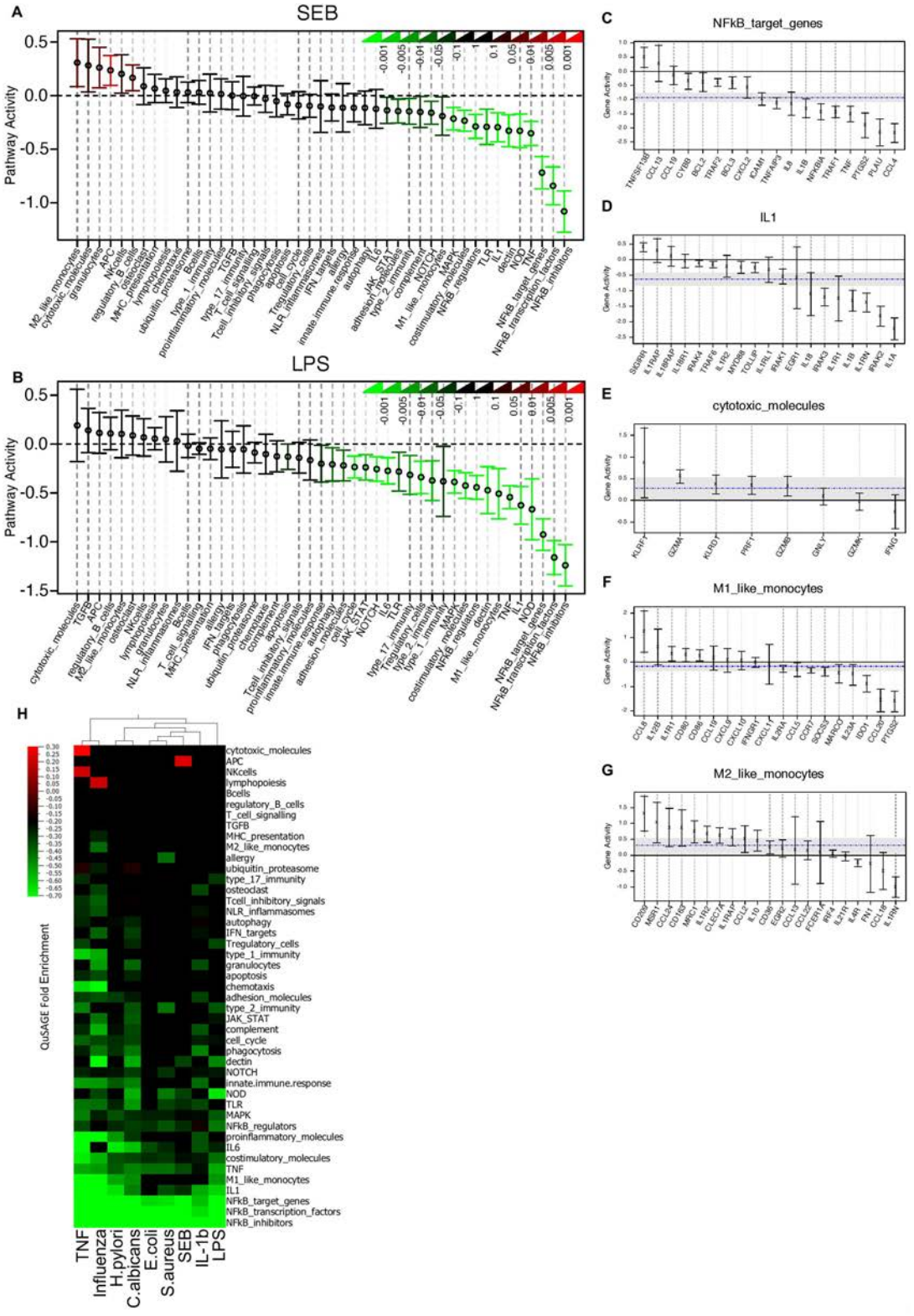

Figure 3 Modular transcriptional repertoire analysis reveals multiple mechanisms of tumour necrosis factor (TNF)-blocker action in spondyloarthritis (SpA). (A, B) Effect of anti-TNF therapy on the activity of 45 gene modules (online supplemental table 5) generated from 456 immune-related genes. Whole-blood cultures were stimulated with SEB (A) or LPS (B). For each gene module, the mean activity fold change and 95% CI are plotted and colour coded according to their FDR-corrected p values (means compared with fold-change zero). CIs overlapping the horizontal dotted line indicate statistically significant increased or decreased module activity at D7 as compared with D0. (C–G) Detailed gene activity in five representative modules with decreased (C, D, E, LPS stimulation) or increased (F, G, SEB stimulation) pathway activity after anti-TNF therapy. The cultures were stimulated with LPS and SEB, respectively. Represented are the mean fold change and 95% CI for individual genes in each module. The horizontal dashed blue line and the grey band indicate the mean differential expression of all genes in the module at D7 versus D0, and the 95% CI. (H) QuSAGE fold enrichment of gene set activity in nine different stimulated cultures at D7 versus D0. For each module, the mean fold change is color coded to indicate increased (red) or decreased (green) module activity. Only changes reaching a significance threshold of FDR≤0.01 are represented. IFN, interferon; IL, interleukin.

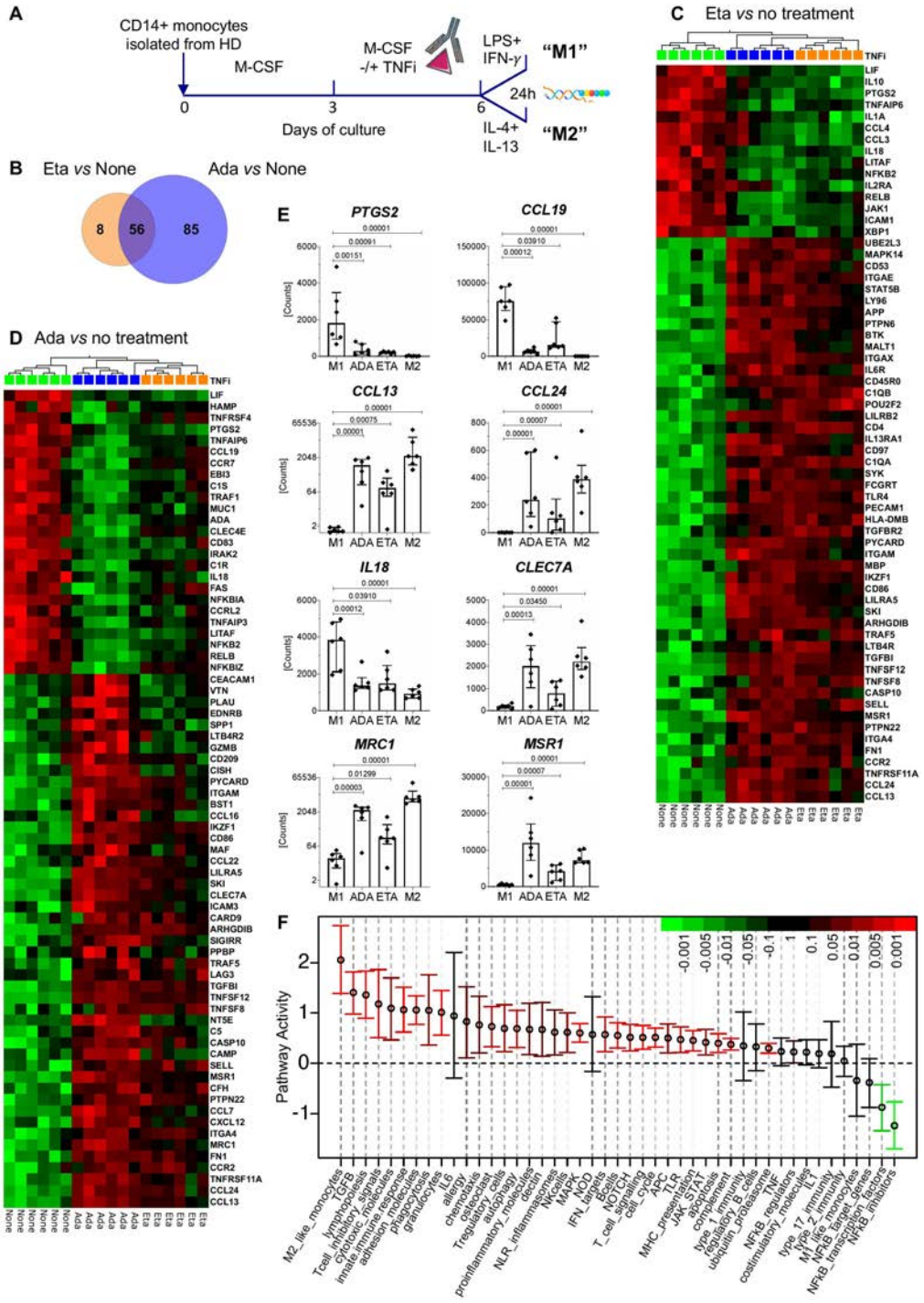

Figure 4 TNF inhibitors (TNFi) have largely overlapping effects on in vitro differentiated M1-type macrophages. (A) Study design. CD14+ cells isolated from healthy donors were differentiated in vitro into macrophages in the presence or absence of etanercept (Eta) or adalimumab (Ada). TNFi were added at day 3 and macrophages were polarised to the M1 subset in the presence or absence of Eta or Ada. Gene expression was analysed with the nCounter Human Immunology v2 panel and with LIMMA (paired sample adjusted p value threshold 0.01). (B) Venn diagram showing the overlap of genes affected by Eta or Ada. Analysis of paired samples with LIMMA, adjusted p value threshold 0.01. (C, D) Heatmaps showing the genes most affected by Eta (orange rectangles) versus no treatment (green rectangles) (C) and Ada (blue rectangles) versus no treatment (D) in macrophages stimulated for 24 hours with LPS and interferon (IFN)-γ ('M1' polarisation). (C) Paired t-test, Eta versus no treatment, adjusted p value threshold 0.01. Included are also gene expression levels for Ada-treated samples for the same genes. (D) Paired t-test, Ada versus no treatment and fold-change threshold of ≥2. Included are also gene expression levels for Eta-treated samples for the same genes. Samples were ordered by hierarchical clustering and genes were ranked by decreasing fold change. (E) Shown are the mRNA levels of eight selected genes from (C) and (D) in untreated M1-polarised macrophages (M1), M1 macrophages treated with Ada, M1 macrophages treated with Eta or untreated M2-polarised macrophages (M2). Symbols represent individual data points, boxes the median and whiskers the IQR. Adjusted p values are those of the LIMMA analysis. (F) Effect of Ada on the activity of 45 gene modules (online supplemental table 5) as in figure 3. For each gene module, the mean activity fold change and 95% CI are plotted and color coded according to their FDR-corrected p values compared with zero. Red and green bars indicate statistically significant increased or decreased module activity, respectively, in M1 polarised macrophages treated with Ada versus no treatment.

associated with M2 macrophages, such *MRC1*, *MSR1* and *CLEC7A* was significantly increased (figure 4E).

TNFi also strongly downmodulated *PTGS2* expression in stimulated M1 macrophages (figure 4E), and affected the mRNA levels of chemokines and their receptors: the expression of *CCL19*, *CCL4* and *CCL3* was downregulated, while *CCL13* and *CCL24* were upregulated by TNFi (figure 4C, D and E). These data are consistent with our results for TNFi treatment in vivo and suggest that TNFi may affect leucocyte recruitment to inflamed joints.

Finally, we confirmed a significant downregulation of NF-κB pathway genes (figure 4C, D and F). These data further support the notion that TNFi affect immune responses by acting on multiple inflammatory pathways and that phagocytic cells are important targets of these effects (figure 4F).

Immune gene expression associated with therapeutic responses to anti-TNF therapy

Finally, we investigated the correlation between therapeutic responses to TNFi and stimulated immune responses in 80 patients with axSpA, before initiation of anti-TNF therapy. Response to therapy was calculated as the delta ASDAS 'improvement score' (ASDAS D0—ASDAS D90).[16 25] Fifty patients (62.5%) had either a major or a clinically important improvement ('responders', delta ASDAS≥1.1), while 30 (37.5%) were non-responders (table 1 and online supplemental table 1). The analysis of whole-blood cultures stimulated with LPS or SEB revealed that 55 genes were differentially expressed between responders and non-responders (table 2 and figure 5A).

To explore if different types of anti-TNF drugs could have an impact on therapeutic responses to TNFi, we compared differential gene expression between responders and non-responders treated with soluble TNFR2 (n=53) to those treated with monoclonal antibodies (n=27). We found a good correlation (R=0.901) for the 55 genes differentially expressed. These data indicate that the type of TNF blockers does not have a major effect on the genes significantly associated with therapeutic responses before treatment (online supplemental figure 10B).

A search of the DICE database[26] showed expression of these genes in different immune cells, including activated T cells, Treg, Th17 and NK cells (figure 5B). Notably, 29 of the genes were expressed specifically in resting classical or non-classical monocytes (figure 5B). These data suggest that several immune cell populations contribute to determine the efficacy of anti-TNF therapy in patients with SpA.

Among the 55 differentially expressed genes, 15 regulate key steps of leucocyte migration and invasion: these include *PLAU* and *PLAUR*, the integrin subunits *ITGB1*, *ITGA5*, *ITGAX*, and *ITGA6*, and the CD2 ligand *CD58* (figure 5B,C and table 2). The importance of leucocyte recirculation as a determinant of therapeutic responses to TNFi is supported by the observation that several genes encoding chemokines and their receptors, such as *CCL20*, *IL8, CXCL1, CXCL2* and *CXCR1* are expressed at higher levels in cultures from patients with SpA responding to TNFi than in non-responders, while *CXCL9* is expressed at higher levels in non-responders (figure 5B–C, table 2 and online supplemental figure 10). Expression of the receptors for the pro-inflammatory cytokines TNF (*TNFRSF1B*), IL-6 (*IL6R*) and IL-1 (*IL1R1*, *IL1R2* and *IL1RAP*) was also substantially higher in responders than in non-responders, as was expression of the IL-1R-associated kinases *IRAK1* and *IRAK3,* and of *NLRP3*, which controls caspase-1-dependent processing of pro-IL-1β and IL-18. These data indicate that the activation status of the

Table 2 Genes differentially expressed between responders and non-responders to TNFi

Gene ID	Log fold-change (R/NR)	P value (R/NR)	Adjusted P value (R/NR)
*PLAUR*_LPS	0.4816	2.86E−06	0.0023
*ITGB1*_LPS	0.2860	5.29E−06	0.0023
*CD14*_LPS	0.5704	1.78E−05	0.0041
*CCL20*_LPS	0.6264	2.04E−05	0.0041
*IL1R1*_LPS	0.7803	2.48E−05	0.0041
*IRAK1*_LPS	0.2964	3.41E−05	0.0041
*IRAK3*_LPS	0.3977	3.49E−05	0.0041
*CLEC5A*_LPS	0.7180	3.8E−05	0.0041
*ITGA5*_LPS	0.2684	0.0001	0.0066
*LTB4R*_LPS	0.5985	0.0001	0.0069
*LTA*_LPS	−0.3366	0.0001	0.0074
*BST1*_LPS	0.5186	0.0001	0.0077
*IL1RAP*_LPS	0.4707	0.0001	0.0083
*CD58*_LPS	0.2690	0.0001	0.0083
*CEBPB*_LPS	0.2989	0.0001	0.0083
*IL8*_LPS	0.5694	0.0002	0.0083
*IFNGR1*_LPS	0.3022	0.0002	0.0097
*IL1R2*_LPS	0.4411	0.0003	0.0121
*CXCL9*_LPS	−2.0206	0.0003	0.0121
*TNFRSF1B*_LPS	0.3157	0.0003	0.0121
*IL6R*_LPS	0.3360	0.0003	0.0121
*NLRP3*_LPS	0.3896	0.0003	0.0121
*CTNNB1*_LPS	0.1495	0.0003	0.0121
*FCGRT*_LPS	0.3159	0.0003	0.0121
*ITGAX*_LPS	0.3600	0.0003	0.0121
*IFNG*_LPS	−1.4398	0.0005	0.0180
*CXCL1*_LPS	0.4515	0.0006	0.0180
*FCGR2A*_LPS	0.2634	0.0006	0.0180
*ITGA6*_SEB	−0.2569	0.0006	0.0180
*PRKCD*_LPS	0.3330	0.0006	0.0187
*ZEB1*_LPS	0.3487	0.0007	0.0201
*CLEC7A*_LPS	0.3795	0.0007	0.0201
*PECAM1*_LPS	0.4050	0.0008	0.0218
*IRAK1*_SEB	0.1988	0.0009	0.0231
*APP*_LPS	0.1938	0.0010	0.0237
*FCER1G*_LPS	0.2902	0.0011	0.0255
*ICAM5*_SEB	0.5363	0.0011	0.0257
*IL8*_SEB	0.3880	0.0011	0.0257
*PLAUR*_SEB	0.3067	0.0012	0.0270
*IL7R*_SEB	−0.1991	0.0012	0.0270
*IGF2R*_LPS	0.2310	0.0013	0.0270
*IKZF3*_LPS	−0.1544	0.0013	0.0276
*TNFRSF8*_LPS	0.3647	0.0014	0.0276
*NFIL3*_LPS	0.2830	0.0015	0.0290
*LIF*_LPS	1.0229	0.0015	0.0292
*MBP*_LPS	0.2114	0.0016	0.0296
*TP53*_LPS	−0.1846	0.0016	0.0296
*CXCL2*_LPS	0.4914	0.0020	0.0371
*CXCR4*_LPS	0.2833	0.0022	0.0398
*ATG7*_LPS	0.2486	0.0024	0.0412
*CRADD*_SEB	0.3238	0.0025	0.0435
*PLAU*_LPS	0.4759	0.0027	0.0452
*SPP1*_SEB	0.4451	0.0028	0.0452
*SKI*_LPS	0.1760	0.0028	0.0452
*CXCR1*_LPS	0.6786	0.0029	0.0452
*TLR2*_LPS	0.2718	0.0031	0.0471
*MAP4K4*_LPS	0.2504	0.0031	0.0471
*DUSP4*_LPS	0.4570	0.0031	0.0471

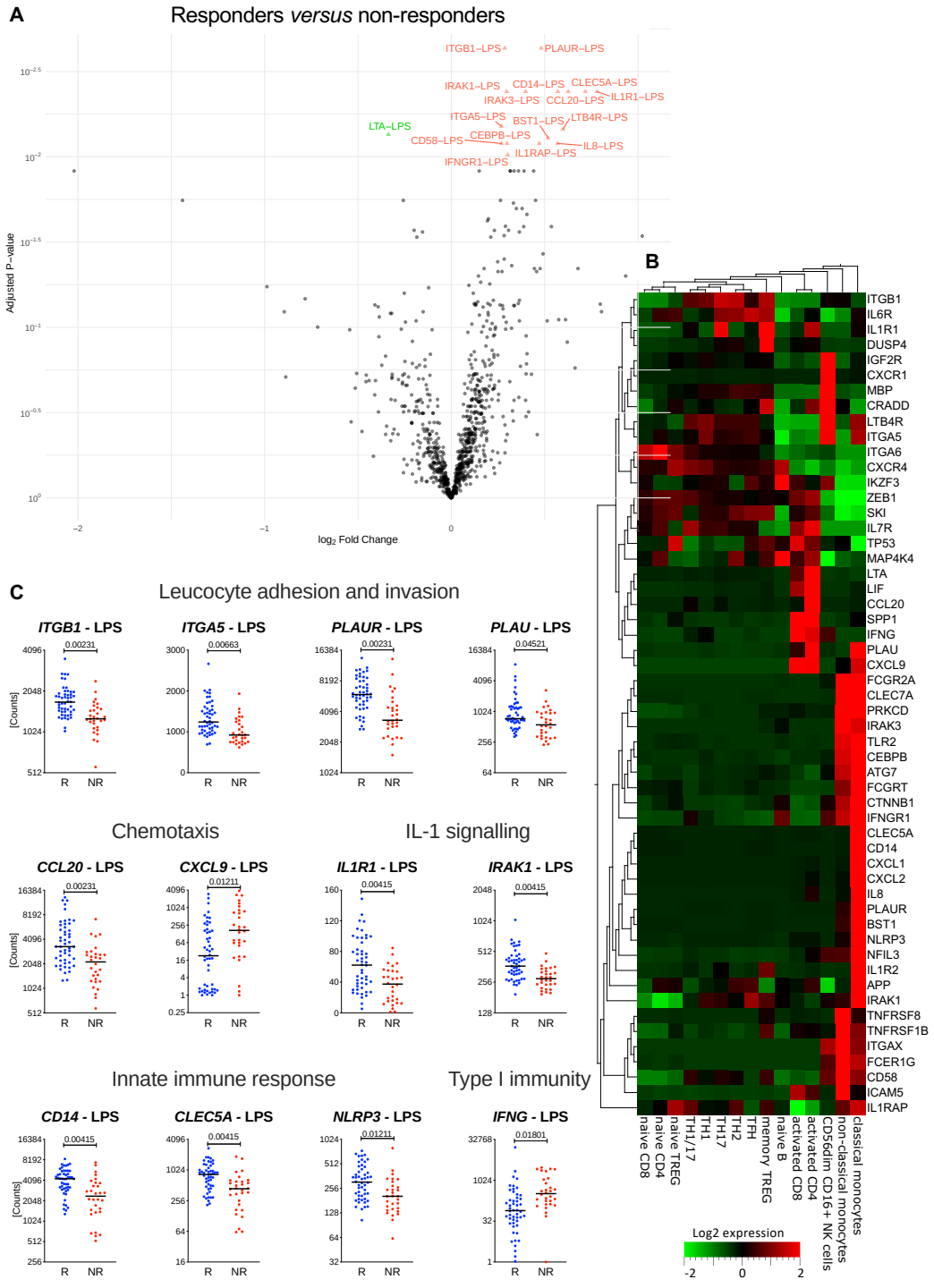

Figure 5 Immune gene expression associated with therapeutic responses to antitumour necrosis factor (TNF) therapy. (A) Volcano plot representation of genes differentially expressed between 50 patients with spondyloarthritis (SpA) responding to anti-TNF therapy and 30 non-responders in whole-blood cultures stimulated with LPS or SEB before initiation of therapy; red triangles: genes higher in responders; green triangle: higher in non-responders (LIMMA analysis, adjusted p value<0.05). Expression levels and fold-change values of the 58 gene-stimulus combinations (corresponding to 55 genes) that are the most differentially expressed between responders and non-responders are reported in table 2. (B). The heatmap shows the expression levels of the differentially expressed genes in different immune cell subpopulations. Gene expression data were extracted from the DICE database (http://dice-database.org/). (C) The expression levels of selected gene-stimulus combinations correlated with treatment response are plotted before treatment initiation (D0). Patients with major or clinically important improvement of disease activity were grouped together as responders and are represented in blue (R, blue, n=50). Non-responders are represented in red (NR, red, n=30). The horizontal black line represents the median. Statistical significance was tested using LIMMA analysis (responders vs non-responders) and adjusted p values are indicated above the graph. IL, interleukin.

IL-1 signalling pathway may influence responsiveness to TNFi. We also noted substantially higher expression in responders of *CLEC5A* (MDL-1, myeloid DAP12-associating lectin-1), an important mediator of autoimmune inflammation in experimental arthritis models[27] (figure 5C and table 2).

DISCUSSION

To investigate immune responses in patients with SpA, we have used highly standardised and robust assays that may be directly translated into a clinical setting. 'TruCulture' assays were designed to preserve physiological cellular interactions and capture immune cell activity without introducing sample collection and manipulation variables.[28] We chose to analyse responses in whole blood, because tissue biopsies cannot be performed routinely in axSpA.

Most of the effects of TNFi could be observed only in stimulated cultures, supporting the notion that TNFi act on activated immune cells, rather than in homeostatic conditions. This may explain the relatively modest changes in gene expression in response to TNFi detected in a recent study of unstimulated PBMCs from patients with axSpA.[29]

Our modular transcriptional repertoire analysis of the stimulation cultures[20] established a hierarchy of signalling pathways affected by anti-TNF therapy, with potential clinical implications.

We found a strong decrease of proinflammatory molecules produced primarily by innate immune cells, pointing to the importance of these cells in SpA pathogenesis. The decreased activity of the NF-κB module underlines the major role of these factors in mediating TNF-blocker functions. However, TNF blockade had only minor effects on the expression and secretion of IL-6, contrary to what observed in RA patients.[30] These data suggest that this cytokine may be more relevant to RA, but less to SpA pathogenesis, consistent with the limited therapeutic efficacy of IL-6-blockade in SpA.[31]

We observed downregulation of the classical, M1-like module and an increase of the non-classically activated, M2-like monocyte gene module activity, consistent with the finding that TNFi can expand a cell population with a M2 macrophage-like appearance in vivo and in vitro.[32 33] Analysis of the effects of TNFi in vitro provided direct evidence that TNFi act directly on macrophage polarisation. These results are consistent with a previous study performed with in vitro differentiated macrophages from patients with rheumatoid arthritis (RA).[34] M2 macrophages, characterised by expression of IL-10, high levels of scavenger and mannose receptors, *IL1R2* and *IL1RN*, are implicated in the resolution of inflammation and orchestrate tissue repair and remodelling.[35 36] Polarisation of monocytes/macrophages towards a M2-like profile may be an additional mechanism by which TNF blockers act on the immune system to regulate inflammatory responses[37] and could also explain the increased risk of opportunistic infections observed for patients treated with TNFi, in particular *M. tuberculosis*.[38]

TNFi strongly downregulated expression of *PTGS2*, the key enzyme in prostaglandin E_2 (PGE_2) biosynthesis and target of non-steroidal anti-inflammatory drugs, the first-line treatment of SpA. PGE_2 is an important early mediator of enthesitis, the hallmark of SpA[39] and COX-2 inhibition may be an important mechanism of TNFi therapeutic action in this disease. PGE_2 induces vasodilation, which may facilitate neutrophil recruitment into the entheseal compartment.[39] We also found that expression of the PGE_2 receptor *PTGER4* (EP4) was downregulated by TNFi. Signalling through EP4 upregulates IL-23R expression promoting human Th17 cell development,[40] and suppresses disease progression in an experimental mouse model of autoimmune encephalomyelitis.[41] Of note, *PTGER4* has been associated with SpA susceptibility, as have been *NFKB1* and *CARD9*,[42] also strongly downregulated by TNFi. Collectively, these data provide evidence that TNFi target the expression of genes closely linked to SpA pathogenesis.

Our findings suggest that TNFi target several immune cell pathways that cooperate to control inflammation. Targeting PGE_2 biosynthesis via *PTGS2* downregulation is of particular relevance for enthesitis, a critical early pathogenic feature of spondyloarthritis, while shifting the balance of macrophages from a proinflammatory phenotype to a proresolving phenotype is important for the resolution of synovitis. MDL-1/CLEC5A was among the most strongly downregulated molecule after TNFi therapy. Dengue virus-mediated activation of MDL-1/CLEC5A can trigger potent induction of TNF, IL-6 and IL-1β and NLRP3 inflammasome activation and shock.[43 44] MDL-1/CLEC5A is also expressed in synovial tissue from RA patients and MDL-1/CLEC5A blockade reduced tissue inflammation and bone erosion in experimental arthritis models.[27] Reduction of MDL-1/CLEC5A expression by TNFi may result in inhibition of bone erosion and inflammatory cytokine production in SpA.

The involvement of multiple pathways in TNF-blocker functions could also explain the difficulties in identifying a genetic marker for treatment response to TNFi.[45] We could not identify a single gene whose expression correlates with responsiveness to TNFi, but rather a set of genes. A limitation is that our study focused on a predefined panel with 594 genes. Genome-wide studies may be necessary to identify unique molecular biomarkers. Nevertheless, our data suggest that high expression of molecules associated with leucocyte invasion and migration as well as IL-1 signalling in stimulated immune cells predisposes to favourable outcome of anti-TNF therapy. Furthermore, this study was performed in patients from France and should be replicated in an independent cohort from different genetic and environmental backgrounds, to support the translational value of our findings.

In conclusion, we suggest that immune response profiling of patients is a powerful approach to define the mechanism of action of biological drugs and may be a useful strategy to establish objective criteria guiding treatment decisions.

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Correction notice This article has been corrected since it published Online First. The author's name has been corrected for Dr Millot and Prof Micelli-Richard.

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Contributors SM, EB and LR designed the study, analysed data, interpreted results and wrote the manuscript. SM, EL, EM, HY-C, DM, CL, NR and SK performed experiments. AG and SH-B-A analysed drug levels and antidrug antibiodies in serum samples. SM, VG, VR, GM, EB and LR performed bioinformatics data analysis. DD provided data from the Milieu Intérieur cohort. JS and FB provided patient samples and clinical data. CM and MD had overall medical oversight, provided patient samples and clinical data, performed clinical data analysis and revised the manuscript. All authors approved the manuscript.

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CLINICAL SCIENCE

10-Year natural course of early hip osteoarthritis in middle-aged persons with hip pain: a CHECK study

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ABSTRACT

Objective To explore the natural course of hip osteoarthritis (OA) in a population of first-time presenters with hip complaints.

Methods Data were collected at baseline and after 2, 5, 8 and 10 years on participants from the Cohort Hip and Cohort Knee study with early symptomatic hip OA. Descriptive statistics were used to analyse the natural course of the hip complaints with respect to clinical signs and symptoms, physical functioning and radiographic osteoarthritis (ROA) features.

Results In total, 588 participants were included with hip complaints and 86% completed the 10-year follow-up. The 10-year follow-up showed that 12% (69 participants) underwent hip replacement (HR), an increase of ROA of the hip (Kellgren and Lawrence score≥2) from 19% to 49%, and an increase in clinical hip OA according to the American College of Rheumatology criteria from 27% to 43%. All Western Ontario and McMaster Osteoarthritis Index subscales and physical activity remained on average constant during the 10-year follow-up for those who did not undergo an HR. The use of pain medication increased from 43% at baseline to 50% after 10 years.

Conclusion One out of nine participants with early hip problems received an HR during the 10-year follow-up. Prevalence of clinical hip OA and hip ROA increased steadily during the 10-year follow-up. Overall, we observed more hip OA, but fewer or stable complaints with respect to clinical signs and symptoms, and physical functioning. So it could be cautiously concluded that after 10 years, first-time presenters with hip complaints either received an HR or their symptoms remained stable.

Key messages

What is already known about this subject?
- The natural course of hip complaints that may be indicating hip osteoarthritis (OA) over time is still poorly characterised.

What does this study add?
- This study provides long-term (10-year) follow-up data about the clinical signs and symptoms of hip OA in people with hip pain.
- One out of nine participants with early hip problems underwent a hip replacement during the follow-up.
- Prevalence of clinical hip OA and radiographic hip OA increased steadily during the 10-year follow-up, but complaints remained stable.

How might this impact on clinical practice or future developments?
- This study provides more information on long-term outcomes to determine the course of progression of hip OA.

INTRODUCTION

Osteoarthritis (OA) of the hip is a common problem in Western society and a common diagnosis in primary care.[1] Hip OA affects 7%–25% of people older than 55 years.[2] The number of affected hips will increase with further ageing of the population.[2] Pain in the hip and hip stiffness are the most common symptoms of hip OA.[3] Consequently, patients are restricted in their activities, which has an impact on the health-related quality of life. For a disease so common and with an enormous impact on the affected patients, remarkably little is known about the natural course of early signs of hip OA. Most previous studies investigated the natural course of hip complaints combined with knee complaints.[4 5] Knee OA is more common than hip OA and has been much more often studied.[6] As a result, the natural course of hip complaints that may be indicating hip OA over time is still poorly characterised.

For many patients, the primary care physician (PCP) is the first physician they consult in their hip OA process. Therefore, it is important to investigate the natural course of hip complaints so that the PCP can start the most relevant non-surgical management, and inform what to expect. The aim of the present study was to describe the natural course of hip complaints with respect to clinical signs and symptoms, physical functioning and radiographic osteoarthritis (ROA) features during the 10-year follow-up of middle-aged first presenters with hip complaints.[7]

PATIENTS AND METHODS

General design

The data for this study were acquired from the Cohort Hip and Cohort Knee (CHECK) study; details on this cohort have been published elsewhere.[8] In short, the CHECK study is a prospective, 10-year follow-up cohort in the Netherlands of 1002 first presenters with hip and/or knee pain. Individuals entered the cohort between October 2002 and September 2005. Inclusion criteria for the CHECK study were (1) stiffness and/or pain in the hip and/or knee, (2) age of 45–65 years, and (3) participants who had not yet consulted their PCP

for these symptoms or (4) the participants' first consultation was within 6 months of entry. Exclusion criteria were (1) other pathological conditions that could explain the existing complaints (eg, other rheumatic disease, previous hip/knee joint replacement, congenital dysplasia, osteochondritis dissecans, intra-articular fractures, septic arthritis, Perthes' disease, ligament or meniscus damage, plica syndrome and Baker's cyst); (2) comorbidity that would not allow physical evaluation during the follow-up; (3) malignancy in the past 5 years; and (4) inability to understand the Dutch language.

Study population

We included participants reporting hip pain at baseline. All participants were divided into subgroups twice; the first subgroups were a subgroup who reported hip pain (yes/no) only at baseline (H group) and a subgroup who reported hip and knee pain (yes/no) at baseline (H&K group). Subsequently, all participants (regardless of whether they reported hip and knee pain at baseline) were divided into a second subgroup based on whether they underwent a hip replacement (HR) during follow-up (HR group) or did not receive an HR (no-HR group).

Outcome variables

Information about pain and other symptoms, physical functioning, education level, height and weight (to calculate body Bass Index (BMI)), comorbidity, quality of life and psychosocial factors was collected at five different points (at baseline, after 2 years (T2), T5, T8 and T10). The information was collected by means of self-reported questionnaires and a physical examination. The Western Ontario and McMaster Osteoarthritis Index (WOMAC) was used to measure pain, stiffness and physical functioning (higher score indicating worse health). Pain intensity was assessed using the Numerical Rating Scale (NRS) for pain intensity (range 0–10, higher scores indicating more pain). The participants were asked to score the pain intensity they experienced in their most painful joint over the past week and to score the present pain intensity in the same joint. At T5, T8 and T10, the participants were asked to report pain intensity related to the left and right hips for the past week. Of these measurements, we used the highest scores as the pain intensity outcome. In the physical examination, hip pain during flexion of the joint (yes/no) and pain during internal rotation of the hip (yes/no) were recorded. In addition, patients were asked whether they had morning

Table 1 Baseline characteristics (mean (SD) or number (%)) of the study population and the subgroups

Baseline characteristics/factors	Total study population	H group	H&K group	HR group	No-HR group
Number of participants	588	170	418	69	518
Age (years), mean (SD)	55.8 (±5.3)	55.7 (±5.6)	55.8 (±5.2)	57.4 (±4.8)	55.6 (±5.3)
Female, n (%)	475 (81)	129 (76)	346 (83)	48(70)	426 (82)
Caucasian, n (%)	578 (99)	169 (99)	409 (98)†	68 (100)	509 (98)
Body Mass Index (kg/m^2), mean (SD)	26.1 (±4.1)†	25.5 (±3.5)†	26.4 (±4.2)†	25.8 (±3.8)†	26.2 (±4.1)†
Education level, n (%)	†	†	†	†	†
► Primary	107 (19)	25 (15)	82 (20)	16 (24)	91 (18)
► Secondary	267 (47)	776 (46)	191 (47)	33 (49)	233 (46)
► Higher	199 (35)	65 (39)	134 (33)	18 (27)	181 (36)
Never smoked, n (%)	175 (30)†	56 (34)†	119 (29)†	29 (43)†	146 (29)†
No use of alcohol, n (%)	125 (22)†	30 (18)†	95 (23)†	17 (25)†	108 (22)†
Use of any pain medication, n (%)	250 (43)†	63 (38)†	187 (46)†	29 (43)†	221 (44)†
Three or more comorbidities, n (%)	152 (26)†	33 (20)†	119 (29)†	5 (7)†	147 (29)†
Baseline NRS (0–10) past week, mean (SD)	3.7 (±2.1)†	3.4 (±2.2)†	3.8 (±2.1)†	4.1 (±2.4)†	3.6 (±2.1)†
Baseline NRS (0–10) present pain, mean (SD)	3.2 (±2.1)†	2.8 (±2.0)†	3.4 (±2.1)†	3.8 (±2.4)†	3.2 (±2.0)†
Morning stiffness in the hip <60 min, n (%)	326 (55)	101 (59)	225 (54)	43 (62)	282 (54)
Knee pain, n (%)	418 (71)	0 (0)	418 (100)	31 (45)	386 (75)
Physically active (>30 min) for three or more times a week, n (%)	316 (55)†	103 (62)†	213 (53)†	34 (52)†	282 (56)†
WOMAC, mean (SD)					
► Pain (0–20)	5.4 (±3.4)†	4.8 (±3.2)†	5.7 (±3.5)†	5.7 (±3.9)†	5.4 (±3.4)†
► Stiffness (0–8)	2.8 (±1.7)†	2.5 (±1.7)†	2.9 (±1.7)†	2.9 (±1.6)†	2.8 (±1.7)†
► Physical function (0–68)	17.2 (±12.0)†	14.7 (±11.1)†	18.3 (±12.2)†	20.1 (±12.5)†	16.9 (±11.9)†
► Total sum score (0–100)	26.4 (±16.8)†	22.6 (±15.4)†	28.0 (±17.1)†	29.9 (±17.9)†	26.0 (±16.6)†
Radiographic severity K/L grade ≥2 either hip, n (%)	110 (19)†	38 (23) †	72 (17)†	37 (54)	73 (14)†
Radiographic severity K/L grade ≥2 either knee, n (%)	76 (13)†	14 (8)†	62 (15)†	12 (17)	64 (13)†
Clinical hip OA,* either hip, n (%)	160 (27)†	51 (30)†	109 (26)†	34 (49)	125 (24)†
Clinical knee OA,* either knee, n (%)	206 (35)†	0	206 (50)†	12 (17)	194 (38)†
Physical examination, n (%)					
► Painful internal rotation, either hip, n (%)	322 (55)†	101 (60)†	221 (53)†	50 (73)	271 (53)†
► Painful flexion, either hip, n (%)	315 (54)†	94 (56)†	221 (54)†	48 (71)†	267 (52)†

Values are mean value±SD or percentages (%).
Participants can be part of two subgroups, for example, H group and HR group.
*According to the clinical criteria of the American College of Rheumatology.
†≤4.3% missing.
H, subgroup who reported hip pain only at baseline ; H&K, subgroup who reported hip and knee pain at baseline; HR, group who underwent a hip replacement during follow-up; K/L, Kellgren and Lawrence; NRS, Numerical Rating Scale; WOMAC, Western Ontario and McMaster Osteoarthritis Index.

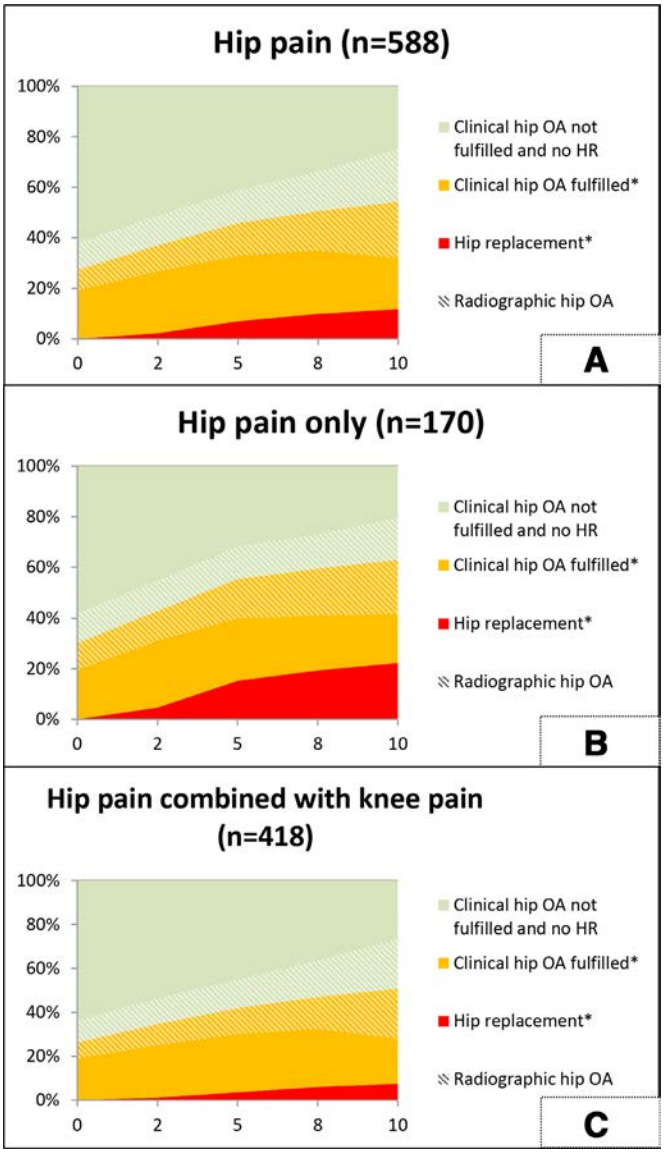

Figure 1 Overview of percentages of participants (reported hip pain at baseline (A), reported only hip pain at baseline (B) and reported both hip and knee pain at baseline (C)) with hip replacement,clinical hip OA according to the ACR criteria and radiographic hip OA according to Kellgren and Lawrence score ≥2 over 10 years of follow-up. *cumulative. A: at t0 4 missing participants, at T2-T10 3 missing participants. B: at t0 1 missing participant. C: at t0 -T10 3 missing participants. OA, osteoarthritis.

stiffness in the hip (yes/no) and if they had hip and knee pain (yes/no).

At baseline, T2, T5, T8 and T10, standardised radiographs were collected of the anteroposterior view, pelvis view or unilateral faux profile view of both hips and of the tibiofemoral joints (both knees). The radiographs were centrally scored for OA features[9] according to the Kellgren and Lawrence (K/L) criteria[10] and for OA features according to criteria described by Altman and Gold.[11] In the hip, all radiograph features showed good interobserver reliability.[9] Radiographic hip or knee OA was defined as K/L grade≥2.[12] Information on HR and/or knee replacements was obtained from radiographs. Clinical hip and knee OA were determined according to the criteria of the American College of Rheumatology (ACR), which for the hip are hip pain and all of the following criteria under 1 or 2: (1) hip internal rotation of ≥15°, pain present on internal rotation of the hip, morning stiffness (≤60 min) and aged >50 years; (2) hip internal rotation of <15° and hip flexion of ≤115°.[13] The ACR criteria for clinical knee OA are knee pain and ≥3 of the following symptoms: (1) aged >50 years, (2) morning stiffness (<30 min), (3) crepitus on active motion, (4) bony tenderness, (5) bony enlargement and (6) no palpable warmth of synovium.[14] If a participant fulfilled these clinical ACR criteria at least once during follow-up, they were classed as a clinical hip/knee OA participant.

Statistical analysis

Descriptive statistics were used to analyse the baseline characteristics and the course of the variables. The last observation carried forward (LOCF) from the last visit prior to HR was used for a subanalysis in the HR group to explore the course of symptoms if, as a thought experiment, HR had not been available (HR group with LOCF). Statistical analyses were performed using SPSS V.24.0 for Windows.

RESULTS

General characteristics

In total, 588 of the 1002 participants reported hip pain at baseline. Of these 588 included participants, 170 participants (29%) reported only hip pain, 418 participants (71%) reported both hip and knee pain and 81 participants were lost to follow-up (online supplemental figure 1). Table 1 summarises the characteristics of the study population at baseline. At baseline, the mean age was 55.8 (SD=5.2) years; the mean BMI was 26.1 (SD=4.1) kg/m^2; and 81% was female. Most prominently, it shows that 19% of the participants had ROA of the hip, and 27% of the participants met the clinical ACR criteria for hip OA. More participants of the H group met the clinical hip OA criteria (30%) and showed ROA (23%) compared with participants of the H&K group at baseline (26% and 17%, respectively). During follow-up, 249 participants (43%) fulfilled the criteria at ≥1 assessment. At baseline, 48 out of 160 participants (30%) with clinical hip OA also showed ROA in at least one hip.

Clinical and radiographic hip OA during follow-up

After 10 year, 131 out of 249 participants (53%) with clinical hip OA had ROA in at least one hip. Of the participants without clinical hip OA at baseline, 62 out of 424 (15%) showed ROA in at least one hip at baseline and after 10 year 122 out of 267 (46%) did so. Most HRs (58 out of a total of 69) took place between T2 and T8. Figure 1A shows the course of these outcomes, taking into account that a participant can only belong to one outcome group at each time point. During follow-up, clinical hip OA and ROA increased and more people received HRs (figure 1A).

Compared with participants of the H&K group, participants of the H group were as likely to meet the clinical hip OA criteria after follow-up (41% vs 43%) and were as likely to show ROA (48% vs 49%). After 10 years, participants of the H group were more likely to undergo an HR compared with participants of the H&K group (22% vs 7%). Figure 1B, C show the course of these outcomes, taking into account that a participant can only belong to one outcome group at each time point.

Clinical signs, symptoms and physical functioning during the 10-year follow-up

Table 2 and online supplemental table 1 summarises the findings from 10 years follow-up. After 10 years, only 51% still reported hip pain and fewer participants reported morning stiffness in the hip (55% at baseline vs 45% after 10 years) than at baseline.

Table 2 Course of pain, physical functioning and radiographic OA features during follow-up, for total study group (n=588)

	Baseline	T2	T5	T8	T10
WOMAC, mean (SD)					
► Pain (0–20)	5.4 (±3.4)	5.2 (±3.5)	5.1 (±4.0)	4.5 (±3.6)	4.7 (±3.8)
► Stiffness (0–8)	2.8 (±1.7)	2.6 (±1.6)	2.8 (±1.8)	2.4 (±1.8)	2.6 (±1.9)
► Physical function (0–68)	17.2 (±12.0)	16.4 (±12.0)	17.6 (±12.0)	16.3 (±13.0)	16.2 (±13.1)
NRS past week, mean (SD)	3.7 (±2.1)	3.7 (±2.3)	3.2 (±2.6)	2.7 (±2.5)	2.9 (±2.6)
Use any pain medication, n (%)	250 (43)	263 (48)	251 (47)	250 (49)	249 (50)
Hip pain, n (%)	588 (100)	374 (68)	301 (57)	267 (54)	247 (51)
Knee pain, n (%)	418 (71)	361 (65)	327 (62)	297 (58)	264 (53)
Morning stiffness (hip) <60 min, n (%)	326 (55)	287 (52)	272 (51)	239 (47)	228 (45)
Physically active (>30 min) for ≥3 times a week, n (%)	316 (55)	319 (60)	292 (56)	296 (58)	276 (56)
Cumulative sum of HR, n (%)	0 (0)	13 (2)	41 (7)	58 (10)	69 (12)
Cumulative sum of KR, n (%)	0 (0)	0 (0)	4 (1)	9 (2)	10 (2)
K/L grade ≥2 either hip, n (%)	110 (19)	128 (22)	151 (28)	183 (35)	253 (49)
Clinical knee OA* either knee, n (%)	206 (35)	278 (47)	323 (55)	349 (59)	366 (62)
Painful internal rotation either hip, n (%)	322 (55)	197 (36)	190 (38)	166 (36)	179 (39)
Painful external rotation either hip, n (%)	160 (35)	86 (17)	115 (20)	86 (15)	89 (20)
Painful flexion either hip, n (%)	315 (54)	227 (42)	192 (39)	149 (32)	159 (35)

Values are mean value±SD, or number (percentages, %).
*According to the clinical criteria of the ACR; once those clinical ACR criteria are satisfied, the case will be seen as clinical hip or knee OA.
ACR, American College of Rheumatology; HR, hip replacement; K/L, Kellgren and Lawrence; KR, knee replacement; NRS, Numerical Rating Scale; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Osteoarthritis index.

In comparison with baseline, lower hip pain intensity over the past week was observed (3.7 (SD=2.1) vs 2.9 (SD=2.6)). Slight differences between baseline and the follow-up were observed in all WOMAC subscales. The use of any pain medication increased during follow-up (43% at baseline vs 50% after 10 years). The number of individuals who were physically active (>30 min≥3 times/week) stayed stable over time (55% at baseline vs 56% after 10 years). During follow-up, the number of participants with clinical hip OA increased, as well as the participants with clinical knee OA (table 2). We observed a decrease in painful movements of the hip: painful internal rotation, external rotation and flexion of the hip are reported more frequently at baseline compared with the follow-up (table 2). Compared with participants of the H group, participants of the H&K group reported slightly higher scores for pain (intensity), stiffness and physical function during follow-up (online supplemental table 1).

Clinical signs, symptoms, physical functioning and HRs

Online supplemental table 2 summarises the findings for the 10-year follow-up of the HR group and no-HR group. In addition (online supplemental table 2) shows the results for HR group with LOCF. Participants of the HR group have higher prevalence of ROA and were more likely to have met the clinical hip criteria at baseline. After 10 years, participants of the HR group reported lower pain intensity (both on the NRS, −1.9 points after 10 years) and on the WOMAC subscale (−2.6 points after 10 years, in analysis without LOCF) and lower scores for physical function (−8.1 points on WOMAC physical function after 10 years, in analysis without LOCF) compared with baseline. Use of any pain medication after 10 years seemed to decrease in persons of the HR group and increased in the no-HR group. Regarding the physical examination results, we observed an increase in painful movements of the hip in participants of the HR group: painful internal rotation, external rotation and flexion of the hip are reported more frequently after 10 years compared with the baseline (online supplemental table S2). However, at T10, only a small number of participants underwent a physical examination in the HR group. Figure 2 shows the course of (hip) pain intensity over the past week, figure 3 the course of ROA and figure 4 the course of clinical hip OA in the years preceding the HR (for the HR group). We observed higher pain intensity for the past week before HR compared with the pain intensity during the follow-up with an HR (figure 2). The percentage with ROA increased in the years before the HR; only a small proportion of participants had severe ROA (19%–35% K/L 3 and 0% K/L 4 obtained from radiographs before HR) (figure 3). Most participants met the clinical hip OA criteria (69%–88%) before undergoing an HR (figure 4).

Figure 2 Overview of self-reported NRS pain score in past week for participants who received an HR, represented per time point when they received an HR. Left of the red box (= when HR is seen on radiograph) pain scores before HR with intervals of 2 or 3 years,and right of the red box the pain scores with HR. The last row represents the weighted average pain scores for all participants.

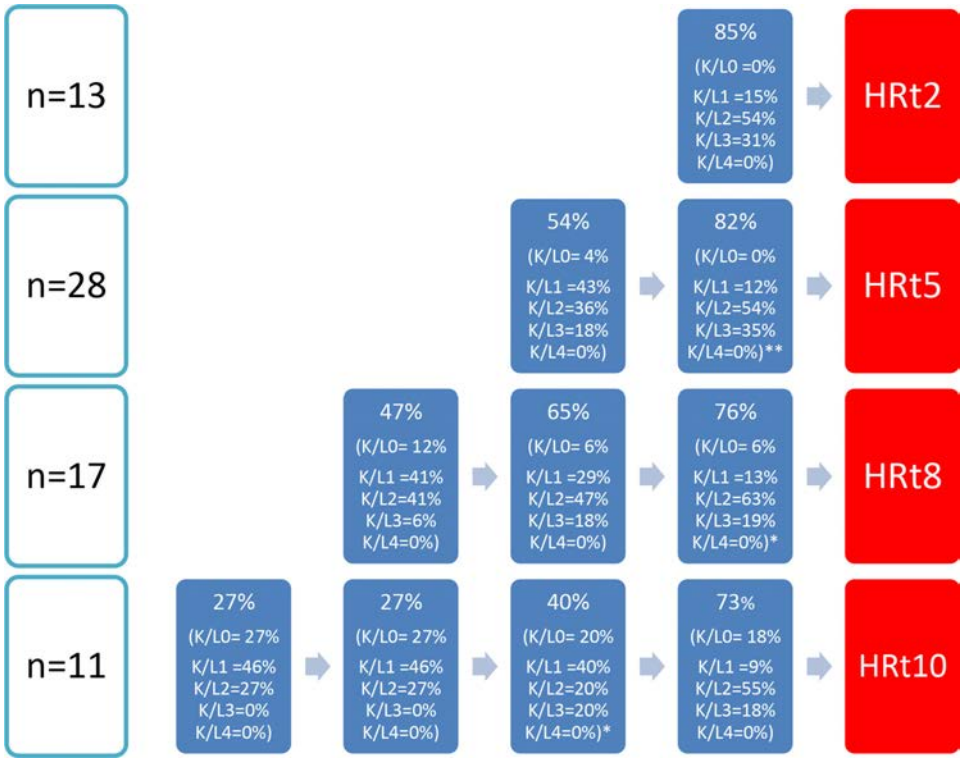

Figure 3 Overview of percentages of participants with radiographic hip OA according to Kellgren and Lawrence score ≥2 and noted in brackets the highest Kellgren and Lawrence score of any hip in time intervals of 2 or 3 years beforeHR. *=1 missing, **=2 missing.

DISCUSSION

We observed that 12% of first-time presenters with hip complaints underwent an HR during 10 years follow-up. Furthermore, the prevalence of both clinical hip OA and ROA increased during follow-up. We observed less participants reporting hip pain, on average stable pain intensity, stable WOMAC pain scores and less reported pain during physical examination after 10 years compared with baseline, for those who did not undergo an HR. On the other hand, more participants were using any pain medication during follow-up. In general, the participants of the HR group had relatively higher pain intensity and higher prevalence of ROA and clinical hip OA before receiving the HR.

The WOMAC subscales and physical activity remained stable over time, which is in line with other longitudinal studies.[15–17] This may be based on regression to the mean, but it is also possible that at a group level, study participants truly do not get worse over time. Another explanation could be the response shift phenomenon,[18] indicating that as time goes on, individuals learn to cope with their chronic disease. Many participants with mild OA have worse periods with more complaints followed by better periods with fewer complaints, and a response shift could have occurred in the self-reported questionnaires. Previous studies using trajectories showed that, on average, the majority remained stable, but they also showed that some of the subjects improved and stayed improved.[19 20] A remarkable result is the decrease in the number of patients reporting hip pain (except for the HR group). This might be due to the fact that all participants started with pain at baseline, and therefore, the number of participant with hip pain could only stay stable (still have pain) or decrease at follow-up measurements. Furthermore, decreasing pain levels during physical examination over a period of 10 years is in line with previous studies[21–23] and is a logical consequence of less hip pain.

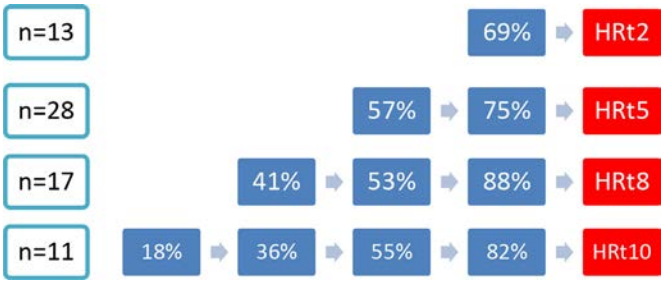

Figure 4 Overview of percentages of participants with clinical hip OA in time intervals of 2 or 3 years before HR.

We observed an increased use of any pain medication during follow-up. This increment could be an additional explanation for the stable WOMAC scores and an even decreasing trend in pain during physical examination. More experience in when to use pain medication and a positive response during flares might influence the overall pain intensity in patients with hip OA. This increase in the use of pain medication is in line with other literature. A study that investigated pain medication for knee OA also showed an increased use of paracetamol and non-steroidal anti-inflammatory drug over 3 years of follow-up.[24] Other studies have shown that the use of (over-the-counter) pain medication among a general population has increased (modestly) over the past decades.[25 26]

In line with a previous study,[27] we showed that patients with hip pain can have ROA without fulfilling the ACR criteria for clinical hip OA and vice versa. Our findings of the number of participants undergoing an HR are similar to those of other studies; a study with a 6-year follow-up reported rates for receiving an HR of 22%.[28] This result is even higher than our findings, despite the shorter follow-up, but the mean age and the amount of ROA at baseline were higher in that particular study. Besides the increase in clinical hip OA, we also showed

an increase in clinical knee OA in patients with hip pain at baseline. This might indicate that we have included some participants who initially had knee problems, as a recent study suggested that hip flexion and internal rotation might be affected by early knee OA.[29] Thereby, we defined clinical hip (and knee) OA from the moment participants fulfilled the clinical ACR criteria; however, it is known that patients intermittently fulfilled the criteria over longer follow-up.[20] It should also be mentioned that the ACR criteria are widely used in epidemiological research but are not validated in primary care.[30]

Twelve percent of the our study population underwent an HR during follow-up. It could be argued that an HR was justified for these participants because of the progression of their pain intensity, and the majority had ROA and/or clinical hip OA preceding the HR. Nevertheless, they still did not have very high levels of pain intensity, and relatively only a small proportion of participants with K/L≥2 had severe ROA. So it could still be considered as mild hip OA. The greatest benefit from joint replacement is expected if the procedure is restricted to patients with more severely affected functional status and more severe ROA.[31] As shown in online supplemental table 2, it seems unlikely that participants of the HR group are suppressing the scores for the total group, because the outcomes for pain and physical function for our total group and the no-HR group are quite similar.

To our knowledge, this is the first study that provides long-term follow-up data about the clinical signs and symptoms of hip OA in people with hip pain. The strengths of the present study are that it is a population-based prospective longitudinal design, with a large sample of persons with early-stage symptomatic hip OA, monitored from the onset of disease management in primary care and a follow-up of 10 years. A limitation of the study is that, although participants were asked where the pain was located, participants were not asked to fill in the WOMAC questionnaire (at each follow-up moment) and NRS (at baseline and T2) for a specific joint. Therefore, we do not know if the NRS score, measured at baseline and at T2 for the participants who reported both hip and knee pain, was really pain due to hip symptoms. To solve this problem as well as we could, we selected participants with only hip pain at baseline as a subgroup, but still misclassification is possible. A second limitation to our study is that we had follow-up assessments every 2 or 3 years, at which we asked the participants about the pain intensity in the past week. So these results are not representative for the entire follow-up time. Future research with more frequent symptom assessment could solve this problem. Finally, pain could be reduced by other treatments. We do not have information on the specific treatments people had.

This study provided more background information about the natural course of hip complaints during 10 years of follow-up in first presenters with hip complaints. In conclusion, we observed that the prevalence of clinical hip OA and ROA increased. After 10 years of follow-up, one out of nine (11.7%) participants had undergone an HR. Overall, we observed more hip OA, but less participants reported hip pain. Complaints with respect to clinical signs and symptoms and physical functioning remained stable. It could be cautiously concluded that after 10 years, first-time presenters with hip complaints either underwent an HR, or their symptoms remained stable or improved slightly. Further research should aim to investigate how the course of pain intensity in individuals changes over time and what factors are associated with the fluctuation of pain intensity.

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CLINICAL SCIENCE

Constitutional morphological features and risk of hip osteoarthritis: a case–control study using standard radiographs

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ABSTRACT

Objectives To evaluate the risk of association with hip osteoarthritis (OA) of 14 morphological features measured on standard antero-posterior pelvis radiographs.

Methods A case–control study of 566 symptomatic unilateral hip OA cases and 1108 controls without hip OA, using the Genetics of OA and Lifestyle database. Unaffected hips of cases were assumed to reflect pre-OA morphology of the contralateral affected hip. ORs with 95% CI adjusted for confounding factors were calculated using logistic regression. Hierarchical clustering on principal component method was used to identify clusters of morphological features. Proportional risk contribution (PRC) of these morphological features in the context of other risk factors of hip OA was estimated using receiver operating characteristic analysis.

Results All morphological features showed right–left symmetry in controls. Each feature was associated with hip OA after adjusting for age, gender and body mass index. Increased sourcil angle had the strongest association (OR: 6.93, 95% CI 5.16 to 9.32). Three clusters were identified. The PRC varied between individual features, as well as between clusters. It was 35% (95% CI 31% to 40%) for all 14 morphological features, compared to 21% (95% CI 19% to 24%) for all other well-established risk factors.

Conclusions Constitutional morphological variation strongly associates with hip OA development and may explain much of its heritability. Relevant morphological measures can be assessed readily on standard radiographs to help predict risk of hip OA. Prospective studies are required to provide further support for causality.

Key messages

What is already known about this subject?

► Several constitutional variants of hip joint shape associate with increased risk of hip osteoarthritis (OA). However, whether these relate to each other, and the overall contribution of morphological variants to risk of hip OA are unknown.

What does this study add?

► Fourteen morphological features of the hip and pelvis, ten of which had not been studied before, were shown to associate with hip OA after adjusting for age, gender and body mass index. The strongest association was with more vertical sourcil angle (SA). Three clusters of features were identified, and the proportional risk contribution to hip OA was 35% for the combined variants, compared with 21% for other recognised risk factors combined.

How might this impact on clinical practice or future developments?

► Although prospective studies are required to provide further support for causality, morphological variation is a strong risk factor for hip OA and may partially explain its heritability. SA measured on standard radiographs may be used as a single surrogate marker to assess morphological risk of hip OA.

INTRODUCTION

Osteoarthritis (OA) is a common complex disorder with multiple interactions between genetic, constitutional and environmental risk factors.[1] Strong genetic contribution to hip OA is supported by 60% heritability in a classic twin study in women,[2] and a fivefold increased prevalence of radiographic hip OA in siblings of people with hip OA requiring total hip replacement.[3] Morphological variation of the hip and pelvis is also emphasised as a potentially important constitutional risk factor for hip OA.[4–9]

It is recognised that rare monogenic abnormalities of bone shape such as severe acetabular dysplasia can cause young-onset hip OA.[10] However, it is possible that more subtle variations in joint and bone morphology, resulting from multiple common gene polymorphisms, may impose biomechanical insult and partially explain genetic predisposition in common hip OA. This is supported by studies showing that mild hip dysplasia,[5] non-spherical femoral head ('pistol grip' deformity)[4 11] and high or low neck shaft angle (NSA)[4 10] are relatively common and associate with increased risk of hip OA. Studies using statistical shape modelling also report associations between variations in proximal femoral shape and risk of hip OA.[12–14] It is also noteworthy that three genetic associations with large joint OA confirmed with genome-wide significance (GDF5,[15 16] FRZB[17 18] and MCF2L[19]) are involved in early skeletal growth. Furthermore, hip OA frequently occurs without OA at other sites,[20 21] supporting the importance of local factors in its development.

Previously we used the Genetics of OA and Lifestyle (GOAL) database to demonstrate that mild acetabular dysplasia (assessed by acetabular depth (AD), centre edge angle (CEA)),[5] non-spherical femoral head shape (assessed by femoral head to femoral neck ratio (FHNR))[4] and both high and low NSA[4] associate with hip OA. Because morphological features can be secondary to hip OA, we undertook measures of the unaffected hip of people with unilateral hip OA under the assumption that this reflects the constitutional morphology of the affected hip prior to hip OA development. This assumption was supported by right–left symmetry in normal controls without hip OA.[4 5] However, these and other morphological features may relate to, or interact with each other to increase risk of hip OA. In addition, the proportional risk contribution (PRC) of local morphological features in the context of overall risk of developing hip OA is unknown. The objectives of this study were to: (1) examine 10 additional morphological features of the hip and pelvis that can be measured readily on plain radiographs, for right–left symmetry and age variation; and (2) measure their risk contributions, both individually and in combination with others, and in the context of other recognised risk factors for hip OA. The new features we assessed were: femoral head diameter (FHD)[22]; femoral neck length (FNL)[23] and femoral neck width (FNW)[6 23 24]; femoral head offset (FHO)[25]; femoral outer shaft diameter (OSD) and inner shaft diameter (ISD); sourcil angle (SA)[26 27]; mid-centre distance (MCD); and pelvic width (PW) and pelvic height (PH).

METHODS

Cases and controls

All participants (566 unilateral hip OA cases and 1108 non-OA controls) were selected from the Nottingham GOAL database, which was a hospital-based case–control study to investigate genetic associations and gene-environmental interaction in people with knee or hip OA. Fifty-nine per cent of unilateral hip OA individuals had right hip OA and 41% had left hip OA. The laterality of unaffected hips was matched in the same ratio to controls. All participants were Caucasian and aged between 45 and 80 years. Details of recruitment, exclusion criteria, questionnaire and clinical and radiographic assessments of participants have been published previously.[4 5 28 29]

Radiographic assessment of hips

A standard protocol was used to obtain antero-posterior (AP) non-weight-bearing radiographs of the pelvis with the participants supine and feet internally rotated 10°.[4] All radiographs were scored previously by a single observer for radiographic features of hip OA, which included minimum joint space width (JSW).[4 5] Radiographic hip OA was defined as JSW ≤2.5 mm.[30] Those participants with unilateral hip OA, that is no symptoms and normal radiographic appearance (JSW >2.5 mm and no other OA features) in the contralateral hip, were included for morphological assessment of the unaffected hip. The asymptomatic control group (all with JSW >2.5 mm and no radiographic features of OA in either hip) underwent morphological assessment of both hips. These controls also had no symptoms or radiographic evidence (Kellgren Lawrence grade <2) of knee OA. The anatomical indices that were measured are described in table 1 and figure 1. Data for four (AD, CEA, FHNR and NSA) of these features had previously been scored by a single observer with good reproducibility,[4 5] and were reused in the current study. The 10 other new features were measured both in normal controls and participants with unilateral hip OA by a different single trained reader (HA) using HIPAX software

Table 1 Descriptions of the morphological landmarks and measurements of the hip joint and pelvic bones examined in this study

Morphological measurements	Descriptions
Centre of femoral head	The equatorial centre of the head was determined by fitting it is geometry within a concentric circle on the Perspex template of the Lequesne arthrometer.[48]
Femoral shaft axis	Two points in the centre of the femoral shaft were measured to be equidistant from the medial and lateral borders, one at the lowest part of the femoral shaft and the other one below the lesser trochanter. The line connecting these two points described the axis of the femoral shaft.
Femoral neck axis	The midpoint of the shortest segment of the femoral neck was measured to be equidistant from the superior and inferior borders. A line passing through the centre of the femoral head and the midpoint of the femoral neck described this axis.
Acetabular depth	The distance between the deepest point of the acetabular roof to a line drawn between the edge of the articular surface of the acetabulum and the upper corner of the symphysis pubis on the same side.[7]
Centre edge angle	The angle between the line from the femoral head centre to the lateral aspect of the acetabulum, and a vertical line drawn from the centre of the femoral head at right angles to the line joining the two femoral head centres.[49]
Femoral head to femoral neck ratio	The ratio of femoral head diameter divided by femoral neck width.[4]
Neck shaft angle	The angle between the femoral shaft axis and femoral neck axis.
Femoral head diameter	The maximum diameter was described by drawing a line through the central point of the femoral head and at a right angle to the femoral neck axis line.
Femoral neck width	This was the minimum femoral neck diameter, determined by drawing a line at the narrowest point of the femoral neck and at a right angle to the femoral neck axis.
Femoral neck length	The distance from the defined centre of the femoral head to the intersection of the femoral neck axis and femoral shaft axis.
Outer shaft diameter of the femur	This was defined as the full diameter of the femoral shaft, which was made at the level of half of the femoral head diameter, distal to the lesser trochanter.
Inner shaft diameter of the femur	This was measured at the level of half of the head diameter distal to the lesser trochanter. This measurement represents the thickness of the medullary canal of the femoral bone.
Mid-centre distance	The distance from the centre of the femoral head to the midline of the pelvic X-ray and perpendicular to this midline point.
Sourcil angle	The angle formed between a line extending from the medial to the lateral edge of the sourcil and a horizontal line.[27]
Femoral head offset	The distance from the centre of the femoral head to the axis of the femoral shaft in a right angle.
Pelvic width	The widest diameter of the pelvic bone on the radiograph.
Pelvic height	The greatest height of the pelvic bone at the centre of the pelvis on the radiograph.

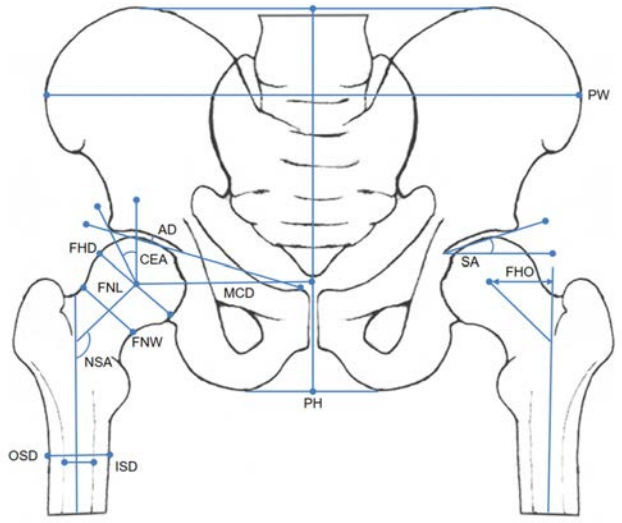

Figure 1 Morphological measurements of the hip and pelvic bones. AD, acetabular depth; CEA, centre edge angle; FHD, femoral head diameter; FHO, femoral head offset; FNL, femoral neck length; FNW, femoral neck width; ISD, inner shaft diameter; MCD, mid-centre distance; NSA, neck shaft angle; OSD, outer shaft diameter; PH, pelvic height; PW, pelvic width; SA, sourcil angle.

(Hipax, Vorstetten, Germany). As in our previous studies, this reader was blind to participant identifiers, demographic and clinical information.

Patient and public involvement

There was no patient and public involvement for this study.

Statistical analysis

The intraobserver reproducibility of measuring the 10 new morphological features was assessed using a random sample of 30 pelvis radiographs on three occasions (beginning, middle and end of study). Interobserver reproducibility was assessed by measuring 30 pelvis radiographs for two previously assessed measures (NSA and FHNR) and comparing results to those of the previous readers.[4] Intraclass correlation coefficient (ICC) was used to determine reproducibility.

Symmetry of the morphological measurements was determined using paired t-test and minimal detectable change (MDC) in the control group.[31] The difference between groups was determined using t-test (continuous data) or χ^2 test (categorical data). Correlations between the measurements were examined using Pearson correlation coefficient. The dose–response relationship of individual morphological measurements in tertiles and risk of OA was examined. Logistic regression model was used to calculate OR and 95% CIs adjusting for confounding factors such as age, gender and body mass index (BMI).

Cluster analysis was undertaken using the hierarchical clustering on principal component (HCPC) method to examine clusters of morphological measurements. HCPC was done using 'factoextra' and 'FactoMineR' packages in R.[32] Distribution of clusters was plotted in the factor map.

The PRC was estimated using receiver operating characteristic (ROC) curves where areas under the curve (AUC) were proportionalised according to risk factors.[33] First, we built the full risk model with all risk factors available in an ROC curve (AUC_f). The full risk model included established risk factors such as age, gender, weight, height, BMI, calcaneal bone mineral density (BMD), finger nodes in at least two rays of each hand, type 3 pattern of index to ring finger (2D:4D) ratio, history of hip injury, manual occupation,[4 29 34] and all 14 morphological features (ie, both the newly assessed and previously measured features in GOAL). Second, we removed the risk factor(s) of interest to examine the contribution of the risk factor(s) removed through the reduction of the ROC curve, that is, the partial AUC (AUC_p). Third, we calculated the PRC using the following formula: $PRC=(AUC_f-AUC_p)/(AUC_f-0.5)$, where 0.5 is the AUC under the diagonal line of the ROC curve indicating no discrimination at all by all included risk factors.[33] Data were analysed using STATA V.15 and R V.3.5. A significance level of $p<0.05$ was set for all analyses.

Table 2 Characteristics of the study participants

	Unilateral hip OA (n=566)	Non-OA controls (n=1108)
Age (years)	67.5±7.2	64.2±8.4**
Women (%)	47.9	46.3
BMI (kg/m^2)	29.3±5.0	27.5±4.6**
Weight (kg)	81.1±16.4	76.9±15.1**
Height (cm)	166.1±9.4	166.9±9.2
Calcaneal BMD	0.9±1.3	0.7±1.2**
Finger nodes (%)	23.1	11.6**
Type 3 2D:4D ratio (%)	41.3	34.2*
History of hip injury (%)	7.1	1.6**
Manual occupation (%)	36.9	33.9

Mean±SD or prevalence are shown.
*p<0.05, **p<0.01.
BMD, bone mineral density; BMI, body mass index; OA, osteoarthritis.

RESULTS

Characteristics of the study participants

Characteristics of study participants are shown in table 2. Of 1674 participants, 566 had unilateral hip OA (cases) and 1108 had no hip OA (normal controls). Gender, height and manual occupation were similar between groups, but cases were older and had higher weight, BMI and BMD than controls. Prevalence of nodal hand OA, type 3 pattern 2D:4D finger ratio and frequency of self-reported hip injury were also higher in the OA group.

Repeatability of measurements

In addition to the excellent reproducibility of the four features reported previously,[4 5] the 10 new features had good intraobserver agreement across the three time points, the ICCs ranging from 0.84 to 0.97 for all features ($p<0.05$). There was also good agreement between the two readers for NSA and FHNR with ICCs of 0.87 and 0.85, respectively ($p<0.05$).

Symmetry and age association in non-OA controls

In the non-OA control group the paired t-test showed that mean differences between left and right sides for most measurements were not statistically significant except for AD, CEA, ISD and MCD. However, the magnitude of these differences was less than MDC_{90} (online supplemental table S1). While age was associated with most morphological features on the left and right, it was not associated with symmetry, that is, the difference between left and right (online supplemental table S2).

Table 3 Morphological features and association with hip OA

	Frequency (%)		OR (95% CI)	
	Cases	Controls	Crude	Adjusted
Acetabular depth				
T1	273 (48.23)	285 (25.75)	1 (referent)	1 (referent)
T2	164 (28.98)	396 (35.77)	0.43 (0.33 to 0.56)**	0.45 (0.35 to 0.59)**
T3	129 (22.79)	426 (38.48)	0.31 (0.24 to 0.41)**	0.30 (0.23 to 0.39)**
P trend			<0.001	
Centre edge angle				
T1	290 (51.24)	277 (25.00)	1 (referent)	1 (referent)
T2	163 (28.80)	443 (39.98)	0.35 (0.27 to 0.45)**	0.33 (0.26 to 0.43)**
T3	113 (19.96)	388 (35.02)	0.27 (0.21 to 0.36)**	0.23 (0.17 to 0.30)**
P trend			<0.001	
Femoral head diameter				
T1	210 (37.10)	348 (31.41)	1 (referent)	1 (referent)
T2	172 (30.39)	386 (34.84)	0.74 (0.58 to 0.95)*	0.58 (0.43 to 0.79)**
T3	184 (32.51)	374 (33.75)	0.81 (0.64 to 1.04)	0.57 (0.39 to 0.84)**
P trend			0.100	
Femoral head to femoral neck ratio				
T1	239 (42.23)	326 (29.48)	1 (referent)	1 (referent)
T2	191 (33.75)	380 (34.36)	0.68 (0.54 to 0.87)**	0.65 (0.50 to 0.84)**
T3	136 (24.03)	400 (36.17)	0.46 (0.35 to 0.60)**	0.41 (0.31 to 0.56)**
P trend			<0.001	
Femoral neck length				
T1	217 (38.75)	321 (30.51)	1 (referent)	1 (referent)
T2	178 (31.79)	359 (34.13)	0.73 (0.57 to 0.94)*	0.71 (0.55 to 0.93)*
T3	165 (29.46)	372 (35.36)	0.65 (0.50 to 0.84)**	0.64 (0.48 to 0.83)**
P trend			0.001	
Inner shaft diameter				
T1	214 (39.05)	314 (31.56)	1 (referent)	1 (referent)
T2	195 (35.58)	318 (31.96)	0.89 (0.70 to 1.15)	0.79 (0.60 to 1.02)
T3	139 (25.36)	363 (36.48)	0.56 (0.43 to 0.73)**	0.44 (0.33 to 0.58)**
P trend			<0.001	
Outer shaft diameter				
T1	201 (36.68)	313 (32.86)	1 (referent)	1 (referent)
T2	176 (32.12)	332 (33.37)	0.86 (0.67 to 1.11)	0.68 (0.51 to 0.90)**
T3	171 (31.20)	336 (33.77)	0.83 (0.64 to 1.07)	0.60 (0.44 to 0.82)**
P trend			0.143	
Pelvic width				
T1	174 (37.26)	346 (31.77)	1 (referent)	1 (referent)
T2	148 (31.69)	370 (33.98)	0.79 (0.61 to 1.03)	0.70 (0.53 to 0.92)*
T3	145 (31.05)	373 (34.25)	0.77 (0.59 to 1.00)	0.60 (0.45 to 0.79)**
P trend			0.054	
Sourcil angle				
T1	90 (16.27)	464 (41.95)	1 (referent)	1 (referent)
T2	158 (28.57)	394 (35.62)	2.06 (1.53 to 2.77)**	2.11 (1.55 to 2.86)**
T3	305 (55.15)	248 (22.42)	6.34 (4.66 to 8.62)**	6.93 (5.16 to 9.32)**
P trend			<0.001	
Femoral head offset				
T1	217 (38.75)	321 (30.69)	1.57 (1.22 to 2.03)**	1.67 (1.28 to 2.19)**
T2	160 (28.57)	373 (35.66)	1 (referent)	1 (referent)
T3	183 (32.68)	352 (33.65)	1.21 (0.93 to 1.56)	1.19 (0.91 to 1.56)
P trend			NA	
Femoral neck width				
T1	184 (32.51)	377 (34.03)	1.04 (0.81 to 1.33)	1.01 (0.73 to 1.37)
T2	178 (31.45)	378 (34.12)	1 (referent)	1 (referent)
T3	204 (36.04)	353 (31.86)	1.22 (0.96 to 1.57)	1.34 (1.01 to 1.79)*
P trend			NA	

Continued

Table 3 Continued

	Frequency (%)		OR (95% CI)	
	Cases	Controls	Crude	Adjusted
Mid-centre distance				
T1	172 (30.39)	386 (34.84)	0.99 (0.77 to 1.28)	1.03 (0.79 to 1.34)
T2	173 (30.57)	385 (34.75)	1 (referent)	1 (referent)
T3	221 (39.05)	337 (30.42)	1.46 (1.14 to 1.87)**	1.43 (1.11 to 1.85)**
P trend			NA	
Pelvic height				
T1	145 (38.87)	320 (31.34)	1.45 (1.08 to 1.94)	1.51 (1.09 to 2.07)*
T2	111 (29.76)	355 (34.77)	1 (referent)	1 (referent)
T3	117 (31.37)	346 (33.89)	1.08 (0.80 to 1.46)	1.05 (0.75 to 1.47)
P trend			NA	
Neck shaft angle				
T1	209 (36.99)	366 (33.18)	1.40 (1.09 to 1.78)**	1.36 (1.05 to 1.75)*
T2	176 (31.15)	431 (39.08)	1 (referent)	1 (referent)
T3	180 (31.86)	306 (27.74)	1.44 (1.11 to 1.85)**	1.50 (1.15 to 1.96)**
P trend			NA	

Logistic regression was adjusted for age, gender and body mass index. For femoral head offset, femoral neck width, mid-centre distance, pelvic height and neck shaft angle, Tertile 2 was used as referent.
**p<0.05, **p<0.01.
NA, not applicable; OA, osteoarthritis; T, tertile.

Risk of hip OA

Table 3 represents the OR of hip OA associated with individual morphological measures. After adjustment for age, gender and BMI, the risk of hip OA increased as the tertiles for AD, CEA, FHD, FHNR, FNL, ISD, OSD, PW decreased. In contrast, SA showed a positive dose response, the risk of hip OA being seven times higher for Tertile 3 versus Tertile 1 (OR: 6.93, 95% CI 5.16 to 9.32, p<0.01).

FNW, MCD, FHO, PH and NSA showed a U-shape association with hip OA. Using Tertile 2 as the referent, the results showed that either the smaller or larger of these measures were associated with increased risk of OA. For example, either high or low NSA associated with greater risk of hip OA, ORs being 1.50 (95% CI 1.15 to 1.96) and 1.36 (95% CI 1.05 to 1.75), respectively. The results by gender are shown in online supplemental table S3.

Clusters of morphological features

The 14 morphological features were associated with each other (online supplemental table S4). Three clusters were identified within the 14 morphological features (figure 2). Cluster 1 included FHNR (non-spherical femoral head). Cluster 2 included SA, NSA, FNW and MCD. Cluster 3 included AD and CEA (ie, mild acetabular dysplasia), FHD, FNL, OSD, ISD, FHO, PW and PH. The contribution of the individual morphological features to each cluster is shown in online supplemental table S5.

Proportional risk contribution

The AUC for the full model including all risk factors was 0.81 (95% CI 0.79 to 0.83), of which 34.95% (95% CI 30.93 to 39.65) was explained by the 14 morphological features, and 21.36% (95% CI 18.62 to 24.21) was explained by all other established risk factors (table 4). Of the 14 morphological features, SA had the highest contribution (PRC=7.12%, 95% CI 6.01 to 8.07). The PRC of cluster 1, 2 and 3 was 2.26% (95% CI 1.80 to 2.46), 7.12% (95% CI 6.31 to 8.42) and 7.44% (95% CI 6.61 to 8.42), respectively.

DISCUSSION

This is the first large study to assess 14 hip and pelvis morphological features, individually and in composite, and their contribution to the risk of hip OA. The right–left symmetry of all measures demonstrated in the normal controls supports the assumption that the unaffected hip of unilateral hip OA cases represents the pre-OA morphology of the affected hip.[4 5] Although age associated with some morphological features, it was not associated with the symmetry, that is, the difference between left and right. The main findings are: (1) all 14 hip morphological features

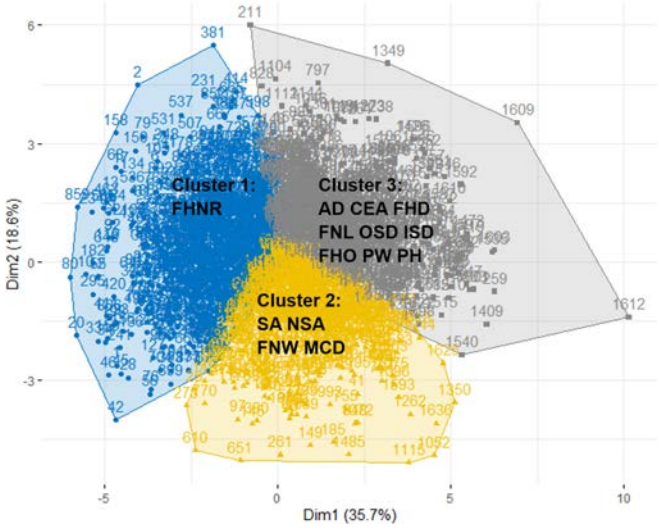

Figure 2 Morphological features were assigned into three clusters: cluster 1 includes FHNR; cluster 2 includes SA, NSA, FNW and MCD; and cluster 3 includes AD, CEA, FHD, FNL, OSD, ISD, FHO, PW, PH. AD, acetabular depth; CEA, centre edge angle; FHD, femoral head diameter; FHNR, femoral head to femoral neck ratio; FHO, femoral head offset; FNL, femoral neck length; FNW, femoral neck width; ISD, inner shaft diameter; MCD, mid-centre distance; NSA, neck shaft angle; OSD, outer shaft diameter; PH, pelvic height; PW, pelvic width; SA, sourcil angle.

Table 4 AUC and PRC of multivariate models

	AUC	95% CI	PRC (%)	95% CI
Full model	0.809	0.785 to 0.833	100	
Partial model without other risk factors	0.743	0.716 to 0.771	21.359	18.619 to 24.211
Partial model without morphological features	0.701	0.672 to 0.730	34.951	30.931 to 39.649
Partial model without SA	0.787	0.762 to 0.813	7.120	6.006 to 8.070
Partial model without FHNR	0.802	0.778 to 0.827	2.265	1.802 to 2.456
Partial model without ISD	0.803	0.777 to 0.827	1.942	1.802 to 2.807
Partial model without CEA	0.804	0.780 to 0.828	1.618	1.502 to 1.754
Partial model without FHD	0.805	0.780 to 0.829	1.294	1.201 to 1.754
Partial model without FHO	0.806	0.782 to 0.830	0.971	0.901 to 1.053
Partial model without FNW	0.808	0.784 to 0.832	0.324	0.300 to 0.351
Partial model without FNL	0.808	0.784 to 0.832	0.324	0.300 to 0.351
Partial model without NSA	0.808	0.784 to 0.832	0.324	0.300 to 0.351
Partial model without MCD	0.808	0.784 to 0.832	0.324	0.300 to 0.351
Partial model without PW	0.808	0.784 to 0.832	0.324	0.300 to 0.351
Partial model without AD	0.808	0.784 to 0.832	0.324	0.300 to 0.351
Partial model without PH	0.809	0.785 to 0.833	0	0
Partial model without OSD	0.809	0.785 to 0.833	0	0
Partial model without cluster 1	0.802	0.778 to 0.827	2.265	1.802 to 2.456
Partial model without cluster 2	0.787	0.761 to 0.812	7.120	6.306 to 8.421
Partial model without cluster 3	0.786	0.761 to 0.811	7.443	6.606 to 8.421

The full model included other risk factors and morphological features.
Other risk factors included age, gender, weight, height, body mass index, calcaneal bone mineral density, finger nodes, type 3 2D:4D finger ratio, history of hip injury and manual occupation.
Morphological features included AD, CEA, FHNR, NSA, FHD, FNL, FNW, FHO, OSD, ISD, MCD, SA, PW and PH.
AD, acetabular depth; AUC, areas under the curve; CEA, centre edge angle; FHD, femoral head diameter; FHNR, femoral head to femoral neck ratio; FHO, femoral head offset; FNL, femoral neck length; FNW, femoral neck width; ISD, inner shaft diameter; MCD, mid-centre distance; NSA, neck shaft angle; OSD, outer shaft diameter; PH, pelvic height; PRC, proportional risk contribution; PW, pelvic width; SA, sourcil angle.

associated with increased risk of hip OA independent of age, gender and BMI, with larger SA being the strongest risk factor; (2) two patterns of associations were observed—dose response and U-shaped curve response (both higher and lower values associating with increased risk); (3) three clusters were identified (figure 2); and (4) the total contribution of the 14 morphological features to risk of hip OA was greater (35%) than the sum of other recognised risk factors (21%).

Our findings of small FHD, wide FNW and short FNL as risk factors for hip OA concur with the previous studies.[6 11 14 22–24] Biomechanically many of these features have a plausible aetiological mechanism. For example, small FHD and/or wide FNW may both encourage 'cam type' impingement of the proximal femur on the acetabulum,[25] as does a non-spherical femoral head.[35] Furthermore, a small femoral head has a smaller surface area for load transmission, thus the force per unit area may be higher and cause increased joint tissue stress. On the other hand, a wide FNW may encourage 'pincer-type' impingement of the femoral head-neck junction against the acetabular rim.[25] The explanation for smaller measurements of both OSD and ISD could relate to the inverse relationship between osteoporosis and OA.[36] Low FHO and wide MCD necessitates a greater abductor muscles force to maintain body balance[37] and the resultant greater stress on the hip may predispose to OA. The association of AD, CEA, FHNR and NSA with hip OA were reported and discussed in our previous studies.[4 5]

Importantly, our findings indicated that of the 14 features studied, increased SA was the strongest individual risk factor for hip OA and showed the highest PRC. Departure of the acetabular sourcil orientation from the horizontal plane will negatively affect the equilibrium of forces across the hip joint,[26] and with bigger SA the femoral head is less covered by the acetabulum, which is consistent with the negative correlation between SA and CEA, so the unit force per surface area is increased. In previous studies, SA related more than other indices with development of OA[38 39] and it is considered a more precise measure for mild dysplasia than CEA.[40] Therefore overall, more vertical SA is a major morphological risk factor and may be used as a single surrogate marker in clinical practice to assess morphological risk of hip OA.

The 14 morphological features were assigned into three clusters. Cluster analysis may uncover relationships between measures. For example, in a case with high NSA (coxa valga), the increased inclination of the weight-bearing surface of the acetabulum (assessed by SA) can increase the compressive forces on the joint and lower the threshold for the onset of OA.[41] The coexistence of less acetabular coverage and shorter femoral neck were reported in one hip shape mode (HSM) derived by statistical shape modelling which positively associated with incident hip OA.[14] But in another HSM, more coverage of the femoral head and wider PW were found to associate with OA,[14] which is inconsistent with our findings. The higher proportion of women and the different definition of PW in that study[14] should be considered when comparing the results with ours. However, the possible explanation for the associations observed for PW and PH are open to speculation. Further prospective study for causality is still required.

The risk contribution of the 14 morphological features (PRC=35%, 95% CI 31% to 40%) was significantly larger than other established risk factors including age, gender, BMI, history of hip injury, physical occupation, nodal OA and 2D:4D finger ratio (PRC=21%, 95% CI 19% to 24%). This suggests that local morphological risk factors may contribute more than systemic factors to development of hip OA. The results align with the

literature for incidence and progression of hip OA[42 43] and may be explained by shared single nucleotide polymorphisms between OA and hip shape.[44 45]

There are several caveats to this study. First, this was a cross-sectional case–control study. Whether these morphological features cause hip OA requires a prospective population-based study. Although we used the unaffected hips of people with unilateral hip OA to determine constitutional pre-OA shape, it is possible that the morphology in the unaffected hip had adapted to altered gait pattern and abnormal loading caused by hip OA on the other side,[46] in accord with Wolff's law which states that bones adapt their mass and shape in response to loading.[47] In addition, the apparently normal hips could have undergone bone remodelling due to early OA before other features such as cartilage loss were evident.[23] Furthermore, we did not account for presence of symptoms or structural OA in other lower limb joints (knees, ankles, feet) of cases which may have affected biomechanical stress on the unaffected hip. Also radiographic assessment is less sensitive to early OA changes than other imaging modalities, such as MRI. We also found that some morphological features changed with age in the control group. Although symmetry was unaffected by age, we cannot be certain that the current features measured in unaffected hips of cases would fully reflect the pre-OA morphology on the affected side before it developed OA many years ago. Second, although we observed symmetry of morphological features in the non-disease control group, this does not exclude the possibility of asymmetry in the cases before they developed unilateral hip OA, or the presence of additional unidentified risk factors on the affected side, or protective factors on the unaffected side. This again requires a prospective cohort study to confirm whether the predisease morphological features are truly symmetrical between the left and right sides, and to determine how many people with the features of interest subsequently go on to develop bilateral hip OA. Third, the GOAL database includes only Caucasian participants so the generalisability of the findings is limited and requires study in other populations. Fourth, we undertook measurements on a single two-dimensional standard AP pelvis radiograph without other views. Although this is conventional and readily applicable to large-scale population studies, it has major limitations for identifying true morphological variations in three-dimensions. A further caveat is that measurement of morphological features was not undertaken blind of hip OA status, since pelvic images were saved on software (HIPAX) that prevents image cropping. Furthermore, despite the use of a standardised protocol, variations in positioning may have affected some assessments, for example, due to anteversion or rotation secondary to pain or deformity in the affected hip.

In conclusion, we have confirmed 14 morphological features that associate with increased risk of hip OA. The risk contribution of these features is more than that of other conventional risk factors combined. SA is the strongest risk factor and could be used as a single surrogate measure of morphological risk in large epidemiological studies or in clinical settings. Future prospective studies are required to provide further support for causality between these features and OA.

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CLINICAL SCIENCE

Machine-learning, MRI bone shape and important clinical outcomes in osteoarthritis: data from the Osteoarthritis Initiative

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ABSTRACT

Objectives Osteoarthritis (OA) structural status is imperfectly classified using radiographic assessment. Statistical shape modelling (SSM), a form of machine-learning, provides precise quantification of a characteristic 3D OA bone shape. We aimed to determine the benefits of this novel measure of OA status for assessing risks of clinically important outcomes.

Methods The study used 4796 individuals from the Osteoarthritis Initiative cohort. SSM-derived femur bone shape (B-score) was measured from all 9433 baseline knee MRIs. We examined the relationship between B-score, radiographic Kellgren-Lawrence grade (KLG) and current and future pain and function as well as total knee replacement (TKR) up to 8 years.

Results B-score repeatability supported 40 discrete grades. KLG and B-score were both associated with risk of current and future pain, functional limitation and TKR; logistic regression curves were similar. However, each KLG included a wide range of B-scores. For example, for KLG3, risk of pain was 34.4 (95% CI 31.7 to 37.0)%, but B-scores within KLG3 knees ranged from 0 to 6; for B-score 0, risk was 17.0 (16.1 to 17.9)% while for B-score 6, it was 52.1 (48.8 to 55.4)%. For TKR, KLG3 risk was 15.3 (13.3 to 17.3)%; while B-score 0 had negligible risk, B-score 6 risk was 35.6 (31.8 to 39.6)%. Age, sex and body mass index had negligible effects on association between B-score and symptoms.

Conclusions B-score provides reader-independent quantification using a single time-point, providing unambiguous OA status with defined clinical risks across the whole range of disease including pre-radiographic OA. B-score heralds a step-change in OA stratification for interventions and improved personalised assessment, analogous to the T-score in osteoporosis.

Key messages

What is already known about this subject?

- There is a huge unmet need for accurate and reliable assessment of osteoarthritis (OA) status.
- MRI has demonstrated much more pathology but has been largely constrained to reader-dependent semiquantitative assessment.
- Machine-learning enables accurate, reader-independent quantification and we have previously demonstrated it can measure a characteristic OA three-dimensional bone shape with good precision.

What does this study add?

- Through application of machine learning, this study has provided a new highly reliable and precise measure of OA status, a quantified 3D femur bone shape termed the B-score.

How might this impact on clinical practice or future developments?

- B-score should enable improved stratification for interventions, accurate classification across the range of OA severity and improved personalised assessment, analogous to the role of the T-score in osteoporosis.

INTRODUCTION

Osteoarthritis (OA) is a serious disease resulting in pain, loss of function and reduced quality of life and represents a major public health problem.[1] The pathophysiology of OA involves multiple tissues, with deterioration of both cartilage and bone considered integral to the OA process.[2] End-stage disease can be successfully treated with joint replacement, but there has been limited progress with interventions that address earlier OA stages.

OA structural pathology has conventionally been assessed using X-rays. Radiographic determination of OA structural status is imprecise due to its dependence on acquisition method and reader reliability.[3] The most common scoring system, the semiquantitative Kellgren-Lawrence grade (KLG, scored 0–4), assesses cartilage and bone as well as (indirectly) meniscal changes.[4] Semiquantitative radiographic assessment has driven our understanding of structure-symptom relationships,[5] demonstrating associations at group, but not at individual patient level.

MRI has enabled detailed understanding of three-dimensional OA structural pathology and revealed multiple pathologies not evident on X-rays. MRI provides direct quantitative assessment of cartilage and bone[6 7] and the most responsive imaging biomarkers. However, there remains a strong need for validated surrogate measures of clinically important outcomes, which provide OA status from a single time point, without longitudinal evaluation.

In areas such as hypertension and diabetes, the provision of a single, quantitative measurement has provided breakthroughs in clinical management and drug discovery. In the management of osteoporosis, the dual-energy absorptiometry-based T-score

replaced imprecise and insensitive measures based on radiographic bone assessment and photon absorptiometry, creating a single standard measure.

In the field of clinical imaging, the appearance of a tissue can be learnt and then applied to automatically find and delineate that tissue in new, unseen images.[8] Importantly, this approach is agnostic, being independent of prior expert opinion. Statistical shape modelling (SSM), a type of supervised machine-learning, employs principal component analysis to reduce complex 3D geometric shapes to a single metric value.[9] Using SSM, we have identified a characteristic OA 3D bone shape, incorporating osteophyte ridge formation and widening and flattening of the articular surfaces. This bone shape predicts radiographic onset of OA,[10] is associated with radiographic structural progression[11] and discriminates knees with OA from non-OA.[12] In each of these studies, the femur had the greatest discrimination and responsiveness, and we have focused this study on femur shape, here termed 'B-score'. To determine the value of B-score as a measure of OA status, we examined its precision, relationship with the existing radiographic standard (KLG) and explored the relationships of both B-score and KLG with clinically important outcomes: pain, function and total knee replacement (TKR) surgery.

METHODS

Quantifying tissue shape

Patient image data

Data were obtained from the Osteoarthritis Initiative (OAI), a multicentre, longitudinal, prospective observational study of knee OA; bilateral knee MR images were collected in a standardised way together with clinical data from 4796 individuals with, or at risk of developing knee OA.[13] Data are publicly available at https://data-archive.nimh.nih.gov/oai/.

High-resolution sagittal 3D dual-echo at steady-state water-excitation (DESS-we) knee MRI images were acquired on recruitment into the OAI and at 1, 2 and 4 year timepoints, using a 3T MRI system (MAGNETOM Trio, Siemens Healthcare, Erlangen, Germany). Image acquisition parameters have been published in detail.[14]

Statistical shape modelling

Femur bones were automatically segmented from DESS-we images using active appearance models (AAMs), a type of SSM trained to search images, provided by Imorphics (Manchester, UK). AAMs are proven technology that can segment knee bone surfaces with submillimetre accuracy.[12 15] AAMs were constructed using a training set, from DESS-we images, selected to provide examples of all stages of OA.[16]

We constructed an 'OA vector', defined as the line passing through the mean shape of a population with OA (OA Group, defined as all knees with KLG ≥2 at all four time points of 0, 1, 2 and 4 years) and a population without OA (Non-OA Group, defined as those with KLG of 0 at each of the same time points).

B-score

Distances along the OA vector are termed 'B-score', with the origin (B-score 0) defined as the mean shape of the Non-OA Group for each gender. 1 unit is defined as 1 SD of the Non-OA Group along the OA vector (positive values towards the OA Group). Representative examples of differences in femur bone shape at various B-scores, and a heat map of the areas which change most with increasing B-score are shown in figure 1. The range of B-scores in the Non-OA Group was defined as the 95% confidence limits of B-scores in this group, being ±1.96; this enabled delineation of the Non-OA range of B-scores in figures and analysis. Expanded details of the methods for AAM search and construction of B-score are provided in online supplemental methods.

Measurement repeatability

All visually acceptable DESS-we images from the OAI retaken on the same day were assessed; a test-retest set (1 week apart) of those with definite OA were also analysed.[12 16] Repeatability

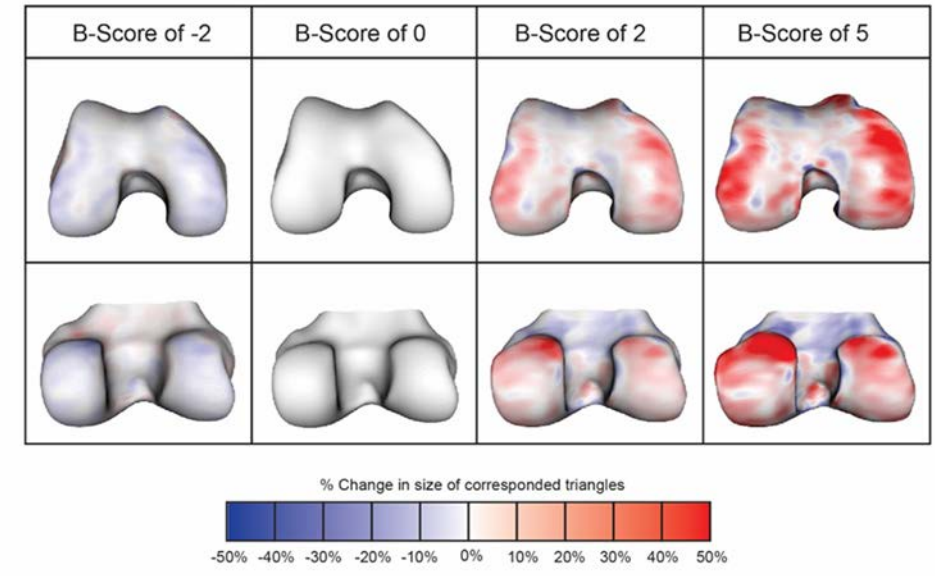

Figure 1 Figure shows change in shape for the anterior femur (top row) and posterior femur (bottom row), for various B-scores. Red indicates where there is an increase in size (locally calculated, based on anatomically corresponded triangles from the shape model), and blue indicates decrease in size (locally); scale shows percentage in area size change of each triangle. Change tends to be greatest around the edge of the cartilage plate (osteophyte region), but it also occurs in central subchondral regions where the bone flattens out.

(smallest detectable difference, SDD) was calculated as the 95% limits of agreement between the two image measurements, using the Bland-Altman method.

KLG reading was performed in the OAI using carefully acquired radiographs, with the knee positioned using a custom-designed frame allowing for a standard knee flexion angle and reporting position of the X-ray source.[17] Two expert readers independently assessed each radiograph; differences were adjudicated by a group including a more senior reader.

STATISTICAL ANALYSIS

All analyses were conducted using SAS V.9.4 (Cary, North Carolina, USA). Values for the associations with clinical outcomes are presented as proportion of the relevant population; referred to throughout as risk of a clinical outcome.

Pain by B-score and KL grade

Pain was assessed using the 7-day pain severity numeric rating scale (NRS, 0–10). Current pain was defined as NRS score at baseline, future pain as the median value of all later timepoints (up to 8 years, average follow-up 5 years). Knees were categorised as moderate pain (score ≥4) and severe pain (score ≥8).[18 19] As a sensitivity analysis, we assessed WOMAC-A pain (0–20 scale, moderate ≥4 and severe ≥8). Logistic regression analyses were performed for current and future pain as defined above against either KLG or baseline B-score, with no additional covariates.

Function by B-score and KL grade

Function was assessed using WOMAC function score (0–68), for the knee with the highest B-score per person. Current function was defined at baseline, future function as the median value at all later timepoints (follow-up as for pain). Moderate functional limitation was defined as ≥20 and severe as ≥35.[20 21] Logistic regression analyses were performed for current and future function as defined above against either KLG or baseline B-score, with no additional covariates.

Total knee replacement by B-score and KL grade

KL grade and B-score were independently assessed to determine predictors of TKR at any point during the follow-up period for an individual knee, defined as having an adjudicated TKR within a follow-up period of up to 8 years. This was assessed by modelling TKR as outcome against B-score and KLG separately using logistic regression models.

Logistic regression of KLG by B-score quartiles

To assess whether B-score provided additional information over KLG, two modelling approaches were considered. In the first, individual KLG groups were subdivided into quartiles based on B-score and assessed for the five clinical outcomes of current and future pain and function, and TKR, using logistic regression. The second approach involved initially modelling each outcome as described previously with KLG, then adding B-score to each model and assessing whether the regression coefficient for B-score was statistically significant and then calculating the resulting area under the curve (AUCs) for the combined models.

Confounders of B-score and risks of clinical outcomes

Potential confounders of the relationship between B-score and the risks of current pain, function and TKR were investigated by adjusting the models for age, sex, ethnicity, body mass index (BMI), alignment, previous knee surgery, non-steroidal anti-inflammatory drugs (NSAIDs) use and smoking status. A description of these variables is shown in the online supplemental methods section.

Table 1 Demographic and baseline characteristics

Parameter	Males N=1992	Females N=2799	Combined N=4791
Knee MRIs in the OAI dataset at baseline	n=1992	n=2799	n=4791
Both right and left	1929 (97)	2713 (97)	4642 (97)
Right only	37 (2)	49 (2)	86 (2)
Left only	26 (1)	37 (1)	63 (1)
Age (y)	n=1992	n=2799	n=4791
Mean (SD)	60.9 (9.5)	61.3 (9.0)	61.2 (9.2)
Median percentile (25th, 75th)	59 (53 to 70)	61 (54 to 69)	61 (53 to 69)
Min, Max	45 to 79	45 to 79	45 to 79
Race	n=1989	n=2797	n=4786
White	1666 (84)	2122 (76)	3788 (79)
Black or African American	276 (14)	595 (21)	871 (18)
Asian	13 (1)	32 (1)	45 (1)
Other non-white	34 (1)	48 (2)	82 (2)
Current cigarette smoker	n=1964	n=2766	n=4730
No	987 (50)	1513 (55)	2500 (53)
Yes	977 (50)	1253 (45)	2230 (47)
Use of NSAIDs at Baseline	n=1983	n=2796	n=4779
Yes	463 (23)	720 (26)	1183 (25)
No	1520 (77)	2076 (74)	3596 (75)
BMI (m/kg^2)	n=1990	n=2797	n=4787
Mean (SD)	28.8 (4.15)	28.5 (5.27)	28.6 (4.84)
Median percentile (25th, 75th)	28.5 (25.7 to 31.5)	28.1 (24.4 to 32.0)	28.2 (25.1 to 31.7)
Min, Max	18.3 to 44.6	16.9 to 48.7	16.9 to 48.7

All values are n (%) unless stated.
*BMI denotes body mass index, MRI magnetic resonance imaging, NSAIDS nonsteroidal anti-inflammatory drugs, and OAI Osteoarthritis Initiative.

RESULTS

Participant characteristics

Table 1 provides demographic and baseline characteristics. More than 96% of OAI participants had both knees assessed (total knees n=9433). Age ranged from 45 to 79 years. Median BMI was 28 kg/m^2 (range, 16.9–48.7).

Repeatability

A total of 139 knees were imaged twice on the same day within the OAI: the repeatability (SDD) of B-score in this group was 0.251 (B-score units). This group was representative of the whole OAI dataset (86 female, KLG 0, 1, 2, 3, 4 as fraction: 33%, 20%, 31%, 12%, 4%, BMI mean (SD) 30.3 (5.23); mean age (SD) 62.7 (9.45). A total of 35 knees were imaged in the test-retest set, at baseline and 1 week: SDD of B-score in these images was 0.254. This represents 2.5% of the likely range of B-scores (−3 to +7 in this study).

Relationship of B-score with KL grade

Distribution of B-score by KLG is shown in figure 2. There was a large range of B scores for each KLG, reflecting the increased measurement sensitivity of the measure, with B-score range increasing with KLG. Mean B-score had a non-linear association with KLG, increasing more rapidly at grades 3 and 4; CIs were wider with increased KLG. For example, the 95% confidence limits of B-score for a KLG3 knee (n=1237) were –0.2 and +6.0. 3.4% of KLG0 knees had B-scores greater than the non-OA range, KLG1:7.9%, KLG2:33.1% KLG3:57.6%,

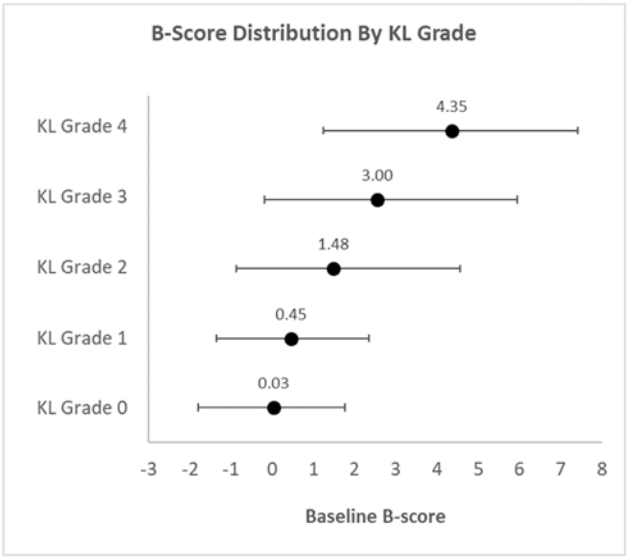

Figure 2 Distribution of B scores by KL grade are displayed for males and females (mean and 95% CIs for each grade). Mean B score for each KL grade is noted above each line.

KLG4:89.3%. Proportions of B-score bins classified by KLG are shown in online supplemental table S4 and online supplemental figure S3.

KLG and clinically important outcomes

The risk of moderate knee pain or limitation of function increased across the range of KLG from around 10% to around 60%; this was not linear, and risk increased more rapidly between KLG 3 and 4 (figure 3). Risks of severe knee pain or severe limitation of function also increased from 2% to 15% and 8% to 35%, respectively. Risk of TKR increased in a curvilinear manner, with risk increasing approximately 2.5-fold for each increase in KLG. Risk of future pain and function are shown in online supplemental figure S1.

B-score and clinically important outcomes

The risks of moderate knee pain or loss of function increased across the range of B-score from around 10% to around 60% and are curvilinear (figure 4 and online supplemental table S1). Risks of severe knee pain or severe function limitation increased similarly. Risk of TKR also increased similarly. Risks of future pain and function are shown in online supplemental figure S1. The distribution of pain, function and other OA-related factors at baseline is shown in online supplemental table S2. AUCs for the relationship of B-score and all five outcomes were comparable with those found for KLG and those outcomes (online supplemental table S3).

Additional information provided by B-score

Within KLG2-4, ORs for all clinical outcomes varied significantly between lowest and highest B-score quartiles ($p<0.001$) (for KLG3 knees, see table 2). No statistically significant differences were found between lowest and highest quartiles in KLG 0 and 1 knees. In terms of discrimination, addition of B-score resulted in improvement in the AUCs in all models, although of small magnitudes (online supplemental table S3), while the regression coefficient for B-score was statistically significant ($p<0.05$) in all models.

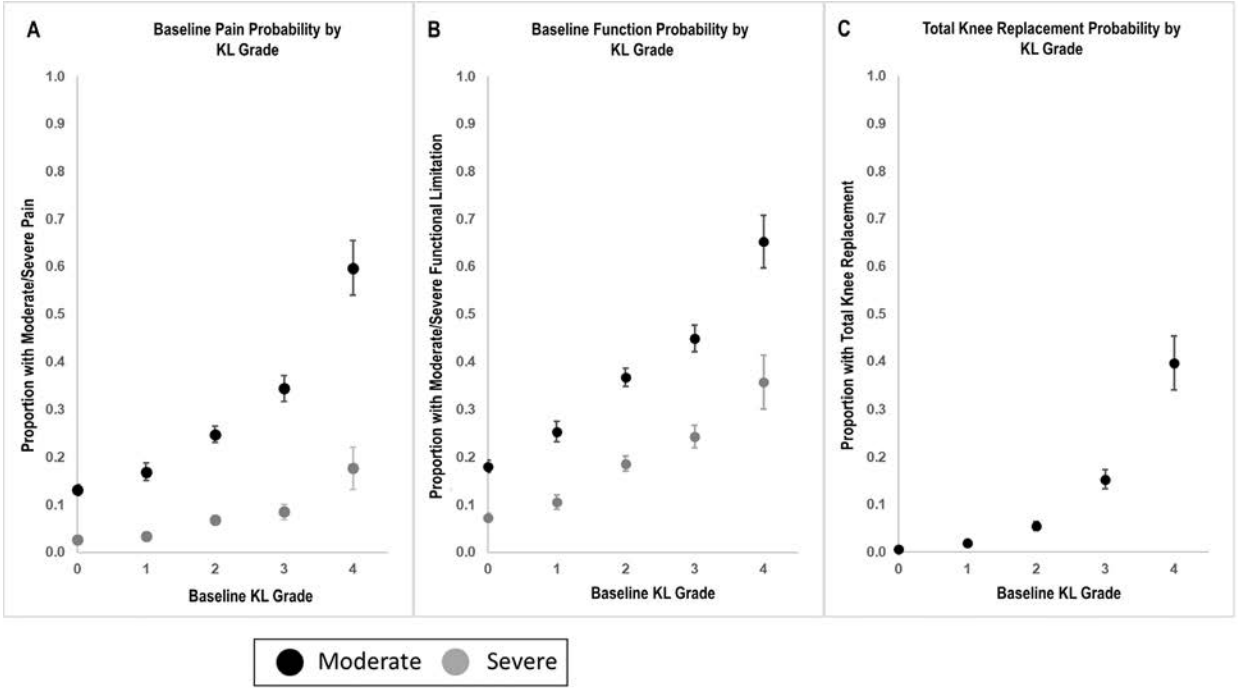

Figure 3 Error bars show 95% confidence limits for each measure. Pain: moderate or greater pain was defined as NRS pain ≥4 on the 10-unit scale (black points); severe pain as NRS pain ≥8 (grey points). Function: moderate or greater limitation of function was defined as function ≥20 on the 68-point WOMAC function scale (black points); severe loss of function was defined as ≥36 (grey points). TKR—risk of total knee replacement over follow-up period (up to 8 years, average follow up 5 years).

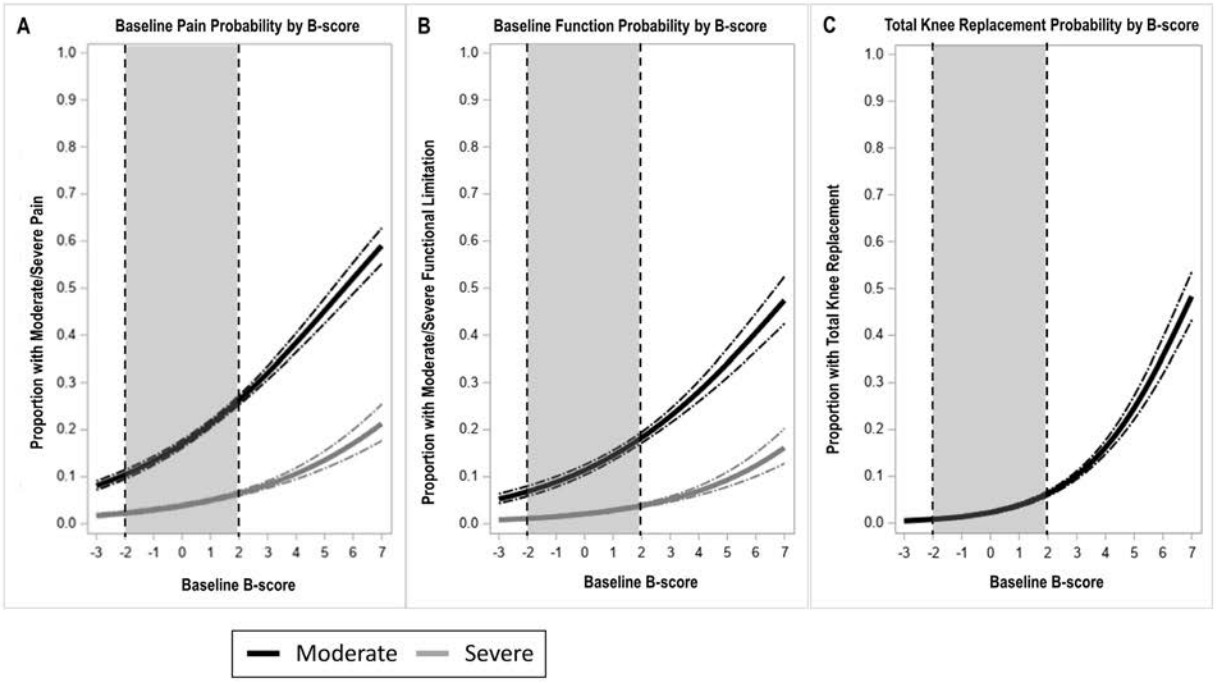

Figure 4 Error bars show 95% CIs for each measure. Moderate or greater pain was defined as NRS pain ≥4 on the 10-unit scale (black lines); severe pain as NRS pain ≥8 (grey lines). Moderate or greater limitation of function was defined as function ≥20 on the 68-point WOMAC function scale (black lines); severe limitation of function was defined as ≥36 (grey lines). TKR—risk of total knee replacement over follow-up period (up to 8 years, average follow-up 5 years). Limits of non-OA group B-scores are provided using a dotted line and greyed area.

Increased discrimination of all risks, using B-score at individual patient level

The increased utility of B-score is demonstrated by considering a KLG3 knee. The mean(CI) risk of a moderately painful knee based on this KLG was 34.4 (31.7 to 37.0)%. B-score within KLG3 knees ranged (95% CI) from 0 to 6; if the knee had a B-score of 0 the risk of a moderately painful knee was 17.0 (16.1 to 17.9)% while for a B-score of 6 it was 52.1 (48.8 to 55.4)%. The risk of a moderate limitation of function for a KLG3 was 20.6 (18.2 to 22.9)% if the knee had a B-score of 0 the risk of moderate function limitation was 11.4 (10.4 to 12.5)% while for a B-score of 6 it was 40.6 (36.6 to 44.6)%. For TKR, KLG3 knee had risks of 15.3 (13.3 to 17.3)%, whereas B-score 0 had negligible risk of TKR 2.3 (2.0 to 2.6)% and B-score six had a risk of 35.6 (31.8 to 39.6)%.

Confounders of, and additional information provided by, B-score

After adjustment for covariates the effect sizes from regression were still classified as 'small' for the risk of pain, function or TKR (online supplemental table S1).

DISCUSSION

Machine-learning has made possible the development of a quantitative measure of OA status; we have termed this the B-score. In this large observational cohort, B-score produced logistic regression models for clinically important outcomes, which were very similar in terms of predictive validity to those of the existing radiographic standard, providing construct validity for this new measure. However, by providing a scalar measure enabling

Table 2 ORs and 95% CIs for B score quartiles among KLG 3 & 4 knees, compared with the lowest B score quartile, for all current and future clinical outcomes

Outcome	B- Score Quartile 2	B-ScoreQuartile 3	B-scoreQuartile 4
Pain moderate - current	1.36 (0.95,1.94)	1.76 (1.24,2.49)***	2.4 (1.69,3.4)***
Pain severe - current	1.43 (0.67,3.05)	3.13 (1.59,6.16)**	3.54 (1.8,6.93)***
Function loss moderate - current	1.67 (1.12,2.51)*	1.91 (1.28,2.86)**	2.35 (1.58,3.49)***
Function loss severe - current	1.22 (0.5,2.99)	2.66 (1.21,5.84)*	2.03 (0.89,4.63)
Pain moderate - future	1.95 (1.33,2.86)***	2.54 (1.74,3.69)***	3.18 (2.18,4.62)***
Pain severe - future	1.25 (0.49,3.21)	3.28 (1.46,7.4)**	3.62 (1.61,8.14)**
Function loss moderate - future	1.61 (0.99,2.62)	2.83 (1.79,4.48)***	3.52 (2.23,5.55)***
Function loss severe - future	1.23 (0.37,4.06)	2.95 (1.05,8.29)*	2.16 (0.73,6.41)
Total knee replacement	1.21 (0.73,2.01)	1.51 (0.93,2.47)	2.58 (1.62,4.09)***

*P<0.05, **p<0.01, ***p<0.001.

at least 40 measurable subdivisions for OA structural change, B-score provides increased discrimination of risk over KLG for all clinically important outcomes. As a fully automated (reader-independent) measurement, B-score allows for rapid analysis of large datasets; and in both clinical trials and routine practice, provides a consistent measurement metric. As a scalar measure (compared with the categorical KLG), B-score permits the use of more powerful statistical methods for analysis.

The primary utility afforded by the precision of B-score is demonstrated by comparison with KLG. We have presented an example for KLG3 in the Results section, demonstrating the benefits conferred by having a range of B-scores within a single KLG. This applies for all KLG, even for a KLG0 knee, (often considered to be normal), for which the mean risk of moderate pain was 12%, while B-score risk range (−2 to +2) was 10%–27%. In day-to-day clinical use, it is unlikely that KLGs can be as consistent and repeatable as those in the OAI, where images are carefully acquired and read. Several studies estimated inter-reader agreement of KLG and found a 'moderate' intraclass coefficient of around 0.5–0.7.[22–24] In practice, this means that a KLG3 knee has an equal chance of being scored as KLG2, 3 or 4. This misclassification profoundly affects the risks exemplified above: a KLG3 knee had a risk of between 13.3% and 17.3% of TKR within 8 years. If the knee is equally likely to be scored as KLG2 or KLG4, then this becomes 4.5%–45.5%, a 10-fold increase in CI.

B-score provides a measure of OA status across the whole range of OA structural severity, including early disease. This is often conceptualised as KLG2, but the findings of the current study show that 31% of those categorised as KLG2 had a B-score within the non-OA range, and KLG0-1 knees included 8% with B-scores above the non-OA range. There is currently no consensus on a definition of 'early' OA, and B-score can provide a valuable measure. We have used the 95% CI of those who almost certainly do not have radiographic OA (B-score of ≤ 1.96), and this seems a well-validated basis for a cut-off point. In clinical trials, B-score would provide a reliable stratification tool and has already shown to be a sensitive outcome measure.[25] A number of therapies, including platelet-rich plasma and hyaluronic acid, are used in early OA,[26] and their effect on OA structural progression can now be meaningfully assessed. Implications for clinical practice require further consideration, and at present may improve assessment of prognosis more than selection of therapy (given our limited non-surgical therapeutic options). However, B-score may initially provide clinical usefulness in situations where MRI is already commonly performed (eg, sporting injuries or 'possible early OA').

It was not the intention of this study to suggest that bone shape pathology is causally related with the clinically important outcomes; bone shape is likely reflecting a broader OA construct. It is widely believed that the clinically important outcomes used in this study are related to age, sex, ethnicity, BMI and alignment, and these covariates are often used as inclusion criteria in OA clinical trials. In this study, these covariates had negligible effects on the ORs of the relationship between B-score, a measure of bone pathology, and clinically important outcomes.

We did perform a number of sensitivity analyses on the choice of symptom cut-points, in the absence of widespread consensus on what constitutes moderate and severe symptoms. As well as using a second tool, (WOMAC pain, see online supplemental figure S2) which showed a similar symptom-structure relationship to the NRS score used in the main paper, we also performed sensitivity testing using values of 7, 8 or 9 as cut-off for 'severe' pain, and 32, 34 or 36 as cut-off points for function loss and found that the choice of any of these cut-off points was not an important effect (data not shown).

The strength of this work includes very large patient numbers, but there are limitations. We have not attempted to explore longitudinal change or relationship to cartilage as we focused on the benefits of this new measure at a single time point, and its clear relationships with clinically important outcomes. Our non-OA group, used to set the scale of B-score, was drawn from the OAI with a population aged 45–80, in contrast to the osteoporosis T-score which uses a reference population of healthy young adults. Although we used the DESS-we MR images in this study and have previously demonstrated that the method is applicable to similar MRI sequences,[27] the method would need validation for other MRI sequences. We used a regression analysis for the risk of TKR, rather than hazard or incident rate analysis, as TKR was a 'rare' outcome in our data set, and also to allow the reader to compare estimates in our study (figures 3 and 4). The machine-learning technology can almost certainly be applied to cheaper imaging methods such as CT. Although the method for B-score determination used in this study is proprietary, several methods for bone shape measurement have been published, and the measurement of bone shape is actively being pursued by multiple groups. The bone shape vector revealed here may not hold for very late stages of the disease, where fewer patient numbers were available in this study. When osteophytes begin to carry load directly, they are likely to remodel and may produce shape changes that are less systematic than those reported here.

In conclusion, machine learning has enabled the development of a new objective, precise single time-point measure, B-score, representing OA status. B-score demonstrated similar relationships to clinically important outcomes as the current radiographic standard, but with the increased precision of B-score (providing approximately 10 times more detail on OA structural status), enabling better risk discrimination for clinically important outcomes. B-score should enable improved stratification for interventions and improved personalised assessment, in the same way that bone mineral density and more specifically, the T-score, has done historically for osteoporosis.

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Competing interests MAB and AB are employees of Imorphics, a wholly owned subsidiary of Stryker Corporation. KK is a consultant for Flexion Therapeutics Inc. OA and BD have no conflicts of interest to disclose. NB is an employee of Flexion Therapeutics. PGC has done consultancies or speakers bureaus for AbbVie, Bristol Myers Squibb, Eli Lilly, EMD Serono, Flexion Therapeutics, Galapagos, GlaxoSmithKline, Novartis, Pfizer, Roche, Samumed and Stryker.

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Provenance and peer review Not commissioned; externally peer reviewed.

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CLINICAL SCIENCE

Efficacy and safety of fasinumab in patients with chronic low back pain: a phase II/III randomised clinical trial

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ABSTRACT

Objectives To study the efficacy and safety of fasinumab in moderate-to-severe, chronic low back pain (CLBP).

Methods In this phase II/III, double-blind, placebo-controlled study, patients with CLBP aged ≥35 years with inadequate pain relief/intolerance to acetaminophen, non-steroidal anti-inflammatory drugs and opioids were randomised to fasinumab 6 or 9 mg subcutaneous every 4 weeks (Q4W), 9 mg intravenous every 8 weeks (Q8W) or placebo. Primary endpoint was change from baseline to week 16 in average daily low back pain intensity (LBPI) numeric rating score. Key secondary efficacy variables included Roland-Morris Disability Questionnaire (RMDQ) and Patient Global Assessment (PGA). The results are based on a modified intent-to-treat analysis of 563/800 planned patients when enrolment was stopped early given emerging signals of joint risk in other osteoarthritis (OA) studies at doses being tested here.

Results Significant placebo-adjusted LBPI reductions at week 16 were observed for fasinumab 9 mg Q4W and Q8W (least squares mean (standard error) −0.7 (0.3); both nominal p<0.05), but not 6 mg (−0.3 (0.3); p=0.39). RMDQ and PGA improvements to week 16 were greatest for fasinumab 9 mg intravenous. Numerically greater efficacy occurred in patients with, versus those without, peripheral OA (pOA) over 16 weeks. Treatment-emergent adverse events (AEs) occurred in 274/418 (65.6%) patients in the combined fasinumab groups and 94/140 (67.1%) placebo patients. Joint AEs, mostly rapid progressive OA type 1, were more frequent in the combined fasinumab groups (19 events in 16 patients (3.8%) vs 1 event in 1 patient (0.7%) for placebo); all except one occurred in pOA patients.

Conclusions Fasinumab highest doses, but not lower dose, improved both CLBP pain and function. Most joint AEs occurred in pOA patients, consistent with earlier findings in symptomatic OA. Further study is needed of patients with CLBP with and without pOA to determine optimal benefit–risk.

Key messages

What is already known about this subject?

- Inadequate relief of chronic pain has a profound effect on an individual's quality of life and is associated with substantial healthcare costs and loss of productivity.

What does this study add?

- There remains an unmet medical need for alternative treatment options that have analgesic efficacy, mitigate the risks associated with current treatment options and provide an acceptable risk/benefit profile.
- Nerve growth factor (NGF) inhibitors have the potential to provide pain relief via a mechanism distinct from that of commonly used analgesic medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, and thus avoid NSAID or opioid adverse effects such as increased risk of cardiovascular events, gastrointestinal toxicity, drowsiness, respiratory depression, dependence and abuse.
- Treatment with NGF inhibitors has been associated with dose-dependent risk of joint damage including rapidly progressive osteoarthritis (OA), that may be more likely in individuals with peripheral OA (pOA) than in those without pOA, and neurologic symptoms, including paraesthesia.
- Higher doses were required to relieve chronic low back pain (CLBP) than was observed in previous studies in patients with pain due to hip and knee OA.

How might this impact on clinical practice or future developments?

- The results observed in this study support continued evaluation of fasinumab as a possible new treatment option for patients with CLBP with inadequate pain control, or who are intolerant to or have a contraindication for existing therapies.
- For future studies in CLBP, consideration will be given to dose of fasinumab to seek the most favourable risk–benefit profile.

INTRODUCTION

Low back pain (LBP) is a major international health problem.[1] According to the Global Burden of Disease 2017 study, LBP ranked highest among other conditions as measured in disability-adjusted life years.[2] Although most patients are believed to recover quickly from acute episodes, recurrence is common.[3] Chronic LBP (CLBP) is defined as pain persisting for ≥3 months.[4] Guidelines recommend initial treatment with non-pharmacological interventions, including exercise and multidisciplinary rehabilitation.[5–8]

If these interventions are inadequate or if CLBP persists, guidelines recommend non-steroidal anti-inflammatory drugs (NSAIDs) as first-line pharmacological treatments and duloxetine and tramadol as second-line treatments.[7] Stronger opioids are an option only if patients fail the afore-mentioned treatments and if the potential benefits outweigh the risks.[7] However, long-term use of both NSAIDs and opioids is limited by tolerability issues and adverse effects, such as gastrointestinal bleeding, cardiovascular events, and the potential for abuse and dependence.[9]

Neurotrophins are a family of polypeptide growth factors that play a role in the proliferation, differentiation, survival and death of neuronal and non-neuronal cells.[10] Nerve growth factor (NGF) was the first neurotrophin identified.[11] It provokes pain,[12 13] is elevated in the synovial fluid of patients with osteoarthritis (OA)[14 15] and its receptors are upregulated in injured and inflamed tissues.[16 17] NGF produced by peripheral tissues binds neurotrophic receptors (low-affinity p75 and high-affinity tropomyosin-related kinase A) on nociceptive neurons to modulate pain.[18 19] NGF inhibitors might, therefore, provide pain relief via a novel mechanism, potentially avoiding the risks of NSAID or opioids.

Fasinumab is a fully human monoclonal antibody shown to reduce pain in OA.[20 21] This study evaluated the efficacy and safety of fasinumab for moderate-to-severe CLBP in patients with intolerance to, or inadequate pain relief from, acetaminophen, oral NSAIDs and opioids.

METHODS

Patient and public involvement

This phase II/III, randomised, double-blind, double-dummy, placebo-controlled study (NCT02620020) was conducted at 105 sites in the USA, Canada and Europe.

Study population

Eligible patients were ≥35 years old with CLBP and history of inadequate pain relief or intolerance to analgesic therapy, including acetaminophen, at least one oral NSAID and at least one opioid (or unwillingness to take opioids), and a diagnosis of moderate-to-severe CLBP (Quebec Task Force category 1: no radiating pain, or Quebec Task Force category 2: proximal radiation above the knee)[22] for ≥3 months prior to screening. An LBP intensity Numeric Rating Scale (LBPI NRS) score ≥4 at both screening and at randomisation (after withdrawal of previous pain medication(s), without requirement for pain flare), and a Patient Global Assessment (PGA) of LBP of fair, poor or very poor at screening were also required. Presence of OA was not exclusionary (see online supplemental methods for full inclusion and exclusion criteria).

Study design and treatments

The study consisted of a screening period (up to 30 days), a 7-day prerandomisation period during which all pain medication except study-provided rescue medication was discontinued, a 16-week randomised treatment period and a 20-week follow-up period. Patients were randomised (1:1:1:1) according to a computer-generated central randomisation scheme and assigned by interactive voice response system, to either: fasinumab 6 mg subcutaneously (SC) every 4 weeks (Q4W), fasinumab 9 mg SC Q4W, fasinumab 9 mg intravenously (IV) every 8 weeks (Q8W) or placebo SC Q4W or IV Q8W. Patients randomised to fasinumab 6 mg or 9 mg SC received a loading dose (extra nominal dose) on day 1 (total dose of 12 or 18 mg, respectively), followed by nominal doses at weeks 4, 8 and 12 (total of four doses). Patients randomised to fasinumab 9 mg IV Q8W were not loaded, receiving IV fasinumab 9 mg on day 1 and week 8 (total of two doses). To maintain treatment blinding, patients received double-dummy placebo injections (IV or SC) on days of dose administration.

Randomisation was stratified by baseline LBPI NRS score (<7,≥7), duration of CLBP (<5 years, ≥5 years) and maximum Kellgren-Lawrence (K-L) score (≤2, >2) at any knee or hip joint at screening.

The primary efficacy endpoint was the change from baseline to week 16 in the average daily LBPI NRS score on an 11-point

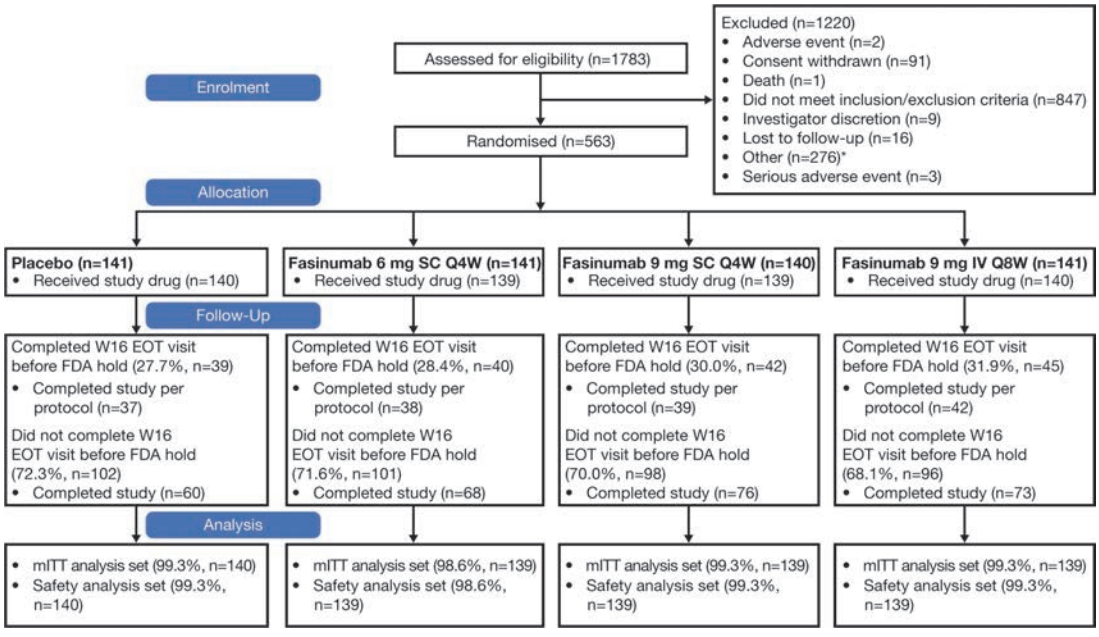

Figure 1 Patient disposition. EOT, end of treatment; FDA, US Food and Drug Administration; IV, intravenous; mITT, modified intent-to-treat; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous; W, week. *Includes, among other reasons: out of screening window, study stopped by sponsor; patients could be excluded for >1 reason.

(0–10) NRS. The average daily LBPI NRS score was defined as the average of daily LBPI NRS scores for the 7 days before and including the nominal visit. Secondary endpoints included change from baseline to weeks 2, 4, 8, 12 and 16 in Roland-Morris Disability Questionnaire (RMDQ) total score and PGA score, and change from baseline to weeks 2, 4, 8 and 12 in LBPI NRS score.

In October 2016, the US Food and Drug Administration (FDA) placed the study on partial clinical hold following a single case of rapidly progressive osteoarthritis (RPOA) that occurred in a patient with knee OA (K-L score 3 at screening), prompting review of study entry criteria. Since patients with concomitant OA could have received fasinumab doses that had been eliminated by the sponsor from an ongoing fasinumab phase III OA study (NCT02683239) due to the rate of arthropathy, the FDA required that the protocol be amended to either exclude patients with peripheral OA (pOA) or to lower the doses to be studied. Since 70% of the target sample (563/800 patients) had already been randomised, investigators and relevant health authorities were notified that the sponsor stopped enrolment and any further dosing. The statistical analysis plan was updated prior to database lock and a final analysis was performed on completion of all protocol-described study visits, to allow assessment of safety and efficacy, including subgroup analyses of the primary and secondary endpoints by pOA status.

Safety assessments

The safety and tolerability of fasinumab compared with placebo was assessed by treatment-emergent adverse events (TEAEs) during treatment (including the day from first dose of study drug to 4 weeks after last dose of SC study drug or 8 weeks after last dose of IV study drug, whichever was later) and post-treatment (up to 20 weeks) adverse events (AEs). Joint and general safety were monitored independently (see online supplemental methods) as previously described.[21]

Statistical analysis

The primary efficacy endpoint, change from baseline to week 16 in average daily LBPI NRS score, was analysed using a mixed-effect model repeated measures approach based on the modified intent-to-treat (mITT) analysis set, according to a prespecified analysis established prior to database lock, in response to the early termination of dosing in the study. The mITT analysis set included all randomised patients who received at least one dose of allocated treatment, including all data available up to 5 weeks (4 weeks visit interval + 1 week allowable visit window) after the last dose of study drug. Analyses were deemed exploratory (all p values are nominal). Further details are provided in the online supplemental methods.

The safety analysis set included all randomised patients who received any study drug. Sensitivity analyses for the primary and secondary endpoints used the full analysis set (all randomised

Table 1 Demography and baseline characteristics (full analysis set)

		Fasinumab				
	Placebo (n=141)	6 mg SC Q4W (n=141)	9 mg SC Q4W (n=140)	9 mg IV Q8W (n=141)	Combined (n=422)	Total (N=563)
Age (years), mean (SD)	58.1 (12.5)	58.2 (11.3)	56.6 (11.0)	55.4 (10.5)	56.7 (11.0)	57.1 (11.4)
Age category, n (%)						
<65 years	93 (66.0)	95 (67.4)	109 (77.9)	117 (83.0)	321 (76.1)	414 (73.5)
≥65 years	48 (34.0)	46 (32.6)	31 (22.1)	24 (17.0)	101 (23.9)	149 (26.5)
Sex, n (%)						
Male	58 (41.1)	56 (39.7)	56 (40.0)	60 (42.6)	172 (40.8)	230 (40.9)
Female	83 (58.9)	85 (60.3)	84 (60.0)	81 (57.4)	250 (59.2)	333 (59.1)
Race, n (%)						
White	127 (90.1)	119 (84.4)	118 (84.3)	116 (82.3)	353 (83.6)	480 (85.3)
Black or African American	13 (9.2)	19 (13.5)	19 (13.6)	21 (14.9)	59 (14.0)	72 (12.8)
Asian	1 (0.7)	2 (1.4)	2 (1.4)	1 (0.7)	5 (1.2)	6 (1.1)
American Indian or Alaska Native	0	1 (0.7)	0	1 (0.7)	2 (0.5)	2 (0.4)
Native Hawaiian or other Pacific Islander	0	0	0	1 (0.7)	1 (0.2)	1 (0.2)
Other	0	0	1 (0.7)	1 (0.7)	2 (0.5)	2 (0.4)
Body mass index (kg/m^2), mean (SD); n	29.7 (4.8); 141	29.0 (5.1); 139	29.6 (4.7); 140	30.1 (4.4); 141	29.6 (4.7); 420	29.6 (4.8); 561
Average daily LBPI NRS score, mean (SD); n	6.5 (1.3); 140	6.5 (1.3); 139	6.7 (1.3); 140	6.5 (1.2); 141	6.5 (1.3); 420	6.5 (1.3); 560
Duration of chronic LBP (years), mean (SD); n	11.8 (10.2); 126	13.6 (12.1); 131	13.7 (13.0); 135	12.7 (10.7); 134	13.3 (12.0); 400	13.0 (11.6); 526
Maximum K-L score at any knee or hip joint at screening, n (%)						
0	25 (17.7)	16 (11.3)	35 (25.0)	25 (17.7)	76 (18.0)	101 (17.9)
1	51 (36.2)	49 (34.8)	35 (25.0)	43 (30.5)	127 (30.1)	178 (31.6)
2	40 (28.4)	52 (36.9)	42 (30.0)	50 (35.5)	144 (34.1)	184 (32.7)
3	21 (14.9)	21 (14.9)	23 (16.4)	18 (12.8)	62 (14.7)	83 (14.7)
4	4 (2.8)	3 (2.1)	5 (3.6)	5 (3.5)	13 (3.1)	17 (3.0)
pOA, n (%)						
Yes	82 (58.2)	94 (66.7)	68 (48.6)	78 (55.3)	240 (56.9)	322 (57.2)
No	59 (41.8)	47 (33.3)	72 (51.4)	63 (44.7)	182 (43.1)	241 (42.8)

Baseline average daily LBPI NRS score was defined as the average of the non-missing daily LBPI NRS scores for 5 days prior to randomisation (from day −4 to day 1). pOA defined by medical history and/or K-L score ≥2 in hip or ≥3 in knee.
IV, intravenous
; K-L, Kellgren-Lawrence; LBP, lower back pain; LBPI NRS, Lower Back Pain Intensity Numeric Rating Scale; pOA, peripheral osteoarthritis; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous.

Table 2 Change from baseline to week 8 and week 16 in the average daily LBPI NRS, RMDQ and PGA of LBP scores (mITT analysis set)

		Fasinumab		
	Placebo (n=140)	6 mg SC Q4W (n=139)	9 mg SC Q4W (n=139)	9 mg IV Q8W (n=140)
	LBPI NRS			
Baseline average daily LBPI NRS score, mean (SD); n	6.5 (1.3); 139	6.5 (1.3); 137	6.7 (1.3); 139	6.4 (1.2); 140
Week 8				
Average daily LBPI NRS score, mean (SD); n	5.3 (2.1); 96	4.7 (2.0); 99	4.3 (2.4); 105	4.1 (2.3); 103
Change from baseline to week 8, mean (SD); n	–1.3 (1.8); 95	–1.9 (1.9); 98	–2.4 (2.2); 105	–2.3 (2.2); 103
LS mean (SE)	–1.2 (0.2)	–1.8 (0.2)	–2.3 (0.2)	–2.2 (0.2)
95% CI	–1.6 to –0.8	–2.2 to –1.4	–2.7 to –1.9	–2.6 to –1.8
Difference versus placebo, LS mean (SE)		–0.5 (0.3)	–1.1 (0.3)	–1.0 (0.3)
95% CI		–1.06 to –0.03	–1.57 to –0.55	–1.48 to –0.47
P value versus placebo		0.04	<0.01	<0.01
Week 16				
Average daily LBPI NRS score, mean (SD); n	4.7 (2.0); 50	4.3 (1.9); 48	4.2 (2.3); 55	3.9 (2.4); 56
Change from baseline to week 16, mean (SD); n	–1.9 (2.1); 49	–2.1 (1.9); 48	–2.6 (2.0); 55	–2.5 (2.2); 56
LS mean (SE)	–1.7 (0.2)	–2.0 (0.2)	–2.5 (0.2)	–2.4 (0.2)
95% CI	–2.19 to –1.29	–2.46 to –1.56	–2.90 to –2.03	–2.83 to –1.97
Difference versus placebo, LS mean (SE)		–0.3 (0.3)	–0.7 (0.3)	–0.7 (0.3)
95% CI		–0.88 to 0.34	–1.32 to –0.12	–1.26 to –0.07
P value versus placebo		0.39	0.02	0.03
	RMDQ			
Baseline RMDQ total score, mean (SD); n	10.9 (5.3); 132	10.8 (5.2); 135	10.7 (5.7); 136	11.7 (5.3): 136
Week 8				
RMDQ total score, mean (SD); n	7.9 (5.6); 100	5.7 (5.2); 101	5.9 (5.6); 105	5.6 (5.4); 104
Change from baseline to week 8, mean (SD); n	–3.2 (4.9); 92	–5.4 (5.3); 97	–4.7 (4.9); 102	–6.2 (5.4); 101
LS mean (SE)	–3.1 (0.5)	–5.3 (0.5)	–5.0 (0.5)	–5.9 (0.5)
95% CI	–3.99 to –2.17	–6.18 to –4.40	–5.86 to –4.10	–6.77 to –5.01
Difference versus placebo, LS mean (SE)		–2.2 (0.6)	–1.9 (0.6)	–2.8 (0.6)
95% CI		–3.42 to –1.01	–3.10 to –0.70	–4.01 to –1.61
P value versus placebo		<0.01	<0.01	<0.01
Week 16				
RMDQ total score, mean (SD); n	6.6 (5.6); 50	5.1 (4.9); 48	4.8 (4.6); 55	5.0 (5.2); 57
Change from baseline to week 16, mean (SD); n	–3.8 (4.5); 46	–6.0 (5.7); 46	–6.2 (4.7); 55	–6.6 (5.6); 55
LS mean (SE)	–3.8 (0.5)	–6.0 (0.5)	–5.8 (0.5)	–6.3 (0.5)
95% CI	–4.88 to –2.76	–7.09 to –4.97	–6.78 to –4.76	–7.30 to –5.28
Difference versus placebo, LS mean (SE)		–2.2 (0.7)	–2.0 (0.7)	–2.5 (0.7)
95% CI		–3.65 to –0.77	–3.36 to –0.54	–3.88 to –1.06
P value versus placebo		<0.01	<0.01	<0.01
	PGA of LBP			
Baseline PGA, mean (SD); n	3.5 (0.7); 140	3.5 (0.7); 139	3.4 (0.8); 139	3.4 (0.7); 140
Week 8				
PGA, mean (SD); n	3.0 (0.8); 100	2.7 (0.8); 101	2.6 (0.9); 105	2.5 (1.0); 104
Change from baseline to week 8, mean (SD); n	–0.5 (0.8); 100	–0.8 (0.9); 101	–0.8 (0.9); 105	–0.9 (1.0); 104
LS mean (SE)	–0.5 (0.1)	–0.8 (0.1)	–0.8 (0.1)	–0.9 (0.1)
95% CI	(–0.65 to –0.33)	(–0.94 to –0.62)	(–0.95 to –0.64)	(–1.05 to –0.74)
Difference versus placebo, LS mean (SE)		–0.3 (0.1)	–0.3 (0.1)	–0.4 (0.1)
95% CI		–0.51 to –0.08	–0.52 to –0.09	–0.62 to –0.19
P value versus placebo		0.01	0.01	<0.01
Week 16				
PGA, mean (SD); n	2.8 (0.8); 50	2.5 (0.9); 48	2.5 (0.9); 55	2.3 (1.0); 57
Change from baseline to week 16, mean (SD); n	–0.7 (0.8); 50	–0.9 (1.1); 48	–0.8 (1.0); 55	–1.0 (0.9); 57
LS mean (SE)	–0.7 (0.1)	–0.9 (0.1)	–0.8 (0.1)	–1.0 (0.1)
95% CI	–0.88 to –0.49	–1.08 to –0.69	–1.03 to –0.65	–1.20 to –0.83
Difference versus placebo, LS Mean (SE)		–0.2 (0.1)	–0.1 (0.1)	–0.3 (0.1)
95% CI		–0.46 to 0.07	–0.41 to 0.11	–0.59 to –0.07
P value versus placebo		0.15	0.26	0.01

Analyses are based on MMRM model with baseline randomisation strata, baseline score, treatment, visit and treatment-by-visit interaction. P values are nominal. Average daily LBPI NRS score was defined as the average of the non-missing daily LBPI NRS scores for the 7 days before and including the nominal visit. The daily LBPI NRS score for the week 16 nominal visit day was missing for all patients because the daily LBPI NRS score was entered each day starting at 18:00 and clinic visits typically occurred during the day with diaries returned at the end of the visit. Therefore, the average LBPI NRS score at week 16 was based on 6 days.
IV, intravenous; LS, least squares; mITT, modified intent-to-treat; MMRM, mixed effect model repeated measures; LBP NRS, Lower Back Pain Numeric Rating Scale; PGA, Patient Global Assessment; Q4W, every 4 weeks; Q8W, every 8 weeks; RMDQ, Roland-Morris Disability Questionnaire; SC, subcutaneous.

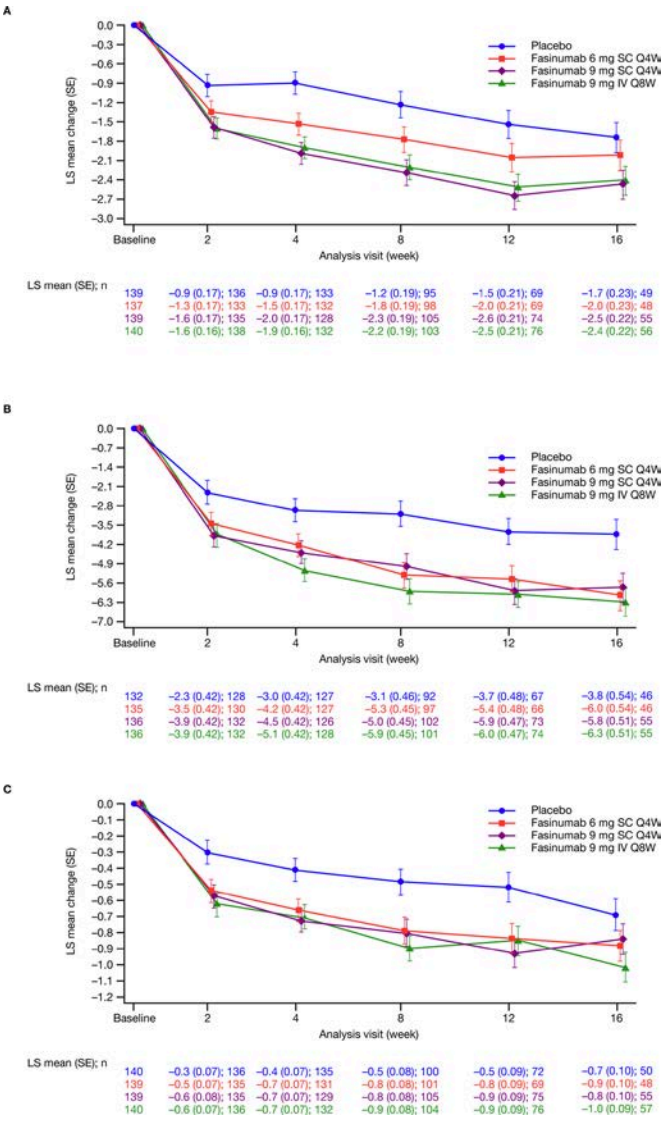

Figure 2 Least squares mean change from baseline in (A) average daily Lower Back Pain Intensity Numeric Rating Scale score (B) Roland-Morris Disability Questionnaire total score (C) Patient Global Assessment of lower back pain score by study visit (modified intent-to-treat analysis set). Analyses are based on mixed effect model repeated measures with baseline randomisation strata, baseline, treatment, visit and treatment-by-visit interaction. IV, intravenous; LS, least squares; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous.

patients). Assuming a significance level of 0.05 and a 20% dropout rate by week 16, an enrolment of 200 patients per treatment group would provide at least 91% power to detect a treatment difference of 0.9 between fasinumab 9 mg SC Q4W and placebo for the primary efficacy endpoint with a common SD of 2.4.

To assess potential differences in efficacy and safety between those with and without pOA at baseline, subgroup analyses were performed on the primary and secondary efficacy endpoints, using medical history and/or radiographic evidence of OA (K-L score ≥2 at the hip or K-L score ≥3 at the knee based on screening radiographs), in line with key components of the American College of Rheumatology OA criteria.[23] Subgroup analyses for the primary efficacy endpoint were also conducted for randomisation strata (baseline LBPI NRS score (<7, ≥7), duration of chronic LBP (≥5 years, <5 years) and maximum K-L score in any knee or hip joint (≤2,>2)).

RESULTS

Overall, 1783 patients were screened; 563 patients were randomised (figure 1). Patient demographic and baseline characteristics were generally balanced across groups (table 1). Most patients (82.2%) had a maximum K-L score at any knee or hip joint of ≤2 at screening; 14.7% and 3.0% of patients had scores of 3 and 4, respectively, (table 1). Of 558 patients who received at least one dose of study drug (safety analysis set), 35.3% to 42.4% of the fasinumab SC groups and 36.4% of the placebo SC group received all planned doses through week 16; corresponding values for IV groups were 54.3% (fasinumab 9 mg Q8W) and 51.4% (placebo) (online supplemental table 1).

Efficacy

Baseline LBPI scores were comparable across treatment groups (table 2). Significant reductions versus placebo in LBPI scores from baseline to week 16 were observed in the fasinumab 9 mg SC Q4W and 9 mg IV Q8W groups (least squares mean (standard error) −0.7 (0.3), nominal p=0.02; and −0.7 (0.3), nominal p=0.03, respectively), but not for the 6 mg SC Q4W group (–0.3 (0.3); nominal p=0.39) (table 2). Pain scores improved as early as week 2 (figure 2A). At week 8, all fasinumab doses provided reductions in LBPI scores versus placebo (least squares mean (standard error) 6 mg SC –0.5 (0.3), nominal p=0.04; 9 mg SC Q4W –1.1 (0.3), nominal p<0.01; 9 mg IV Q8W –1.0 (0.3), nominal p<0.01) (table 2). Mean baseline RMDQ (10.7 to 11.7) and PGA (3.4 to 3.5) scores were comparable across groups. RMDQ reductions were observed as early as week 2 in all fasinumab groups versus placebo and maintained to week 16, with the greatest reductions in the 9 mg IV group (table 2 and figure 2B,C). Placebo-adjusted changes in RMDQ at week 16 were –2.2 to –2.5 across fasinumab groups (all nominal p<0.01). Placebo-adjusted changes in PGA at week 16 (–0.1 to –0.3) reached significance only for fasinumab 9 mg IV (nominal p=0.01).

Subgroup analyses

In patients with (57.2%) and without (42.8%) pOA, placebo-adjusted improvements in LBPI scores were greatest in the 9 mg dose groups from week 2 through week 16 (online supplemental table 2 and online supplemental figure 1). Improvement versus placebo was generally numerically greatest in patients with, versus those without, pOA over the 16 week treatment period, with greater separation seen between the pOA subgroups at earlier time points when more patient data were available. A similar pattern was observed for RMDQ and PGA scores (online supplemental table 2 and online supplemental figures 2 and 3). Placebo-adjusted LBPI scores from baseline to week 16 were consistent across randomisation strata (data not shown).

Safety

On treatment, the percentages of patients with ≥1 TEAE were similar between placebo (67.1%; n=94) and combined fasinumab groups (65.6%; n=274), and across the fasinumab dose groups (online supplemental table 3). The system organ class (SOC) with the highest incidence of TEAEs while on treatment was musculoskeletal and connective tissue disorders (16.0% for combined fasinumab groups and 22.1% for placebo) (table 3). Arthralgia was the only TEAE reported in >10% of patients in any treatment group, with a similar incidence in the placebo and

Table 3 TEAEs with >3% incidence by system organ class and preferred term during the on-treatment period (safety analysis set)

Primary system organ class preferred term	Placebo (n=140)	Fasinumab 6 mg SC Q4W (n=139)	Fasinumab 9 mg SC Q4W (n=139)	Fasinumab 9 mg IV Q8W (n=140)	Fasinumab Combined (N=418)
TEAEs, n	90	79	115	85	279
Patients with at least one TEAE, n (%)	52 (37.1)	41 (29.5)	63 (45.3)	56 (40.0)	160 (38.3)
Musculoskeletal and connective tissue disorders, n (%)	31 (22.1)	15 (10.8)	25 (18.0)	27 (19.3)	67 (16.0)
Arthralgia	17 (12.1)	15 (10.8)	16 (11.5)	21 (15.0)	52 (12.4)
Pain in extremity	12 (8.6)	3 (2.2)	5 (3.6)	4 (2.9)	12 (2.9)
Back pain	7 (5.0)	0	4 (2.9)	5 (3.6)	9 (2.2)
Nervous system disorders, n (%)	18 (12.9)	17 (12.2)	26 (18.7)	18 (12.9)	61 (14.6)
Headache	9 (6.4)	9 (6.5)	9 (6.5)	9 (6.4)	27 (6.5)
Paraesthesia	4 (2.9)	6 (4.3)	9 (6.5)	9 (6.4)	24 (5.7)
Dizziness	4 (2.9)	5 (3.6)	6 (4.3)	3 (2.1)	14 (3.3)
Hypoaesthesia	4 (2.9)	4 (2.9)	7 (5.0)	3 (2.1)	14 (3.3)
Infections and infestations, n (%)	12 (8.6)	15 (10.8)	18 (12.9)	14 (10.0)	47 (11.2)
Nasopharyngitis	8 (5.7)	9 (6.5)	8 (5.8)	10 (7.1)	27 (6.5)
Urinary tract infection	0	4 (2.9)	5 (3.6)	2 (1.4)	11 (2.6)
Upper respiratory tract infection	4 (2.9)	2 (1.4)	5 (3.6)	2 (1.4)	9 (2.2)
Gastrointestinal disorders, n (%)	6 (4.3)	7 (5.0)	11 (7.9)	4 (2.9)	22 (5.3)
Diarrhoea	4 (2.9)	4 (2.9)	5 (3.6)	3 (2.1)	12 (2.9)
Nausea	2 (1.4)	4 (2.9)	7 (5.0)	1 (0.7)	12 (2.9)
General disorders and administration site conditions, n (%)	0	1 (0.7)	6 (4.3)	2 (1.4)	9 (2.2)
Oedema peripheral	0	1 (0.7)	6 (4.3)	2 (1.4)	9 (2.2)

TEAEs included any AEs reported during the on-treatment period (the day from first dose of study drug to 4 weeks after the last dose of SC study drug or 8 weeks after the last dose of IV study drug).
MedDRA (V.18.0) coding applied.
A patient who reported two or more TEAEs with the same preferred term is counted only once for that term.
A patient who reported two or more TEAEs with different preferred terms within the same system organ class is counted only once in that system organ class.
For system organ classes, the table is sorted by decreasing frequency in combined fasinumab group. Within each system organ class, preferred terms are sorted by decreasing frequency count in combined fasinumab group.
IV, intravenous; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous; TEAE, treatment-emergent adverse event.

combined fasinumab groups (12.1% and 12.4%, respectively). TEAEs of paraesthesia were more frequent for the combined fasinumab groups than for placebo (5.7% vs 2.9%); most of these events were of mild-to-moderate severity. During the post-treatment follow-up period, the overall incidence of AEs in the combined fasinumab groups (29.9%) was similar to that for placebo (27.9%) (online supplemental table 4).

In total, 16 serious AEs (SAEs) occurred in 14 patients (placebo, n=4 (2.9%); combined fasinumab groups (n=10 (2.4%)) during the on-treatment period (online supplemental tables 3 and 5). Three SAEs were considered related to study drug, two of which were in the fasinumab 9 mg groups (haemorrhagic stroke and meniscus injury). In total, 35 SAEs occurred in 31 patients in the post-treatment follow-up period (online supplemental table 6); most were in the SOC of musculoskeletal and connective tissue disorders, all of which occurred in the fasinumab groups (5 patients, 3.6%, in the 9 mg IV group, and 3 patients, 2.2%, in each SC group; 11 total patients, 2.6%). Within this SOC, the most frequent SAE was RPOA. One patient (fasinumab 6 mg) with a history of smoking died of small cell lung cancer during the post-treatment follow-up period (considered unrelated to study drug).

AEs of special interest included sympathetic nervous system dysfunction and adjudicated arthropathies (AAs). No confirmed cases of the former were observed. There were 20 joints with AAs in 17 patients (table 4). All except one AA were detected outside of the prespecified on-treatment period (online supplemental figure 5), and all but one occurred in the fasinumab groups. Of the 20 joints with AAs, 19 were in patients in the pOA subgroup (table 4), and most (15/20) occurred in joints with screening K-L scores of ≥2 at the knee or hip (online supplemental table 7); in 3 knee joints, the screening K-L score was 0 or 1; in 2 shoulder joints, K-L score was not assessed but screening radiographs documented 1 with moderate OA and 1 with severe OA). Adjudicators could report more than one AA category per joint. Most AAs were categorised as RPOA (ie, RPOA type 1 or 2) and among these, 14 joints had RPOA1 (X-ray joint space narrowing; cartilage loss by MRI) solely; 2 joints in two patients (6 mg SC and 9 mg IV) had an RPOA2 (bone fragmentation or destruction; one with RPOA1); 1 joint had subchondral insufficiency fracture (SIF) as the sole finding (9 mg IV); and three joints had SIFs in conjunction with RPOA1. Only two AAs (one RPOA1; (9 mg SC), and one RPOA2; (9 mg IV)) were detected on imaging prompted by symptoms; others were detected on scheduled images. No primary osteonecrosis was observed.

Four joint replacements (knee) were performed in four patients. Two of these occurred following detection of an AA (9 mg SC, RPOA1; 9 mg IV, RPOA2). For the remaining two, preoperative imaging did not detect AA. In one case (9 mg SC), joint replacement was pursued to address functional consequences of pre-existing OA; in the other case (placebo), it was based on need for revision surgery related to historical hemiarthroplasty.

An increase in mean alkaline phosphatase (ALP) occurred over time in all three fasinumab groups (figure 3). The extent of the increase was similar across groups and small compared with baseline values. A small number of patients had increases in ALP above the upper limit of normal (ULN; 150 U/L): placebo (n=3), fasinumab 6 mg (n=2), 9 mg SC (n=4) and 9 mg IV (n=3), none

Table 4 Summary of AAs across the treatment period and post-treatment follow-up period (safety analysis set)

		Fasinumab			
	Placebo	6 mg SC Q4W	9 mg SC Q4W	9 mg IV Q8W	Combined
All patients, n	140	139	139	140	418
Patients with positive adjudications, n (%)	1 (0.7)	5 (3.6)	4 (2.9)	7 (5.0)	16 (3.8)
Total number of adjudications	46	80	89	110	279
Number of joints with positive adjudications, n (% of total adjudications) and JR outcome	1 (2.2)	7 (8.8)	4 (4.5)	8 (7.3)	19 (6.8)
RPOA1	1	5	3	6	14*
RPOA1, RPOA2†	0	1	0	0	1
RPOA1, SIF†	0	2	1	0	3
RPOA1 → JR	0	0	1	0	1
RPOA2 → JR	0	0	0	1	1
SIF	0	0	0	1	1
Patients with pOA, n	82	92	68	78	238
Patients with positive adjudications, n (%)‡	1 (1.2)	5 (5.4)	4 (5.9)	6 (7.7)	15 (6.3)
Total number of adjudications	41	60	56	88	204
Number of joints with positive adjudications (% of total adjudications) and JR outcome	1 (2.4)	7 (11.7)	4 (7.1)	7 (8.0)	18 (8.8)
RPOA1	1	5	3	5	13#
RPOA1, RPOA2†	0	1	0	0	1
RPOA1, SIF†	0	2	1	0	3
RPOA1 → JR*	0	0	1	0	1
RPOA2 → JR	0	0	0	1	1
SIF	0	0	0	1	1
Patients without pOA, n	58	47	71	62	180
Patients with positive adjudications, n (%)‡	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)	1 (0.6)
Total number of adjudications	5	20	33	22	75
Number of joints with positive adjudications (% of total adjudications)	0	0	0	1 (4.5)	1 (1.3)
RPOA1	0	0	0	1	1

pOA defined by medical history and/or K-L score ≥2 in hip or ≥3 in knee.
*Two RPOA1 events (6 and 9 mg Q4W groups; both in patients with pOA) were reported two times (as a sole finding and as RPOA1, SIF).
†More than one adjudicated arthropathy category could have been reported simultaneously in a single joint.
‡Per cent values calculated using the number of patients in each subgroup as denominator.
IV, intravenous; JR, total joint replacement; K-L, Kellgren-Lawrence; pOA, peripheral osteoarthritis; Q4W, every 4 weeks; Q8W, every 8 weeks; RPOA1, rapid progressive OA type 1; RPOA2, rapid progressive OA type 2; SC, subcutaneous; SIF, subchondral insufficiency fracture.

of which met the prespecified definition for potential clinical significance (≥1.5×ULN). During the 20-week post-treatment follow-up period, mean ALP values returned towards baseline (figure 3). A similar pattern was observed for patients with and without pOA (online supplemental figure 4).

Treatment-emergent antidrug antibody (ADA) responses occurred in five patients (1.3%) on fasinumab and one patient (0.8%) on placebo. All ADA-positive patients exhibited low titre responses, and none was neutralising. A positive ADA response did not affect concentrations of fasinumab.

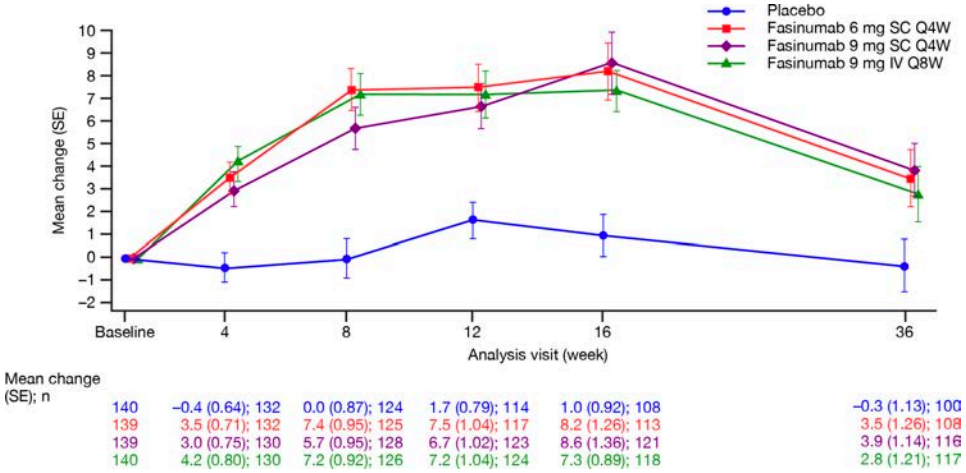

Figure 3 Mean change from baseline in alkaline phosphatase (U/L) (safety analysis set). IV, intravenous; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous.

DISCUSSION

In this study, fasinumab provided improvements in CLBP, function and overall patient assessment of benefit. Although these outcome measures were focused on assessment at the end of the 16-week treatment period (primary endpoint), improvements were noted across most parameters and dose regimens as early as week 2. A key limitation of the study is that because of the FDA hold and early termination of dosing, results are based on an incomplete cohort (35%–56% receiving all planned doses of study drug); data for fewer patients than originally planned were available for efficacy and safety analyses, and p values were considered nominal. Exposure data are limited because of the relatively short treatment duration (16 weeks) and because not all subjects received all planned doses. Moreover, the pOA subgroup analyses were exploratory (a formal diagnosis of OA per American College of Rheumatology (ACR) criteria was not required at study entry and patients were not stratified for OA), but provided an opportunity to address emerging concerns about AA risk in pOA patients as the development programme matured. The use of loading doses in the fasinumab SC groups should also be considered when interpreting the efficacy and safety results. Although the loading dose may have influenced SC treatment effect at earlier time points, it did not seem to impact differences in effect noted across the 9 mg SC and IV dose groups, though this possibility cannot be excluded for the 6 mg SC group.

Although cross-study comparisons are imprecise, the placebo-adjusted treatment effect of fasinumab at endpoint, which ranged from –0.3 for fasinumab 6 mg SC Q4W to –0.7 for 9 mg SC Q4W and IV Q8W, is broadly consistent with studies in patients with CLBP of another NGF inhibitor, IV or SC tanezumab, which reported week-16 placebo-adjusted treatment effects of –0.3 for 5 mg and –0.4 to –0.8 for 10 mg.[24 25] The efficacy of fasinumab in the current study also appears comparable or slightly better than most potent opioids (placebo-adjusted treatment effect of –0.4 was reported in a systematic review and meta-analysis),[26] and maximal doses of NSAIDs (treatment effect of –0.4 for naproxen was reported in the IV tanezumab trial).[25]

CLBP can be caused by various aetiologies including chronic muscular pain, discogenic pain and facet joint OA. However, prior studies have been unable to deconvolute the various components that might contribute to pain in different patients. Our study provided an opportunity to evaluate responses across subgroups without and with pOA, known to be associated with facet joint OA.[27] Since studies focused on OA had suggested a dose-related risk of arthropathy,[20] analysis by pOA status was also an opportunity to uncover differential safety patterns in the treatment of CLBP. Patients with pOA generally experienced greater placebo-adjusted improvement in pain and function than those without, in part driven by greater resolution of pain in the placebo group of the non-pOA subgroup, and was particularly evident at earlier timepoints (4 and 8 weeks), when more patient data were available for assessment. These findings might reflect differential components of pain in these two subgroups. For example, a greater proportion of patients without pOA may have had CLBP caused by factors other than OA of the spine, such as proximal radiculopathy (ie, included in Quebec Task Force category 2). NGF inhibitor therapy has shown no benefit in patients with pain caused by radiculopathy (ie, sciatica).[28]

The incidence of TEAEs was similar between placebo and fasinumab. However, patients treated with fasinumab had higher rates of AAs across all doses studied. All but one AA occurred in patients with concomitant pOA, suggesting that pOA patients may be more predisposed than those without to risk of arthropathy at the high fasinumab doses used in this CLBP study. These findings are consistent with studies that reported higher rates of arthropathy at the highest doses of fasinumab and tanezumab, beyond those producing maximal treatment benefit in OA pain.[20 25 29–31] Across a higher dose range studied in CLBP here, there was no clear difference across doses in the frequency of AA events, even when focusing only on the pOA subgroup.

Elevations in ALP with fasinumab treatment were observed in a phase IIb/III study in patients with OA of the knee or hip.[20] In the current study, mean ALP elevations (peak at week 16) were lower than observed in the previous OA study, even in the pOA subgroup. ALP levels returned towards normal during the post-treatment follow-up, as has been previously reported.[20] It is unclear whether these small changes in ALP associated with treatment represent bone turnover or a more independent effect on enzyme production or enzymatic activity.

Despite dosing being prematurely terminated, all fasinumab doses provided improvements versus placebo in measures of pain (average daily LBPI NRS score), function (RMDQ) and overall patient assessment (PGA) over the first 8 weeks of the study. Significant pain improvement was maintained over 16 weeks for both fasinumab 9 mg groups, but not for 6 mg. Further studies will be needed to determine whether the robust efficacy shown at week 8 is sustained for longer durations at lower doses. Although the treatment benefit in this study was numerically greater in the pOA subgroup, the rates of AA in these patients were substantially higher. This study, therefore, validated concerns about the use of fasinumab in CLBP subjects with concomitant OA, whose benefit–risk at the highest doses was unacceptable. For patients without pOA, low rates of AA were observed at these high doses, though treatment effect was more modest. In these patients, since their back pain may be dominated by mechanisms other than OA, fasinumab may be less likely to provide benefit. Hypothetically these patients may also need even higher doses, and joint AEs would need to be carefully balanced against treatment benefits. Further studies with longer treatment and follow-up are needed to inform benefit–risk.

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Data availability statement Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing once the product and indication has been approved by major health authorities (e.g., FDA, EMA, PMDA, etc), if there is legal authority to share the data and there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/

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EPIDEMIOLOGICAL SCIENCE

Influence of COVID-19 pandemic on decisions for the management of people with inflammatory rheumatic and musculoskeletal diseases: a survey among EULAR countries

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ABSTRACT

Objectives To investigate how the first wave of COVID-19 pandemic influenced decisions of rheumatologists and health professionals in rheumatology regarding the management of patients with inflammatory rheumatic and musculoskeletal diseases (RMDs).

Methods An English-language questionnaire was developed by a EULAR working group and distributed via national rheumatology societies of EULAR countries, EMEUNET and individual working group members. Responses were collected using an online survey tool. Descriptive statistics were calculated.

Results We analysed 1286 responses from 35/45 EULAR countries. Due to containment measures, 82% of respondents indicated cancellation/postponement of face-to-face visits of new patients (84% of them offering remote consultation) and 91% of follow-up visits (96% with remote consultation). The majority of respondents (58%) perceived that the interval between symptom onset and first rheumatological consultations was longer during containment restrictions than before. Treatment decisions were frequently postponed (34%), and the majority (74%) of respondents stated that it was less likely to start a biological disease modifying anti-rheumatic drug (DMARD)/targeted synthetic DMARD during the pandemic, mainly because of patients' fear, limited availability of screening procedures and decreased availability of rheumatological services. Use of (hydroxy)chloroquine (HCQ) and tocilizumab (TCZ) for the COVID-19 indication was reported by 47% and 42% of respondents, respectively, leading to a shortage of these drugs for RMDs indications according to 49% and 14% of respondents, respectively.

Conclusion Measures related to containment of COVID-19 pandemic led to a perceived delay between symptom onset and a first rheumatological visit, postponement of treatment decisions, and shortage of HCQ and TCZ, thereby negatively impacting early treatment and treat-to-target strategies.

Key messages

What is already known about this subject?

- Containment measures have been established in several European countries to prevent exponential growth of the infectious rate with the novel SARS-CoV-2 causing COVID-19.
- (Hydroxy)chloroquine (HCQ) or tocilizumab (TCZ) have been used for treatment of some patients with COVID-19.

What does this study add?

- This study investigated from a public health perspective to what extent COVID-19 affected decisions of rheumatologists and health professionals in rheumatology concerning the management of patients with inflammatory rheumatic and musculoskeletal diseases (RMDs).
- Rheumatology services were partially or completely closed in the majority of EULAR countries leading to cancellation/postponement of face-to-face visits.
- The perceived interval between symptom onset and first rheumatological consultations was longer during containment restrictions than before.
- Treatment decisions were frequently postponed and it was less likely to start a biological disease modifying anti-rheumatic drug (DMARD)/targeted synthetic DMARD during the pandemic.
- Use of HCQ and TCZ for the COVID-19 indication led to a shortage of these drugs for RMDs patients.

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INTRODUCTION

The novel SARS-CoV-2 and COVID-19 is a highly contagious disease that has reached Europe at the beginning of 2020 and has been causing high morbidity and mortality.[1–3] Containment measures have been established in most European countries in order to prevent exponential growth of the infection.[3] To what extent these measures influenced early diagnosis and treatment of patients with inflammatory rheumatic and musculoskeletal diseases (RMDs) is unknown.

While the majority of patients with COVID-19 has a favourable outcome, some of them develop severe pneumonia eventually leading to respiratory failure along with other organ manifestations and

Key messages

How might this impact on clinical practice or future developments?

- Telemedicine and other care strategies should be researched more intensively in order to maintain high-quality of care even when face-to-face visits are not feasible.
- Future off-label use of drugs for COVID-19 indication outside a clinical trial should be discouraged as it might led to shortage of the respective substance for patients with RMDs.
- Prioritising strategies for face-to-face visits and investigations should be developed in order not to delay diagnosis and treatment and to guarantee adequate monitoring of disease activity and safety of patients with inflammatory RMDs also during future waves of COVID-19 or other pandemics caused by highly contagious infectious agents.

sepsis.[1] COVID-19 appears to have at least two distinct disease phases: a phase characterised by the immune response against the virus aiming at eliminating the pathogen, and in some patients, a subsequent phase of severe 'cytokine release syndrome' instead of the expected phase of convalescence.[4] Some of the most severe complications of COVID-19 seem indeed to be caused by an exaggerated response of the immune system. Immunomodulatory agents commonly prescribed in rheumatology such as (hydroxy)chloroquine (HCQ) or tocilizumab (TCZ) have been used for treatment of patients with COVID-19.[5–7] Whether the off-label use of these drugs in COVID-19 induces a shortage of supply and whether this has an impact on treatment decisions in patients with RMDs is elusive so far.

Looking at the current situation from a public health perspective, there are several questions that arise: (1) have the 'treat to target' and 'early diagnosis' paradigms for patients with inflammatory RMDs been still feasible during the COVID-19 crisis?; (2) have patients been less likely to initiate TCZ or other biologicals or have they been switched from TCZ to therapies with other modes of action in order to save drugs for patients with COVID-19?; (3) has a shortage of medication led to patients having to stop HCQ or TCZ?

This EULAR project was designed to clarify how and to what extent COVID-19 affected decisions of rheumatologists and health professionals in rheumatology (HPR) concerning the management of patients with RMDs from a public health perspective. The knowledge gained from this study will help to prepare for future waves of COVID-19 and other pandemics caused by highly contagious infectious agents.

METHODS

An English-language questionnaire was developed by a EULAR working group composed of rheumatologists, a methodologist, experts in public health, and an HPR. The questionnaire contained 37 questions organised in three broad sections: (1) professional background, (2) influence of containment measures on the organisation of care for patients with inflammatory RMDs and (3) drugs used both in rheumatology and to treat COVID-19. The majority of questions were in the multiple-choice format recognising the possibility that multiple not mutually exclusive strategies might have been applied (eg, which patient groups have been prioritised during closure for a face-to-face or remote visit). The survey also contained a few single choice (eg, for age and sex) or open-ended questions.

The survey was distributed via EULAR secretariat and EULAR scientific member societies (No.: 45), delegates of the EULAR Standing Committee on Epidemiology and Health Services Research, and EMEUNET using emails, newsletters and social media. The working group members also personally contacted physicians and HPR from different countries, requesting them to answer and disseminate the questionnaire (snow-ball principle). The questionnaire was accompanied by an explanatory letter regarding the purpose of the survey. The answers were collected via an online survey tool (SurveyMonkey) from 13 May till 17 June 2020. At least one reminder was sent by EMEUNET and individual working group members. Online supplemental file 1 provides the full questionnaire and additional details on the execution of the survey. Ethical approval was not required because the study did not involve patients; all responses were anonymous.

The target audience of the survey were rheumatologists and other physicians or HPR from EULAR countries who have been directly involved in care of patients with inflammatory RMDs, however; the survey was open to all physicians/HPR.

Descriptive and summary statistics were applied to the questionnaire responses. Absolute and relative frequencies were calculated and depicted in tabular and graphical form. Data are presented as number (nominator) and percentage of all available responses to each question (denominator) throughout the manuscript. The denominator may change from question to question for the following reasons: (1) questions and individual answers could have been skipped, (2) some questions could have been answered with 'not applicable' or 'do not know', which were detracted from the denominator as indicated, (3) specific subgroup analyses were conducted. Since the majority of questions were in the multiple-choice format, the sum of nominators from individual questions may exceed the corresponding denominator.

RESULTS

A total of 1428 responses were collected from 58 countries (see online supplemental table 1 for number of responses from all countries): 1286 (90%) were from 35 out of the 45 EULAR countries, 15 (1%) came from Africa, 10 (0.7%) from Asia, 8 from North-America (0.6%), 7 from South-America (0.5%), 2 (0.1%) from Australia/New Zealand, 1 (0.1%) from Andorra whereas 99 (7%) have not specified the country of practice.

In this paper, only results for EULAR countries are presented (n=1286). Ten (22%) EULAR countries provided no and 19 (56%) more than 10 responses. Demographic data of respondents are summarised in table 1. The number of responses per question ranged from 663 to 1286. To support the interpretation of results in relation to the country-specific impact of COVID-19, we summarised data on infections with SARS-CoV-2, mortality and containment measures in EULAR countries as per April 2020 in online supplemental table 2.

Influence of containment measures on organisation of care for patients with inflammatory RMDs

General organisation of rheumatology care

Partial closure of rheumatology services guaranteeing, for example, only emergency visits was reported by 622/1094 (57%, 192 skipped the question) of respondents, 19 (2%) indicated that rheumatology services were suspended completely at least temporarily, 265 (24%) reported both, partial and complete closure and only 188 (17%) indicated no closure. Partial closure typically lasted between 5 and 8 weeks (43% of

Table 1 Demographics of respondents from EULAR countries (n=1286)

		Number of responses	Percentage of responses
Professional background	Rheumatologist (or other specialist primarily managing patients with inflammatory RMDs)	966	75.1
	Rheumatologist in training	145	11.3
	Healthcare professional in rheumatology	163	12.7
	Other*	12	0.9
Primary affiliation	University hospital	648	50.4
	Community based hospital	375	29.2
	Private practice	231	18.0
	Other	32	2.5
Responses according to countries	Romania	143	11.1
	Italy	121	9.4
	Netherlands	114	8.9
	Germany	110	8.6
	France	109	8.5
	Spain	80	6.2
	Denmark	78	6.1
	Austria	76	5.9
	UK	70	5.5
	Greece	69	5.4
	Switzerland	55	4.3
	Portugal	46	3.6
	Croatia	36	2.8
	Turkey	33	2.6
	Sweden	31	2.4
	Ireland	19	1.5
	Finland	17	1.3
	Norway	15	1.2
	Hungary	13	1.0
	Slovenia	8	0.6
	Belgium	5	0.4
	Albania	4	0.3
	Georgia	4	0.3
	Israel	4	0.3
	Lebanon	4	0.3
	Cyprus	3	0.2
	Czech Republic	3	0.2
	Latvia	3	0.2
	Montenegro	3	0.2
	Russian Federation	3	0.2
	Bulgaria	2	0.2
	Serbia	2	0.2
	Belarus	1	0.1
	San Marino	1	0.1
	North Macedonia	1	0.1
Age ranges	<30	4.7%	
	30–39	24.9%	
	40–49	29.5%	
	50–59	26.2%	
	60–69	12.8%	
	≥70	2.0%	
Gender	Male	475	37.0
	Female	807	62.9
	Other	2	0.2
Number of patients with inflammatory RMDs normally seen in a week by the respondent	<30	449	35.2
	30–59	552	43.2
	60–99	192	15.0
	≥100	84	6.5

*Specialists in rehabilitation, physicians primarily working for pharma or health insurance, specialist in nuclear medicine, dermatologist, nephrologists, internists, retired rheumatologists.
RMD, rheumatic and musculoskeletal disease.

those who reported partial closure), whereas complete closure was normally not longer than 1–4 weeks (48% of those who reported complete closure). See figure 1 for data on duration of partial and complete closure according to different EULAR countries. A median of 26.4% (±34.1%) of total working time of respondents (ie, workforce) was reallocated to other services such as emergency department, infectious disease clinic, COVID-19 unit or similar.

Due to complete and/or partial closure of rheumatology services, 899/1094 (82%) physicians/HPR indicated cancellation or postponement of at least some face-to-face visits of new patients with (suspected) RMDs, 84% of those who had to cancel/postpone visits offered remote consultation at least for some of these visits (see tables 2 and 3 for details). Concerning follow-up visits, 991/1094 (91%) responded to have cancelled/postponed visits with 96% of them offering remote consultation. The frequency of postponement/cancellation of face-to-face visits of new patients and follow-up visits in relation to the duration of partial and complete closure is detailed in figure 2. Accordingly, the percentage of postponed/cancelled visits increased along with the duration of closure.

Remote consultations were conducted by different health workers: 924/1030 (90%) respondents indicated that rheumatologists and/or other specialists performed this activity, 302 (29%) and 223 (23%) stated that specialists in training and HPR, respectively, were (also) involved. Phone (966/1005, 96%) and/or email (n=498, 50%) were among the techniques most commonly used to consult with patients, whereas video (n=241, 24%) or mobile applications (n=44, 4%) were less frequently applied. Respondents stated that patients with suspected inflammatory RMDs (458/1029, 45%), those with previously unstable or active disease (n=563, 55%) or those with ongoing intravenous drug therapy (n=448, 44%) were prioritised for a face-to-face visit. They also indicated that patients receiving biological disease modifying anti-rheumatic drugs (bDMARDs) or targeted synthetic DMARDs (tsDMARDs) (319/1031, 31%) as well as those with unstable disease (n=234, 23%) were prioritised for a remote consultation. No specific prioritisation plan was reported by 277/1029 (27%) for face-to-face visits and by 434/1031 (42%) respondents for remote consultations.

Influence of changed care on principles of early diagnosis and treat to target

The majority of respondents had the impression that the intervals between symptom onset and first rheumatological visits were longer during COVID-19 related closure as compared with the months before (599/1031, 58%, with 26% of those 599 physicians/HPR stating that it was considerably longer).

A minority of respondents (153/1030, 15%) answered that they were contacted more frequently by patients for a suspected flare as compared with before the crisis. Patients with a suspected flare were managed using multiple approaches: most physicians/HPR indicated that a face-to-face visit (723/927, 78% to whom the question was applicable) or a remote consultation (n=553, 60%) were offered. Day-care or in-patient care, referral to the emergency department or consultation with another specialist were rare options (each<10%). The majority of respondents (678/1029, 66%) felt that disease activity of patients with inflammatory RMDs they consulted during closure was not different from that in the preceding period.

Cancellation or postponement of non-urgent tests either by the service provider or by patients themselves were reported by 699/1030 (68%) and 426 (41%) respondents, respectively. Also, 34% of physicians/HPR (299/873 to whom the question was applicable) indicated that treatment decisions were frequently postponed and 62% (n=542) stated that patients' management was mainly based on history and clinical examination without additional tests.

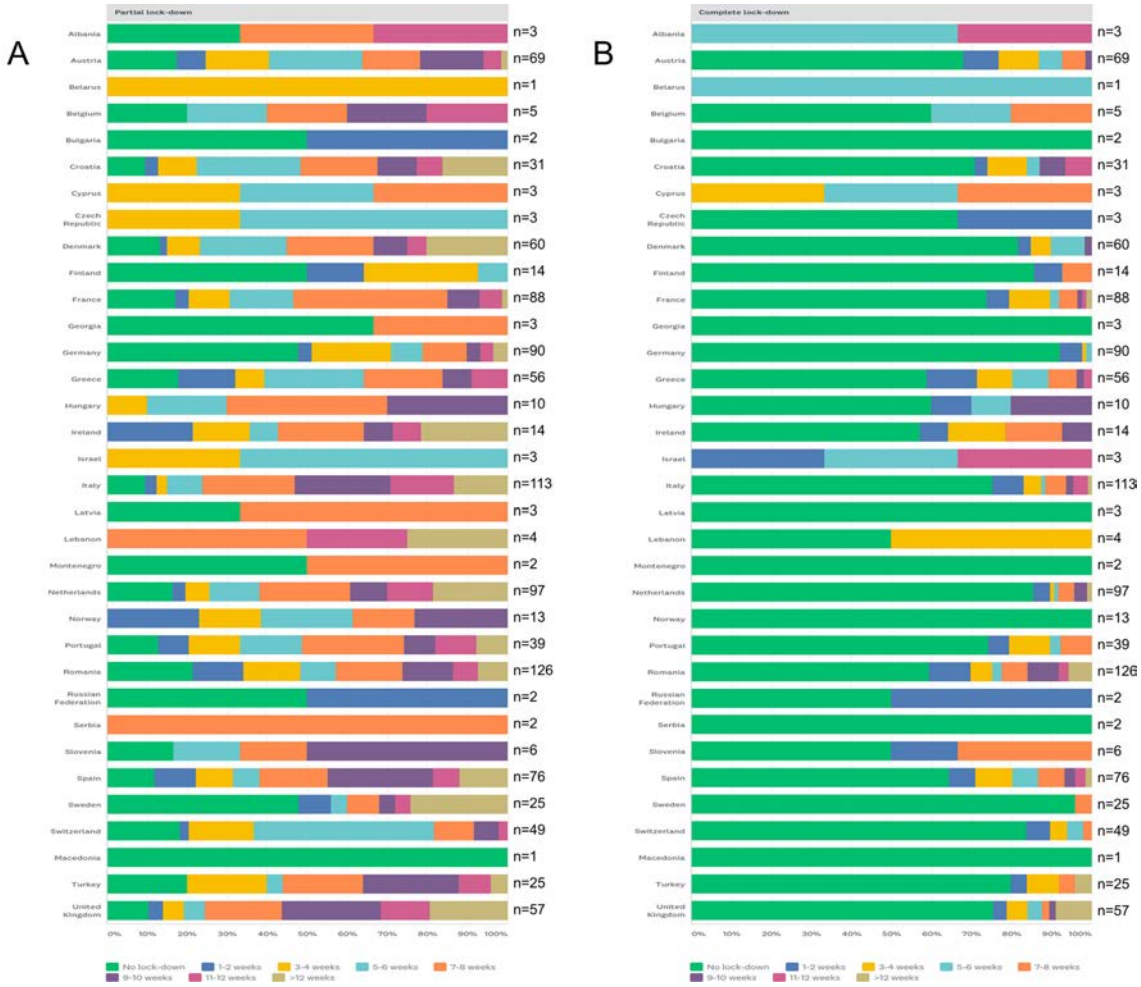

Figure 1 Partial and complete closure of rheumatology services in EULAR countries. Figures indicate the percentage of respondents indicating the number of weeks with partial (A) or complete (B) closure according to different countries.

Drugs used in rheumatology and to treat COVID-19

The use of HCQ for COVID-19 indications was reported by 466/1003 (47%) respondents. HCQ was particularly prescribed to patients admitted to the hospital (351 of those 442 who felt knowledgeable to answer this question, 79%) or to the intensive care unit (n=234, 53%), but also to those managed on an outpatient basis (184, 42%). Only a minority of respondents used HCQ for prophylaxis in health workers and/or other individuals (38/1003, 4%) as well as in patients with RMDs (mean 2%±9% of RMDs patients, n=914 responses). A shortage of HCQ was noted by 492/999 (49%) of respondents with large differences between countries (see figure 3). Consequently, this drug had to be stopped in a mean of 10% (±18%) of RMDs patients (n=811 responses). The majority of physicians/HPR (738/996, 74%) stated that they were less likely to start a bDMARD or tsDMARD in RMDs patients during COVID-19 crisis mainly because of patient's fear to start such a treatment (n=569, 57%), limited availability of screening procedures (n=284, 29%) and/or decreased availability of rheumatological services (n=270, 27%).

Treatment of patients with COVID-19 with TCZ was reported by 423/1005 (42%) respondents, either in the setting of a clinical trial (178 of those 423 who indicated the use of TCZ in their hospital or practice, 42%) or off-label outside a study (n=245, 58%). TCZ was mainly administered to patients admitted to the intensive care unit (64% of those reporting use of TCZ for COVID-19). A shortage of TCZ was noted by 134/980 (14%) respondents, mainly in Italy and Spain as outlined in figure 3. Overall, shortage or expected shortage of TCZ only rarely influenced the decision to start this drug in rheumatoid arthritis (RA)

Table 2 Cancellation or postponement of face-to-face visits of new patients, according to the extent of closure of the rheumatology services

	No closure	Complete closure	Partial closure	Complete and partial closure	Total
No cancellation	67 (35.6)	2 (10.5)	102 (16.4)	24 (9.1)	195 (17.8)
With remote visit	24 (12.8)	4 (21.1)	94 (15.1)	17 (6.4)	139 (12.7)
Without remote visit	14 (7.4)	2 (10.5)	96 (15.4)	33 (12.5)	145 (13.3)
With and without remote visits	83 (44.1)	11 (57.9)	330 (53.1)	191 (72.1)	615 (56.2)
Total	188 (100)	19 (100)	622 (100)	265 (100)	1094 (100)

Data indicate the number (percentages) of respondents indicating cancellation/postponement of face-to-face visits of new patients with (suspected) rheumatic and musculoskeletal diseases with or without remote consultations.

Table 3 Cancellation or postponement of follow-up face-to-face visits, according to the extent of closure of the rheumatology services

	No closure	Complete closure	Partial closure	Complete and partial closure	Total
No cancellation	48 (25.5)	2 (10.5)	39 (6.3)	14 (5.3)	103 (9.4)
With remote visit	35 (18.6)	4 (21.1)	115 (18.5)	26 (9.8)	180 (16.5)
Without remote visit	4 (2.1)	2 (10.5)	21 (3.4)	15 (5.7)	42 (3.8)
With and without remote visits	101 (53.7)	11 (57.9)	447 (71.9)	210 (79.2)	769 (70.3)
Total	188 (100)	19 (100)	622 (100)	265 (100)	1094 (100)

Data indicate the number (percentages) of respondents indicating cancellation/postponement of follow-up face-to-face visits of patients with rheumatic and musculoskeletal diseases with or without remote consultations.

or giant cell arteritis, or to change treatment in patients with stable disease as depicted in table 4. In Italy and Spain however, preference of another bDMARD/tsDMARD, postponement of treatment with TCZ, as well as change of therapy in stable patients was commonly considered (online supplemental table 3).

Other bDMARDs/tsDMARDs used to treat patients with COVID-19 were sarilumab (58 of those 728 who felt knowledgeable to answer this question, 8%), baricitinib (n=55, 8%), canakinumab (n=20, 3%) and/or anakinra (n=103, 14%).

A recommendation for patients with RMDs to decrease or stop nonsteroidal anti-inflammatory drugs (NSAIDs) even when they did not have symptoms of COVID-19 in order to decrease the possible risk for a worse outcome of this disease was made by 151/998 (15%) and 15 (2%) of respondents, respectively. Similarly, 226/1000 (23%) and 1 (0.1%) recommended to decrease or stop glucocorticoids, respectively.

DISCUSSION

The magnitude of the impact of COVID-19 on both management decisions and quality of care of patients with RMDs has been unknown. The most worrisome findings, although not unexpected, are the fact that the lag between symptom onset to first rheumatological visits was increased during COVID-19 related closure, and that treatment decisions, particularly those to start a new b/tsDMARD were postponed mainly because of patients' concerns to start a new treatment during the pandemic, but also due to limited availability of rheumatological services and/or screening tests. COVID-19 thus impacts heavily on two fundamental principles of rheumatology management, namely those of early diagnosis and treat to target.[8 9] While we know from previous studies that long-term non-adherence to these strategies results in worse clinical and structural outcomes, the question to what extent a short-term interruption due to an infectious crisis impacts patients' disease course is still unclear.[8 10] See box 1 for the lessons learnt from this wave of the COVID-19 pandemic.

EULAR provisional recommendations for the management of RMDs in the context of SARS-CoV-2 suggest to consider withholding face-to-face visits temporarily or transforming them into a remote visit in phase of closure when the rheumatic disease is stable.[11] According to the results of our survey, rheumatology service providers compensated for cancelled/postponed face-to-face visits using telemedicine, and many of them developed standard operating procedures to prioritise patients for face-to-face visits. Recent publications also indicate rapid development of telemedicine during the first wave of the pandemic,[12–14] however, it seems that patients' acceptance of telemedicine is only moderate yet.[15 16] Besides, we have insufficient data on the effectiveness of telemedicine in rheumatology and need to know more about how and when telemedicine might efficaciously replace live visits.[17] Given the expected increase in the prevalence of inflammatory and non-inflammatory RMDs in future due to an ageing population and other reasons, and the expected insufficient growth of workforce in rheumatology,[18 19]

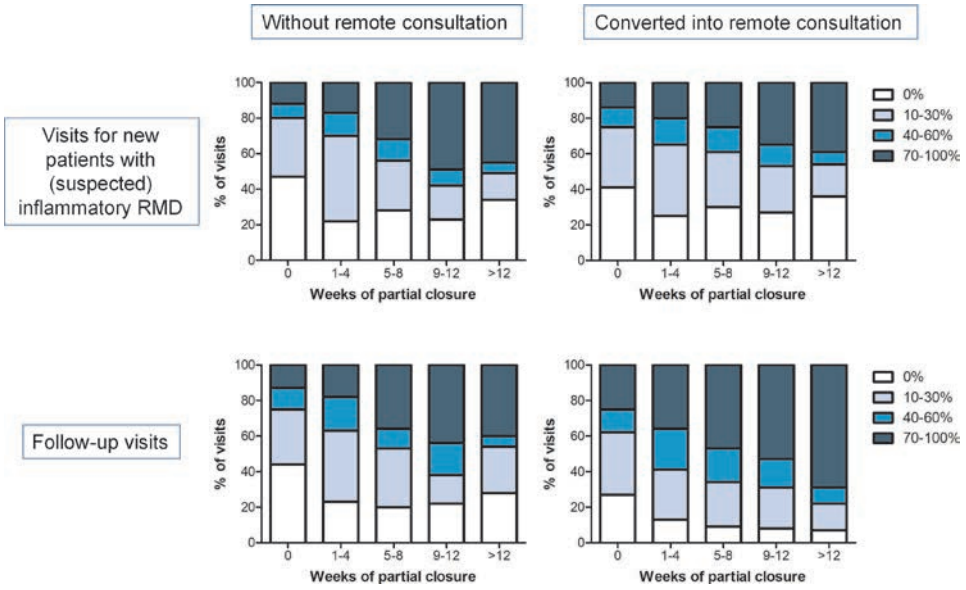

Figure 2 Postponement/cancellation of face-to-face visits according to the duration of closure of rheumatology services. Figures indicate the cumulative percentage of respondents (Y axis) indicating the proportion of face-to-face visits (4 categories represented by the colours) of new patients and follow-up visits postponed/cancelled with or without remote consultation in relation to the duration of partial and/or complete closure of rheumatology services in weeks.

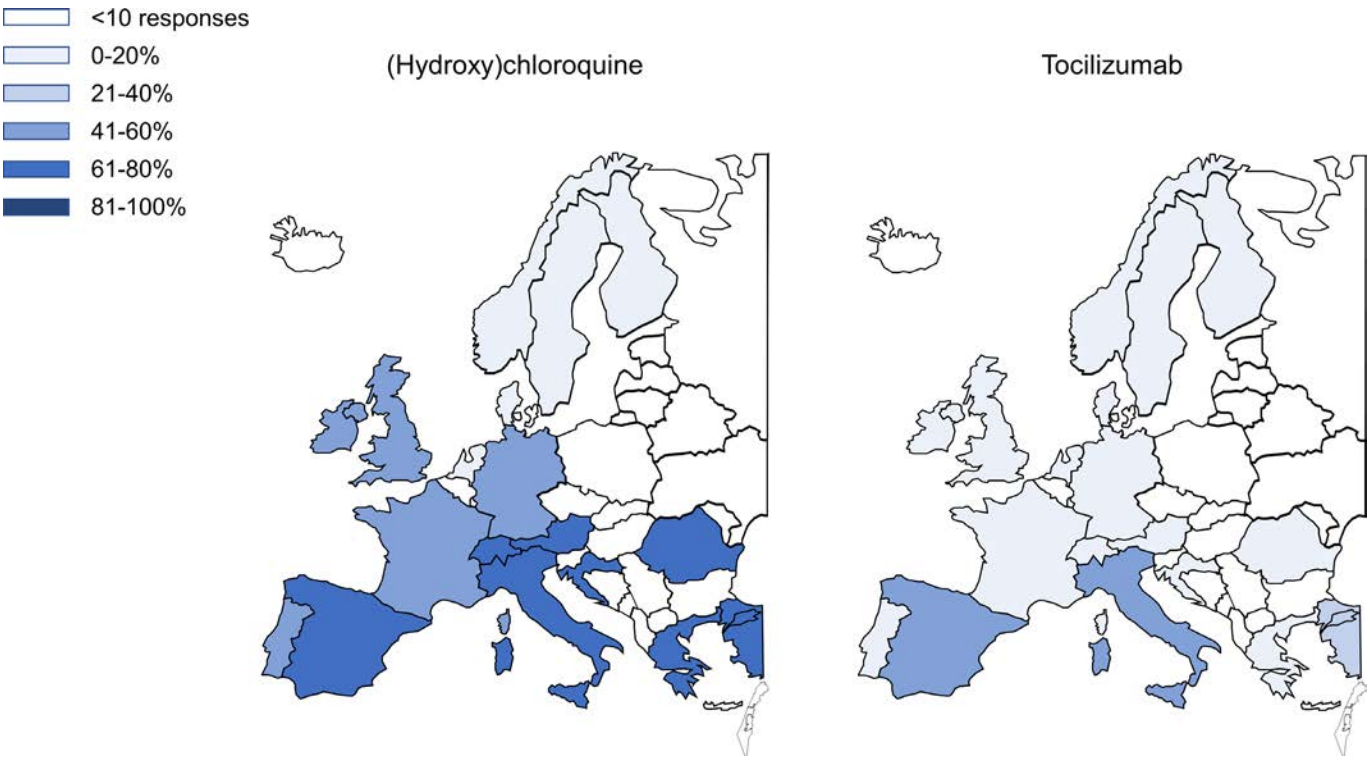

Figure 3 Shortage of (hydroxy) chloroquine (HCQ) and tocilizumab (TCZ) in EULAR countries. Figures indicate the percentage of respondents indicating a shortage of HCQ and/or TCZ according to countries. Only data for EULAR countries with >10 responses are shown.

telemedicine and strategies to better prioritise visits are essential to maintain high quality of care in RMDs, irrespective of additional waves of the COVID-19 pandemic.

Another lesson we learnt from this crisis is that we need to better address patients' concerns and fears about possible risks of immunosuppression in order not to delay treatment of new or active patients. Till today, there is no convincing evidence suggesting that patients with RMDs (regardless of whether or not they are taking DMARDs) are at an increased risk for COVID-19 infection and course as compared with the general population.[20 21] Many advisories, including official government bodies nevertheless considered these patients at risk with corresponding communications to patients' societies, which might have further increased patients' concerns to adhere to hospital visits and immunosuppressive therapy.[22–26]

Another observation is that management of RMD patients during closure was mainly based on patient's history and clinical examination, given that non-urgent tests were either not available or not desired by patients. Some of these tests such as imaging are important to inform rheumatologists who establish a diagnosis and to aid monitoring of disease status and disease activity.[27–29] Similarly, laboratory tests are essential to guarantee patients' safety in case a new DMARD is considered but also for those who are on stable drug treatment.[30] Investigations performed in the office as part of the clinical visit (eg, ultrasound conducted by the rheumatologist) or on a domestic

Table 4 Influence of shortage/expected shortage of tocilizumab on treatment decisions in rheumatoid arthritis and giant cell arteritis

Influenced decision to start tocilizumab de novo			
Rheumatoid arthritis		**Giant cell arteritis**	
	n=707*		n=663*
No influence	599 (85%)	No influence	614 (93%)
Preference of another bDMARD/tsDMARD	76 (11%)	Preference of MTX or another csDMARD	24 (4%)
Postponement of treatment with TCZ	32 (5%)	Postponement of treatment with TCZ	19 (3%)
		Sarilumab used off-label	6 (1%)
Influenced decision to modify treatment with tocilizumab in patients with stable disease			
	n=925*		n=788*
No influence	683 (74%)	No influence	709 (90%)
Switch of intravenous to subcutaneous TCZ	191 (21%)	Switch of intravenous to subcutaneous TCZ	65 (8%)
Prolongation of administration interval	28 (3%)	Prolongation of administration interval	10 (1%)
Change of TCZ to another DMARD	5 (0.6%)	Change of TCZ to another DMARD	2 (0.3%)
Change of TCZ to sarilumab	18 (2%)	Stopped treatment with TCZ	2 (0.3%)

*Total number of answers to this question.
MTX; methotrexate; b, biological; cs, conventional synthetic; DMARD, disease modifying anti-rheumatic drug; TCZ, tocilizumab; ts, targeted synthetic.

Box 1 Lessons learnt from this wave of COVID-19 pandemic

- Patient communication needs to be improved in order to address patients' concerns about the risk of infection and course of new viral epidemics such as COVID-19, particularly if a new disease modifying anti-rheumatic drug therapy is planned.
- Telemedicine and other models of care should be regularly assessed and researched more intensively in order to maintain high-quality of care even when face-to-face visits are not feasible.
- Off-label use of drugs for COVID-19 indication outside a clinical trial might lead to shortage of the respective substance for patients with rheumatic and musculoskeletal diseases (RMDs) and should be discouraged.
- Prioritising strategies for face-to-face visits and investigations such as laboratory testing, imaging and others should be developed in order not to delay diagnosis and treatment, and to guarantee adequate monitoring of disease activity and safety of patients with RMDs.

basis (eg, blood tests) might be preferable over those requested from another department or hospital service, in order to reduce (patients' concerns about) the contact to other patients and hospital-based structures.

HCQ was used for the COVID-19 indication according to almost half of respondents for inpatients and outpatients and occasionally for prophylaxis. The common use of this drug in this off-label indication led to a shortage in several countries and consequently, about 10% of patients with RMDs had to stop it at least temporarily. A shortage of TCZ occurred mainly in Italy and Spain, two countries who were heavily affected by the COVID-19. Clinicians might have been pressured to try every drug with possible efficacy in critically ill patients, however, the use of HCQ and TCZ for COVID-19 was not based on solid data rather than on theories about the mode of action, case series and small observational studies.[31–33] Recent studies indicate that HCQ is not beneficial for COVID-19,[34 35] and some evidence suggests that it might perhaps increase mortality when combined with azithromycine.[36] Patients with inflammatory RMDs, particularly those with connective tissue disease, might be at a considerable risk of flare when they run out of HCQ.[37] A comparable problem arises for TCZ: while a change to another bDMARD/tsDMARD (at least in RA) might be considered in case of drug shortage, this is definitely not desirable due to the risks of intolerance and lack of efficacy. Our survey indicates that in fact, this has been performed only occasionally in clinical practice. While there is some evidence from observational studies and non-randomised trials that TCZ helps to reduce the mortality of patients with COVID-19 who develop severe (autoinflammatory) pneumonia,[38] the randomised phase III (COVACTA) trial comparing TCZ with placebo in patients with severe COVID-19 associated pneumonia failed its primary endpoint of improved clinical status, as well as the key secondary endpoint of reduced mortality.[39] Almost 60% of those 423 physicians/HPR who stated that TCZ had been used in their hospital/practice for patients with COVID-19 indicated off-label use of this drug outside a clinical trial, an ethically questionable approach that is discouraged by EULAR.[11]

NSAIDs, which have been concerned to upregulate ACE 2 receptors and to increase the susceptibility to the virus,[40] and glucocorticoids, which might negatively affect virus clearance,[41] should not automatically be stopped in patients with RMDs according to the EULAR task force.[11] Even patients with symptoms of COVID-19 who are chronically treated with glucocorticoids should continue this treatment.[11] Interestingly, 23% of respondents advised their patients to reduce the glucocorticoid dose and 15% that of NSAIDs, presumably not to expose patients to unnecessary risk during the pandemic. Discontinuation of these drugs, however, was the exception.

Our study is limited by the descriptive nature and by a potential responder bias. There were more responses from Romania and the Netherlands, countries with a relatively small population, than from the UK, Spain, France or Germany. We followed the same dissemination strategy of the survey in every country, so any imbalance in the number of responses compared with the expected target population may be due to factors beyond our control (eg, different communication strategies of national societies). Furthermore, owing to its anonymous nature, the survey could have been completed by different healthcare providers within the same centre, and we were unable to contact respondents to solve any data inconsistency. Two respondents, for example, indicated no cancellation of first or follow-up visits despite complete closure of their rheumatology service. While there might be a plausible explanation for this answer (eg, patients were sent to another rheumatologist), we were unable to clarify it. We did not ask to stratify the responses on prioritisation strategies according to diagnosis, acknowledging that the diagnosis (eg, inflammatory arthritis vs systemic RMDs) might have had an impact on these strategies.

Our study reflects experiences and opinions of physicians and HPR from EULAR countries and despite its limitations, this survey provides important insights into management decisions concerning patients with inflammatory RMDs during the COVID-19 outbreak. Retrieval of empiric data to respond to the questions raised was certainly not feasible during this wave of the pandemic.

In conclusion, measures related to containment of the COVID-19 pandemic led to a perceived delay between symptom onset and a first rheumatological visit, a postponement of treatment decisions, and a shortage of drugs used to treat RMDs patients and those with COVID-19 such as HCQ and TCZ. Important lessons we have learnt are the need to better address patients' concerns about the risk of infection and course of COVID-19, particularly in case a new DMARD is planned. Telemedicine and prioritising strategies should be researched more extensively in order to maintain high quality of care even when face-to-face visits and other investigations, such as laboratory testing or imaging, are not feasible, for example, during a future wave of the COVID-19 pandemic.

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Contributors All authors designed the study and contributed to data collection. CD analysed the data and drafted the first version of the manuscript. All coauthors critically interpreted the results, discussed the findings together, critically reviewed the manuscript and approved its final version.

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CLINICAL SCIENCE

Severity of COVID-19 and survival in patients with rheumatic and inflammatory diseases: data from the French RMD COVID-19 cohort of 694 patients

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ABSTRACT

Objectives There is little known about the impact of SARS-CoV-2 on patients with inflammatory rheumatic and musculoskeletal diseases (iRMD). We examined epidemiological characteristics associated with severe disease, then with death. We also compared mortality between patients hospitalised for COVID-19 with and without iRMD.

Methods Individuals with suspected iRMD-COVID-19 were included in this French cohort. Logistic regression models adjusted for age and sex were used to estimate adjusted ORs and 95% CIs of severe COVID-19. The most significant clinically relevant factors were analysed by multivariable penalised logistic regression models, using a forward selection method. The death rate of hospitalised patients with iRMD-COVID-19 (moderate–severe) was compared with a subset of patients with non-iRMD-COVID-19 from a French hospital matched for age, sex, and comorbidities.

Results Of 694 adults, 438 (63%) developed mild (not hospitalised), 169 (24%) moderate (hospitalised out of the intensive care unit (ICU) and 87 (13%) severe (patients in ICU/deceased) disease. In multivariable imputed analyses, the variables associated with severe infection were age (OR=1.08, 95% CI: 1.05–1.10), female gender (OR=0.45, 95% CI: 0.25–0.80), body mass index (OR=1.07, 95% CI: 1.02–1.12), hypertension (OR=1.86, 95% CI: 1.01–3.42), and use of corticosteroids (OR=1.97, 95% CI: 1.09–3.54), mycophenolate mofetil (OR=6.6, 95% CI: 1.47–29.62) and rituximab (OR=4.21, 95% CI: 1.61–10.98). Fifty-eight patients died (8% (total) and 23% (hospitalised)). Compared with 175 matched hospitalised patients with non-iRMD-COVID-19, the OR of mortality associated with hospitalised patients with iRMD-COVID-19 was 1.45 (95% CI: 0.87–2.42) (n=175 each group).

Conclusions In the French RMD COVID-19 cohort, as already identified in the general population, older age, male gender, obesity, and hypertension were found to be associated with severe COVID-19. Patients with iRMD on corticosteroids, but not methotrexate, or tumour necrosis factor alpha and interleukin-6 inhibitors, should be considered as more likely to develop severe COVID-19. Unlike common comorbidities such as obesity, and cardiovascular or lung diseases, the risk of death is not significantly increased in patients with iRMD.

Trial registration number ClinicalTrials.gov Registry (NCT04353609).

Key messages

What is already known about this subject?

- As stated by recent European League Against Rheumatism guidelines, there is no evidence that patients with inflammatory rheumatic and musculoskeletal diseases (iRMD) are at higher risk of SARS-CoV-2 infection than individuals without iRMD, nor have a worse prognosis with a diagnosis of COVID-19.
- In patients with iRMD, glucocorticoid therapy at doses ≥10 mg/day of equivalent (prednisone) is associated with higher odds of hospitalisation and anti-tumour necrosis factor (TNF) with decreased odds.

What does this study add?

- Patients with iRMD are more likely to develop severe SARS-CoV-2 infection when they have comorbidities already identified as risk factors of severe COVID-19 infection in the general population, such as older age, male gender, obesity, and hypertension.
- Regardless of the dose, corticosteroids were associated with severe infection, whereas methotrexate, and TNFα and interleukin-6 (IL-6) inhibitors were not. Anti-TNF use was associated with less frequent hospitalisation.
- When matched for common comorbidities, the population with iRMD may not have more frequent death compared with the population with non-iRMD.

How might this impact on clinical practice or future developments?

- In addition to common risk factors for severe SARS-CoV-2 infection, patients with iRMD on any dose of corticosteroid should be considered as particularly fragile and at high risk for developing severe disease, whereas patients on methotrexate and TNFα and IL-6 inhibitors are not.
- A potential risk of more severe COVID-19 in patients with interstitial lung disease or treated by rituximab justifies further research.

INTRODUCTION

In December 2019, COVID-19, caused by the SARS-CoV-2, emerged from Wuhan, China.[1 2] Beginning 1 February 2020, France had a total of six confirmed cases and was under nationwide lockdown by 17 March,[3] and now has just over 344 000 confirmed cases and over 30 000 deaths (as of 5 October 2020),[4] with a mean age 68 years for hospitalised patients and 79 years for those who

died.[5] Stay-at-home restrictions in France decreased hospitalisations nearly 11-fold[5]; however, there remains an urgent need for safe, effective COVID-19 therapies.

There is a concern that patients undergoing immunosuppressive therapy for inflammatory rheumatic and musculoskeletal diseases (iRMD) could be more vulnerable to SARS-CoV-2 infection and hospitalisation than the general population, particularly in those patients with comorbidities such as diabetes, chronic obstructive pulmonary disease, and renal failure.[6 7] Several recent studies in patients with iRMD[8–10] and inflammatory bowel diseases (IBD)[11] suggested an increased risk for hospitalisation and severe disease when using glucocorticoids, although no effect on severity or mortality was found with biological disease-modifying anti-rheumatic drug (DMARD) use. A decreased risk for severe COVID-19 was suggested in such populations with respect to anti-tumour necrosis factor alpha (TNFα) drugs.[11 12] Although these studies indicate that the incidence of immune-mediated inflammatory disease among patients with COVID-19 was consistent with the general population and not associated with worse outcomes, population size was a major limitation. Recent European League Against Rheumatism (EULAR) guidelines suggested that patients with RMD are not at greater risk for developing SARS-CoV-2 infection or more severe disease,[13] but as additional information is obtained through ongoing research and clinical trials, recommendations are continually updated.

Taken together, to provide optimal care and ensure positive clinical outcomes in patients with RMD who contracted SARS-CoV-2 infection, it is imperative to understand how these diseases, their comorbidities and the use of immunotherapies may affect progression to severe COVID-19 or death. The primary objective of the current study, by analysing a French cohort of 694 patients with iRMD and COVID-19, was to investigate the frequency of severe infection and predictive factors associated with disease severity. The secondary objectives were to identify predictive factors associated with death and to compare the death rate in patients with moderate to severe COVID-19 with and without RMD.

METHODS

Study design and patients

This is an observational, multicentre, French national cohort study in which patients of all ages with confirmed iRMD (table 1) and highly suspected/confirmed diagnosis of COVID-19 were enrolled. All eligible patients/representatives were informed. The study was performed in accordance with the principles of the Declaration of Helsinki. Positive diagnosis of COVID-19 included biological confirmation (PCR/serology), presence of ground-glass opacities in CT scan, or anosmia or sudden ageusia in the absence of rhinitis or nasal obstruction, or typical clinical

Table 1 Descriptive table of diagnoses according to severity of COVID-19*

Classification, n (%)	Overall (n=694)	Patients with mild infection (n=438)	Patients with moderate infection (n=169)	Patients with severe infection (n=87)	Survivors (n=617)	Non-survivors (n=58)
Chronic inflammatory arthritis						
Rheumatoid arthritis	213 (30.7)	129 (29.5)	55 (32.5)	29 (33.3)	187 (30.3)	20 (34.5)
Axial and peripheral spondyloarthritis	165 (23.8)	135 (30.8)	25 (14.8)	5 (5.8)	161 (26.1)	1 (1.7)
Psoriatic arthritis	70 (10.1)	52 (11.9)	12 (7.1)	6 (6.9)	64 (10.4)	3 (5.2)
Non-systemic idiopathic juvenile arthritis	2 (0.3)	2 (0.5)	0	0	2 (0.3)	0
Other inflammatory arthritis	14 (2.0)	7 (1.6)	5 (3.0)	2 (2.3)	13 (2.1)	1 (1.7)
Autoinflammatory diseases						
Still's disease	5 (0.7)	1 (0.2)	2 (1.2)	2 (2.3)	4 (0.7)	1 (1.7)
Periodic fever syndromes†	15 (2.2)	8 (1.8)	5 (3.0)	2 (2.3)	13 (2.1)	2 (3.5)
Systemic idiopathic juvenile arthritis	3 (0.4)	2 (0.5)	1 (0.6)	0	3 (0.5)	0
Other autoinflammatory diseases	4 (0.6)	2 (0.5)	1 (0.6)	1 (1.2)	3 (0.5)	1 (1.7)
Vasculitis						
Giant cell arteritis and polymyalgia rheumatica	30 (4.3)	8 (1.8)	10 (5.9)	12 (13.8)	21 (3.40)	9 (15.5)
Behcet's disease	7 (1.0)	3 (0.7)	3 (1.8)	1 (1.2)	6 (1.0)	1 (1.7)
Vasculitis associated with cytoplasmic antineutrophil antibodies	17 (2.5)	4 (0.9)	4 (2.4)	9 (10.4)	10 (1.6)	7 (12.1)
Takayasu's arteritis	1 (0.1)	1 (0.2)	0	0	1 (0.2)	0
Other vasculitis (including Kawasaki's disease)	10 (1.4)	5 (1.1)	5 (3.0)	0	9 (1.5)	0
Systemic autoimmune diseases						
Systemic lupus erythematosus	46 (6.6)	32 (7.3)	11 (6.5)	3 (3.5)	42 (6.8)	2 (3.5)
Systemic sclerosis	25 (3.6)	17 (3.9)	6 (3.6)	2 (2.3)	23 (3.7)	2 (3.5)
Primary Sjögren syndrome	17 (2.5)	7 (1.6)	7 (4.1)	3 (3.5)	15 (2.4)	2 (3.5)
Inflammatory myopathy (including dermatomyositis, polymyositis)	12 (1.7)	6 (1.4)	3 (1.8)	3 (3.5)	8 (1.3)	3 (5.2)
Undifferentiated connective tissue disease	3 (0.4)	3 (0.7)	0	0	1 (0.2)	0
Mixed connective tissue disease	4 (0.6)	0	3 (1.8)	1 (1.2)	3 (0.5)	1 (1.7)
Other						
Sarcoidosis	15 (2.2)	6 (1.4)	5 (3.0)	4 (4.6)	12 (1.9)	2 (3.5)
Eye inflammation (including uveitis)	3 (0.4)	2 (0.5)	1 (0.6)	0	3 (0.5)	0
IgG_4-related disease	3 (0.4)	1 (0.2)	1 (0.6)	1 (1.2)	3 (0.5)	0
Other	10 (1.4)	5 (1.1)	4 (2.4)	1 (1.2)	10 (1.6)	0

*Total number of survivors and non-survivors as presented excludes 19 patients whose status at day 21 was unknown at the time of data cut-off.
†Includes TNF receptor-associated periodic syndrome, cryopyrin-associated periodic syndromes, familial Mediterranean fever, and mevalonate kinase deficiency.
TNF, tumour necrosis factor.

signs of COVID-19 (cough, fever, nose/throat symptoms, digestive symptoms without any other diagnosis, influenza syndrome in a patient with recent close contact with a known COVID-19 positive patient). Patients were informed about the objective of the study, and patient consent was obtained for the use of medical data, which was carried out according to French law and good clinical practices. Approval from an ethics committee was not required according to French law.[14] The study was performed in compliance with MR-004,[15] received permission from Lille University Hospital, was declared to the Commission Nationale de l'Informatique et des Libertés (reference DEC20-107).

To compare the death rate resulting from moderate to severe COVID-19 between the population with iRMD and non-iRMD, the Lille University Hospital COVID-19 Research Network (LICORNE) was used. This includes 335 patients with COVID-19 hospitalised in the Lille University Hospital between 24 February and 17 April 2020 for moderate to severe COVID-19. Among them, 256 patients were selected as potential controls, to match to the moderate to severe (hospitalised/intensive care unit (ICU)/death) patients from the French RMD COVID-19 cohort. All patients with iRMD and control patients received care from the same national health system.

Data collection

All cases of highly suspected/confirmed patients with iRMD-COVID-19 were reported retrospectively. The individual data regarding iRMD diagnosis/specific treatments were captured from rheumatologists, internal medicine physicians or paediatric physicians via one national data entry portal. All treating physicians are members of the FAI^{2}R/SFR/SNFMI/SOFREMIP/CRI/IMIDIATE consortium. Data collected from the patient's medical record included demographics and clinical information such as onset of iRMD and current treatments, presence of comorbidities, details of COVID-19 diagnosis, management and outcome with an evaluation of the vital status assessment at least 21 days after the first clinical sign of COVID-19. The main diagnosis was selected for analysis, which justified the management and the choice of treatments. To ensure secure transmission of data, information was collected from the investigating physician via the electronic case report form or a provided file. Data cut-off was on 18 May 2020. Before freezing, the final database was monitored to collect missing data, validate the evolution of COVID-19, remove duplicate or erroneous reports, and check data consistency. All deaths were verified by Eric Hachulla and Christophe Richez to ensure complete data were obtained and if missing, to collect data directly by contacting the physician.

Outcomes

The primary endpoint was the frequency of severe infection in patients with iRMD and predictive factors associated with disease severity. The severity of COVID-19 was assessed and classified according to the care needed for each patient: mild=ambulatory; moderate=hospitalised out of ICU; and severe=ICU or deceased. The secondary objectives were to identify predictive factors associated with death and to compare the death rate in patients with moderate to severe COVID-19 with and without inflammatory iRMD.

Statistical analysis

Categorical variables were expressed as numbers (percentage) and quantitative variables as mean±SD. Comparisons of severe versus mild or moderate patients and survivors versus non-survivors were made using logistic regression models (in case of cell frequency <5, a penalised logistic regression (Firth method)[14] was used), with and without adjustment on pre-specified factors (age and sex). No statistical comparisons were done for categorical variables with a frequency <10 in the overall sample. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated as effect size. Factors associated with severity and hospitalisation status in the age-sex adjusted analyses ($p<0.05$) were introduced into multivariable penalised logistic regression models with a forward stepwise selection procedure (entrance criterion=0.05) to limit overfitting. To avoid case deletion in multivariable analyses, missing data for candidate predictors were imputed by multiple imputations using the regression-switching approach (chained equations, n=10 imputations).[16] The imputation procedure was performed under the missing-at-random assumption using all candidate predictors, with logistic regression (binary, ordinal or multinomial) models for categorical variables. Rubin's rules were used to combine the estimates derived from multiple imputed data sets.[17] Multivariate analysis was performed in available cases (without missing data on candidate predictors) as sensitivity analysis. French RMD COVID-19 cases and LICORNE controls were matched for age, sex, and comorbidities (cardiac disease, diabetes, hypertension, body mass index/BMI, and renal failure) using a propensity score estimation, calculated using a multivariable logistic regression model. Choice of these confounders was based on published literature.[18 19] The two groups were matched (1:1) using an optimal algorithm with calliper width of 0.2 SD of logit for propensity score.[20 21] To evaluate the bias reduction, absolute standardised differences were calculated before and after matching. An absolute standardised difference >10% was interpreted as a meaningful difference.[22] OR for death (iRMD vs controls) was estimated using a mixed logistic regression. All statistical tests were performed at the two-tailed α level of 0.05 using SAS software, V.9.4.

Patient and public involvement

Patients were not directly involved in the design, recruitment, or conduct of the study.

RESULTS

Patient characteristics

We collected a total of 758 records and the final evaluation of COVID-19 severity (primary endpoint) was available for 694 patients (the 13 children were not included in the statistical analysis and are described separately). COVID-19 diagnosis was confirmed in 59% of cases based on PCR or serology (408/694). Of those patients with confirmed COVID-19, approximately 47% (193/408) had a mild, 34% (138/408) moderate, and 19% (77/408) severe infection. In the other patients, COVID-19 diagnosis was confirmed by typical CT scan in 6% (46/694), anosmia/ageusia in 14% (96/694), and typical clinical symptoms in 21% (144/694).

Patients were mainly women (66.6%, 462/694) with a mean age of 56.1±16.4 years, and 51.6% (358/694) were over the age of 55 years (figure 1). Seventy-one percent of the population had at least one comorbidity (492/694), with hypertension (n=182, 26.3%), obesity with a BMI over 30 kg/m^2 (n=146, 21%), respiratory disease (n=99, 14.3%), and cardiovascular disease (n=85, 12.3%) as the most common. Chronic inflammatory arthritis diseases were the most frequent diagnoses in the cohort (66.9%, 464/694), mostly rheumatoid arthritis (RA) and spondyloarthritis, followed by systemic autoimmune diseases

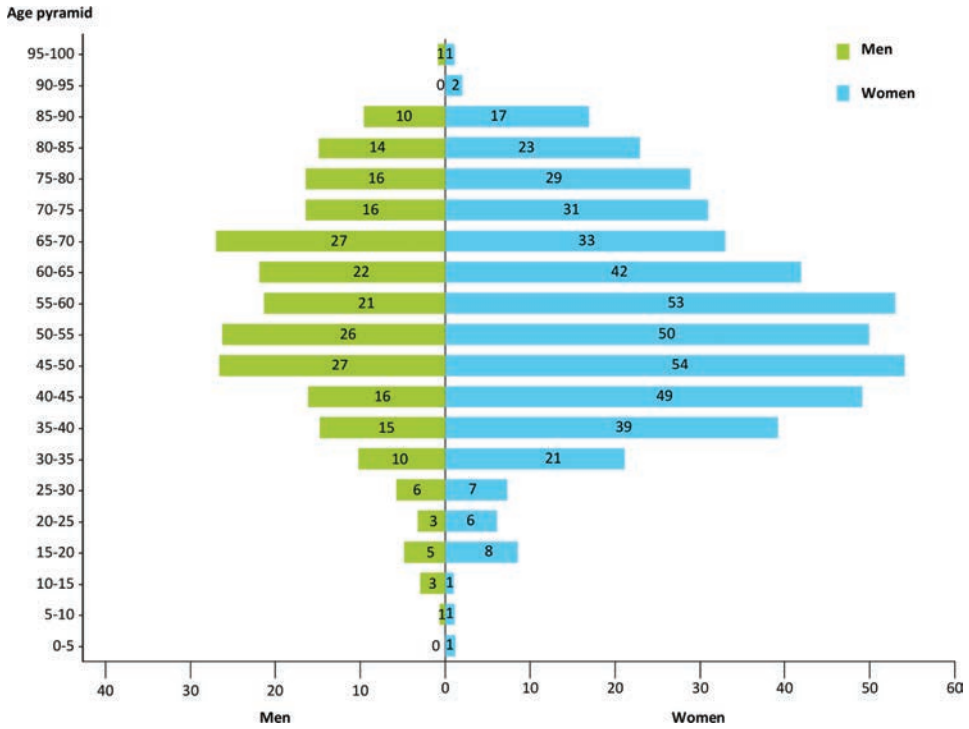

Figure 1 Age-pyramid including the 694 adult patients used in the statistical analysis as well as the 13 children.

(15.4%, 107/694). A detailed description of all iRMD diagnoses included in the cohort is presented in table 1.

Development of severe disease

The frequency of severe COVID-19 in patients with iRMD with confirmed or highly suspected diagnosis of symptomatic COVID-19 was 12.5% (87/694). Age was a driver of disease severity, as only 11 patients between 18 and 54 years developed severe COVID-19, whereas this number increased to 20 in patients between 65 and 74 years (adjusted OR (aOR)=6.46, 95% CI: 2.97–14.06), and to 45 in patients over 75 years (aOR=19.82, 95% CI: 9.69–40.52). There were no severe paediatric cases. When adjusted for age and sex, among the most common comorbidities correlated with severe disease were morbid obesity (BMI ≥40 kg/m^2) (aOR=4.10, 95% CI: 1.28–13.11), diabetes (aOR=2.14, 95% CI: 1.12–4.12), and hypertension (aOR=2.30, 95% CI: 1.34–3.96). Interestingly, interstitial lung disease (aOR=2.87, 95% CI: 1.06–7.80) and chronic renal failure (aOR=3.22, 95% CI: 1.51–6.90) were also associated with disease severity. Severe disease was observed more frequently in patients with vasculitis (aOR=2.25, 95% CI: 1.13–4.41) and autoinflammatory diseases (aOR=7.88, 95% CI: 1.39–37.05), compared with patients with chronic inflammatory arthritis. These results are summarised in table 2. Morbid obesity, diabetes, hypertension, and chronic renal failure were still correlated with severe disease when the analysis was focused only on patients with PCR-confirmed COVID-19 (online supplemental table 1). While not significant, there was also an association with interstitial lung disease (aOR=2.64, 95% CI: 0.94–7.36). In the PCR-confirmed population, severe disease was still observed more frequently in patients with vasculitis (aOR=2.39, 95% CI: 1.14–4.98), compared with patients with chronic inflammatory arthritis.

Regarding treatments for iRMDs, more frequent severe disease was observed with the use of corticosteroids (aOR=2.25, 95% CI: 1.33–3.79), mycophenolate mofetil (aOR=7.67, 95% CI: 1.73–28.04) and rituximab (aOR=4.34, 95% CI: 1.77–10.63). It should be noted that use of TNFα blockers (n=202, aOR=0.44, 95% CI: 0.19–1.04), interleukin-6 (IL-6) inhibitors (n=26, aOR=0.63, 95% CI: 0.12–2.28), methotrexate (n=252, aOR=0.63, 95% CI: 0.37–1.08) and hydroxychloroquine (HCQ) (n=57, aOR=1.06, 95% CI: 0.31–2.96) were not associated with severe COVID-19 (table 3). When the same analysis was performed on the population with PCR-confirmed COVID-19, rituximab was still identified as a contributor to the development of severe disease (aOR=6.35, 95% CI: 2.23–18.11) (online supplemental table 2). However, there was no significant increase in the development of severe disease with the use of corticosteroids (aOR=1.72, 95% CI: 0.98–3.04) nor significant decrease in the development of severe disease with the use of TNF blockers (aOR=0.42, 95% CI: 0.16–1.15).

Similar results were observed in patients with RA (n=213) with respect to the severity of disease including age (aOR age ≥75=16.89, 95% CI: 4.90–88.60), hypertension (aOR=3.36, 95% CI: 1.23–8.60), and use of corticosteroids (aOR=2.57, 95% CI: 1.01–6.52) or rituximab (aOR=5.97, 95% CI: 1.18–27.63) (online supplemental tables 3 and 4).

Results of the multivariable analysis are presented in table 4. Due to the number of events (87 patients with severe infection), the analysis was limited to no more than seven variables, which were selected based on clinical expertise. Older age (OR=1.08, 95% CI: 1.05–1.10), female gender (OR=0.45, 95% CI: 0.25–0.80), BMI (OR=1.07, 95% CI: 1.02–1.12), hypertension (OR=1.86, 95% CI: 1.01–3.42), and use of corticosteroids (OR=1.97, 95% CI: 1.09–3.54), mycophenolate mofetil (OR=6.6, 95% CI: 1.47–29.62) and rituximab (OR=4.21, 95% CI: 1.61–10.98) were significantly associated with COVID-19 severity. Results of the imputed analysis are similar compared with the available case analysis (see table 4).

Table 2 Association between demographic and clinical characteristics and severity of COVID-19

	All patients (n=694)	Patients with mild infection (n=438)	Patients with moderate infection (n=169)	Patients with severe infection (n=87)	OR (95% CI)*	P value	aOR (95% CI)*†	P value†
Age (years)						<0.001		<0.001
18–54	336 (48.4)	268 (61.2)	57 (33.7)	11 (12.6)	1.00 (ref.)	–	1.00 (ref.)	–
55–64	138 (19.9)	95 (21.7)	32 (18.9)	11 (12.6)	2.56 (1.08–6.05)	0.032	2.58 (1.09–6.12)	0.032
65–74	107 (15.4)	52 (11.9)	35 (20.7)	20 (23.0)	6.79 (3.14–14.71)	<0.001	6.46 (2.97–14.06)	<0.001
≥75	113 (16.3)	23 (5.3)	45 (26.6)	45 (51.7)	19.55 (9.62–39.73)	<0.001	19.82 (9.69–40.52)	<0.001
Mean±SD	56.1±16.4	50.6±13.9	61.8±16.1	72.4±13.8				
Female gender	462 (66.6)	309 (70.6)	109 (64.5)	44 (50.6)	0.46 (0.29–0.73)	<0.001	0.45 (0.27–0.75)	0.002
Comorbidities‡								
Respiratory disease (all)	99 (14.3)	53 (12.2)	25 (14.8)	21 (24.1)	2.15 (1.25–3.71)	0.006	1.61 (0.87–2.99)	0.13
Interstitial lung disease	26 (3.8)	10 (2.3)	7 (4.1)	9 (10.3)	3.99 (1.72–9.26)	0.001	2.87 (1.06–7.80)	0.038
COPD	28 (4.0)	14 (3.2)	6 (3.6)	8 (9.2)	2.96 (1.26–6.95)	0.013	1.08 (0.42–2.76)	0.88
Asthma	52 (7.5)	32 (7.3)	14 (8.3)	6 (6.9)	0.90 (0.37–2.18)	0.82	1.24 (0.46–3.33)	0.67
Cardiac disease (all)	85 (12.3)	22 (5.0)	31 (18.3)	32 (36.8)	6.06 (3.61–10.18)	<0.001	1.78 (0.97–3.28)	0.064
Coronary heart diseases	68 (9.8)	15 (3.4)	25 (14.8)	28 (32.2)	6.70 (3.86–11.65)	<0.001	1.86 (0.97–3.56)	0.063
Stroke	25 (3.6)	7 (1.6)	10 (5.9)	8 (9.2)	3.50 (1.46–8.38)	0.005	1.68 (0.63–4.47)	0.30
Diabetes	62 (9.0)	12 (2.8)	29 (17.2)	21 (24.1)	4.38 (2.44–7.85)	<0.001	2.14 (1.12–4.12)	0.022
Obesity						0.050		0.043
<30	459 (75.9)	303 (78.7)	105 (71.9)	51 (68.9)	1.00 (ref.)	–	1.00 (ref.)	–
30–39.9	126 (20.8)	74 (19.2)	35 (24.0)	17 (23.0)	1.25 (0.69–2.25)	0.46	1.47 (0.76–2.82)	0.25
≥40	20 (3.3)	8 (2.1)	6 (4.1)	6 (8.1)	3.43 (1.26–9.32)	0.016	4.10 (1.28–13.11)	0.017
Hypertension	182 (26.3)	71 (16.3)	60 (35.5)	51 (58.6)	5.13 (3.21–8.19)	<0.001	2.30 (1.34–3.96)	0.003
Cancer	33 (4.8)	13 (3.0)	13 (7.7)	7 (8.0)	1.95 (0.82–4.64)	0.13	0.83 (0.31–2.21)	0.71
Chronic renal failure	42 (6.1)	11 (2.5)	12 (7.1)	19 (21.8)	7.07 (3.66–13.65)	<0.001	3.22 (1.51–6.90)	0.003
No. of patients with at least one comorbidity	492 (71.1)	274 (62.8)	136 (80.5)	82 (94.3)	7.80 (3.11–19.54)	<0.001	3.52 (1.35–9.17)	0.010
Disease history§						<0.001		0.023
Chronic inflammatory arthritis	464 (66.9)	325 (74.2)	97 (57.4)	42 (48.3)	1.00 (ref.)	–	1.00 (ref.)	–
Autoinflammatory diseases	12 (1.7)	5 (1.1)	4 (2.4)	3 (3.4)	3.66 (0.89–12.07)	0.053	7.88 (1.39–37.05)	0.014
Vasculitis	65 (9.4)	21 (4.8)	22 (13.0)	22 (25.3)	5.14 (2.80–9.32)	<0.001	2.25 (1.13–4.41)	0.020
Systemic autoimmune diseases	122 (17.6)	73 (16.7)	35 (20.7)	14 (16.1)	1.33 (0.69–2.45)	0.38	1.64 (0.80–3.25)	0.17

Values are presented as frequency (percentage) unless otherwise indicated.
*ORs were calculated for patients with severe infection, using patients with mild or moderate infection as reference.
†Adjusted for age and sex.
‡Two missing values for comorbidities except for obesity where 89 values are missing.
§Penalised logistic regression (Firth method) was used due to low number of patients (n<5) in an analysed group.
aOR, adjusted OR; COPD, chronic obstructive pulmonary disease.

Hospitalisation status

Hospitalisation status of the whole population (n=694) was also affected, and was more frequently related to older age (aOR age ≥75=15.51, 95% CI: 9.11–26.40) as well as the presence of coronary heart disease (aOR=2.73, 95% CI: 1.40–5.30), diabetes (aOR=5.37, 95% CI: 2.66–10.85), hypertension (aOR=1.99, 95% CI: 1.33–2.98), and chronic renal failure (aOR=2.76, 95% CI: 1.26–6.04) (online supplemental table 5). Use of corticosteroids (aOR=2.76, 95% CI: 1.90–4.02) and TNFα inhibitors (aOR=0.35, 95% CI: 0.22–0.55) also affected hospitalisation status and were harmful or protective, respectively (online supplemental table 6). Within the multivariable imputed analysis, age (OR=1.05, 95% CI: 1.04–1.07), diabetes (OR=4.33, 95% CI: 2.07–9.07), BMI (OR=1.06, 95% CI: 1.02–1.10), use of corticosteroids (OR=1.94, 95% CI: 1.24–3.05) and colchicine (OR=3.34, 95% CI: 1.14–9.79) remain associated with a higher risk of hospitalisation. Use of TNF inhibitors (OR=0.55, 95% CI: 0.32–0.95) and female gender (OR=0.65, 95% CI: 0.43–0.99) were associated with less frequent hospitalisation (online supplemental table 7).

Paediatric cases

Thirteen patients were paediatric cases and are described in table 5.

Survival

Fifty-eight patients in our cohort died, resulting in an overall death rate of 8.3%, which corresponds to 22.6% of death in the hospitalised subgroup (58/256) (table 6). Of 335 patients in the LICORNE cohort (patients with non-RMD COVID-19), only 175 controls were matched for age, sex and comorbidities (cardiac disease, diabetes, hypertension, BMI and renal failure) (online supplemental table 8). By matching patients to the LICORNE cohort, a death rate of 25.1% (95% CI: 18.7–31.6) was observed in the French RMD COVID-19 compared with 18.9% (95% CI: 13.1–24.7, respectively) with an OR of 1.45 (95% CI: 0.87–2.42; n=175 in each group). In the iRMD COVID-19 cohort, death was more frequent in patients aged ≥55 years (aOR (55–64)=5.54, 95% CI: 1.62–23.13; aOR (65–74)=6.70, 95% CI: 1.95–28.07; aOR (≥75)=59.02, 95% CI: 21.79–221.45), and with the presence of interstitial lung disease (aOR=3.82, 95% CI: 1.27–11.49), coronary heart disease (aOR=2.18, 95% CI: 1.05–4.53), diabetes (aOR=2.89, 95% CI:

Table 3 Association between rheumatic disease treatments and severity of COVID-19

	All patients (n=694)	Patients with mild infection (n=438)	Patients with moderate infection (n=169)	Patients with severe infection (n=87)	OR (95% CI)*	P value	aOR (95% CI)*†	P value†
Rheumatic or inflammatory disease treatments‡								
Corticosteroid	215 (31.1)	88 (20.1)	76 (45.2)	51 (59.3)	3.93 (2.46–6.26)	<0.001	2.25 (1.33–3.79)	0.002
Daily prednisone ≥10 mg or equivalent	73 (34.3)	28 (31.8)	22 (29.3)	23 (46.0)	1.93 (1.01–3.68)	0.048	1.69 (0.83–3.45)	0.15
NSAIDs§	73 (10.5)	61 (13.9)	10 (6.0)	2 (2.3)	0.22 (0.05–0.66)	0.022	0.50 (0.10–1.58)	0.31
Colchicine	24 (3.5)	12 (2.7)	8 (4.8)	4 (4.7)	1.56 (0.48–4.09)	0.41	3.18 (0.77–11.24)	0.090
Hydroxychloroquine§	57 (8.2)	40 (9.1)	13 (7.7)	4 (4.7)	0.56 (0.18–1.37)	0.26	1.06 (0.31–2.96)	0.91
Methotrexate	252 (36.4)	164 (37.4)	62 (36.9)	26 (30.2)	0.73 (0.45–1.19)	0.20	0.63 (0.37–1.08)	0.096
Leflunomide	27 (3.9)	19 (4.3)	8 (4.8)	0	NA	NA	NA	NA
Sulfasalazine	9 (1.3)	5 (1.1)	4 (2.4)	0	NA	NA	NA	NA
Mycophenolate mofetil/ mycophenolic acid§	16 (2.3)	9 (2.1)	4 (2.4)	3 (3.5)	1.84 (0.47–5.54)	0.33	7.67 (1.73–28.04)	0.004
Azathioprine§	9 (1.3)	5 (1.1)	3 (1.8)	1 (1.2)	NA	NA	NA	NA
IgIV§	7 (1.0)	3 (0.7)	2 (1.2)	2 (2.3)	NA	NA	NA	NA
Biologics								
Anti-TNF	202 (29.2)	170 (38.8)	25 (14.9)	7 (8.1)	0.19 (0.09–0.41)	<0.001	0.44 (0.19–1.04)	0.060
Anti-IL-6§	26 (3.8)	19 (4.3)	5 (3.0)	2 (2.3)	0.70 (0.14–2.21)	0.61	0.63 (0.12–2.28)	0.54
Rituximab	34 (4.9)	16 (3.7)	7 (4.2)	11 (12.8)	3.72 (1.74–7.93)	<0.001	4.34 (1.77–10.63)	0.001
Anti-IL-17a§	27 (3.9)	19 (4.3)	6 (3.6)	2 (2.3)	0.67 (0.14–2.12)	0.57	2.34 (0.45–8.21)	0.24
Anti-IL-1§	8 (1.2)	3 (0.7)	3 (1.8)	2 (2.3)	NA	NA	NA	NA
Abatacept§	18 (2.6)	10 (2.3)	7 (4.2)	1 (1.2)	0.59 (0.07–2.39)	0.55	0.37 (0.04–1.80)	0.31
JAK inhibitor§	21 (3.0)	13 (3.0)	4 (2.4)	4 (4.7)	1.84 (0.56–4.91)	0.27	1.94 (0.54–5.98)	0.28
Other biologic	16 (2.3)	11 (2.5)	5 (3.0)	0	NA	NA	NA	NA

Values are presented as frequency (percentage) unless otherwise indicated.
Not applicable (NA) when <10/694 patients or when 0 patients with severe infection.
*ORs were calculated for patients with severe infection, using patients with mild or moderate infection as reference.
†Adjusted for age and sex.
‡Two patients with missing information for treatments.
§Penalised logistic regression (Firth method) was used due to low number of patients (n<5) in an analysed group.
aOR, adjusted OR; IgIV, immunoglobulin intravenous; IL, interleukin; NSAIDs, non-steroidal anti-inflammatory drugs; TNF, tumour necrosis factor.

1.39–6.02), hypertension (aOR=3.08, 95% CI: 1.56–6.08) or chronic renal failure (aOR=5.22, 95% CI: 2.22–12.31). In addition, systemic autoimmune diseases were more frequently associated with death (aOR=2.65, 95% CI: 1.15–5.95) compared with chronic inflammatory arthritis (table 6). Regarding treatments, use of corticosteroids (aOR=2.64, 95% CI:1.36–5.12), colchicine (aOR=8.21, 95% CI: 1.60–37.97), mycophenolate mofetil (aOR=14.20, 95% CI: 2.26–70.24) or rituximab (aOR=4.04, 95% CI: 1.35–12.04) was associated with a higher frequency of death, whereas a reduced hazard was observed in patients taking methotrexate for iRMD (aOR=0.34, 95% CI: 0.16–0.70) (table 7). Of note, the use of TNFα or IL-6 inhibitors was not associated with death (aOR=0.74, 95% CI: 0.22–2.01 and aOR=0.50, 95% CI: 0.05–2.38, respectively). A detailed description of all fatalities is available in online supplemental table 9.

Treatments used in French patients with iRMD who contracted SARS-CoV-2 infection

With respect to COVID-19-specific treatment used in the French iRMD-COVID-19 cohort, among the total population, 18.6% (129/694) received antiviral or immunomodulating therapies, which increased to 30.2% (51/169) with moderate infection and 37.9% (33/87) with severe infection. HCQ, alone or in

Table 4 Multivariable analyses for disease severity

	Imputed analysis* (n=694)			Available case analysis (n=601)		
Variable	n/N	OR (95% CI)	P value	n/N	OR (95% CI)	P value
Age (years)	87/694	1.08 (1.05–1.10)	<0.001	73/601	1.08 (1.05–1.10)	<0.001
Female gender	44/462	0.45 (0.25–0.80)	0.007	37/395	0.43 (0.24–0.78)	0.005
BMI	87/694	1.07 (1.02–1.12)	0.006	73/601	1.07 (1.02–1.12)	0.007
Hypertension	51/182	1.86 (1.01–3.42)	0.047	42/162	1.83 (0.99–3.37)	0.054
Corticosteroids	51/216	1.97 (1.09–3.54)	0.024	45/188	2.04 (1.13–3.67)	0.018
Mycophenolate mofetil/mycophenolic acid	3/16	6.60 (1.47–29.62)	0.014	3/14	6.51 (1.45–29.23)	0.015
Rituximab	11/34	4.21 (1.61–10.98)	0.003	10/32	4.60 (1.75–12.11)	0.002

ORs were calculated using multivariable penalised logistic regression models (Firth method), using a forward selection method, with patients with mild or moderate infection as reference. Only variables selected by the model are presented. Full model included age, sex, interstitial lung disease, diabetes, BMI, hypertension, chronic renal failure, disease history, corticosteroids, mycophenolate mofetil/mycophenolic acid and rituximab.
n/N indicated the number of events/number of cases.
*ORs and p value were calculated after multiple imputations (m=10) to handle missing data.
BMI, body mass index.

Table 5 Treatment and outcomes of paediatric patients

	Type of RMD	Age/gender	Comorbidities including BMI	RMD treatment		Outpatient management (Y/N)	COVID-19 treatment	COVID outcome	Other comments	SARS-CoV-2 PCR/ serology
				Corticosteroid	DMARD					
Pt 1	Autoimmune bullous dermatosis	4/F	Asthma/17		IgIV, RITU	Y: increase of IgIV dosage	0	Benign		PCR+
Pt 2	Non-systemic JIA	17/M	None/NA		NSAID, MTX, ADA	Y: stop NSAID, MTX and ADA	0	Benign		ND
Pt 3	Non-systemic JIA	7/F	None/14		MTX, ADA	Y: stop ADA and MTX	0	Benign	Relapse of the JIA, recurrent herpes labialis	ND
Pt 4	Non-systemic JIA	14/M	None/18			N	0	Benign	Herpes zoster recurrence	ND
Pt 5	FMF	17/F	None/21		Colchicine	N	0	Benign		Serology+
Pt 6	FMF	16/M	None/23		Colchicine, ADA	N	0	Moderate		PCR+
Pt 7	Systemic–onset JIA	16/F	Smoking/22		TOCI	Y: stop TOCI	0	Benign	Anaemia	PCR+
Pt 8	SLE	17/F	Smoking; obesity/45		Hydroxychloroquine	N	0	Benign	Joint relapse	PCR+
Pt 9	Sarcoidosis and uveitis	13/F	None/20	Prednisone 20 mg/day		N	0	Benign	Relapse of orbital pain	PCR+
Pt 10	Non-systemic JIA	16/M	None/22		NSAID, MTX, ETA	Y: stop NSAID	0	Benign		PCR–
Pt 11	Non-systemic JIA	12/M	None/23			N	0	Benign		ND
Pt 12	Non-systemic JIA	11/M	None/16		ETA	N	0	Benign		ND
Pt 13	Cryopyrinopathy	9/M	None/21			N	0	Benign		ND

ADA, adalimumab; BMI, body mass index; DMARD, disease-modifying anti-rheumatic drug; ETA, etanercept; FMF, familial Mediterranean fever; IgIV, immunoglobulin intravenous; JIA, juvenile idiopathic arthritis; MTX, methotrexate; NA, not applicable; ND, not detected; NSAID, non-steroidal anti-inflammatory drug; RITU, rituximab; RMD, rheumatic and musculoskeletal diseases; SLE, systemic lupus erythematosus; TOCI, tocilizumab.

Table 6 Association between demographic and clinical characteristics and survival*

	Survivors (n=617)	Non-survivors (n=58)	OR (95% CI)†	P value	aOR (95% CI)†‡	P value‡
Age§ (years)				<0.001		<0.001
18–54	327 (53.0)	3 (5.2)	1.00 (ref.)	–	1.00 (ref.)	–
55–64	126 (20.4)	7 (12.1)	5.55 (1.62–23.14)	0.009	5.54 (1.62–23.13)	0.009
65–74	98 (15.9)	7 (12.1)	7.13 (2.08–29.79)	0.003	6.70 (1.95–28.07)	0.004
≥75	66 (10.7)	41 (70.7)	58.39 (21.65–218.44)	<0.001	59.02 (21.79–221.45)	<0.001
Mean±SD	53.9±15.3	76.6±12.6				
Female gender	418 (67.8)	30 (51.7)	0.51 (0.30–0.88)	0.015	0.48 (0.25–0.89)	0.020
Comorbidities¶						
Respiratory disease (all)	82 (13.3)	15 (25.9)	2.27 (1.21–4.27)	0.011	1.64 (0.78–3.43)	0.19
Interstitial lung disease	18 (2.9)	8 (13.8)	5.31 (2.20–12.81)	<0.001	3.82 (1.27–11.49)	0.017
COPD	21 (3.4)	6 (10.3)	3.26 (1.26–8.44)	0.015	0.95 (0.32–2.81)	0.93
Asthma§	48 (7.8)	3 (5.2)	0.74 (0.20–1.99)	0.60	1.15 (0.27–3.72)	0.83
Cardiac disease (all)	56 (9.1)	27 (46.6)	8.69 (4.85–15.60)	<0.001	1.87 (0.93–3.76)	0.081
Coronary heart diseases	41 (6.7)	25 (43.1)	10.61 (5.77–19.49)	<0.001	2.18 (1.05–4.53)	0.037
Stroke	19 (3.1)	6 (10.3)	3.62 (1.39–9.46)	0.009	1.52 (0.51–4.56)	0.46
Diabetes	43 (7.0)	18 (31.0)	6.00 (3.17–11.32)	<0.001	2.89 (1.39–6.02)	0.005
Obesity§				0.053		0.072
<30	419 (77.3)	33 (66.0)	1.00 (ref.)	–	1.00 (ref.)	–
30–39.9	108 (19.9)	13 (26.0)	1.56 (0.78–2.97)	0.19	1.95 (0.88–4.18)	0.093
≥40	15 (2.8)	4 (8.0)	3.64 (1.07–10.29)	0.026	3.77 (0.86–15.09)	0.070
Hypertension	133 (21.6)	40 (69.0)	8.05 (4.47–14.51)	<0.001	3.08 (1.56–6.08)	0.001
Cancer	25 (4.1)	6 (10.3)	2.73 (1.07–6.94)	0.036	1.05 (0.35–3.11)	0.93
Chronic renal failure	22 (3.6)	18 (31.0)	12.13 (6.02–24.44)	<0.001	5.22 (2.22–12.31)	<0.001
No. of patients with at least one comorbidity§	419 (68.1)	57 (98.3)	17.96 (4.83–159.20)	<0.001	5.61 (1.41–50.93)	0.043
Disease history§				<0.001		0.039
Chronic inflammatory arthritis	427 (69.2)	25 (43.1)	1.00 (ref.)	–	1.00 (ref.)	–
Autoinflammatory diseases	10 (1.6)	2 (3.5)	3.99 (0.74–14.77)	0.069	8.98 (0.94–63.49)	0.040
Vasculitis	47 (7.6)	17 (29.3)	6.18 (3.10–12.13)	<0.001	2.09 (0.93–4.56)	0.070
Systemic autoimmune diseases	105 (17.0)	12 (20.7)	1.99 (0.95–3.97)	0.059	2.65 (1.15–5.95)	0.020

Values are presented as frequency (percentage) unless otherwise indicated.
*Total number of survivors and non-survivors as presented excludes 19 patients whose status at day 21 was unknown at the time of data cut-off.
†ORs were calculated for non-survivors, using survivors as reference.
‡Adjusted for age and sex.
§Penalised logistic regression (Firth method) was used due to low number of patients (n<5) in an analysed group.
¶Two missing values for comorbidities except for obesity where 83 values are missing.
aOR, adjusted OR; COPD, chronic obstructive pulmonary disease.

combination with azithromycin, was the most used therapy, in 9.4% (65/694) of the patients. Routinely available antiviral therapies (ritonavir in combination with lopinavir or darunavir) were mainly administered to hospitalised patients (10.5%; 27/256). Use of anti-cytokine therapies (tocilizumab and anakinra) was rare (0.6%) (online supplemental table 10).

DISCUSSION

The current observational, multicentre, French cohort study examined the frequency of severe COVID-19 and factors associated with outcomes of SARS-CoV-2 infection in patients with iRMD. Though similar in objective to the Global Rheumatology Alliance Study,[23] the present investigation analysed a larger patient population with iRMD from a single country and monitored individual data for at least 21 days after the first clinical sign of disease to confirm evolution of COVID-19 and retrieve missing data. While the results do not suggest causality, they inform on treatment options for COVID-19 in patients with iRMD.

Underlying immune dysfunction and treatment with immunosuppressive agents raised the possibility of an increased COVID-19 severity in patients with iRMD. In addition to age (≥75 years), comorbidities such as chronic respiratory disease, cardiovascular disease, diabetes, hypertension, obesity (BMI ≥40 kg/m^2), and renal failure increased the risk for severe COVID-19, again reflecting the observed trend in subjects with non-rheumatic diseases.[6 7] In the present study, death was observed more frequently in patients with iRMD, but this difference in the frequency of mortality did not reach statistical significance. Systemic autoimmune diseases (mainly systemic lupus, systemic sclerosis, Sjögren syndrome and myositis) and vasculitis were found to be independent factors for severe infection and/or mortality, suggesting that a history of drug-induced immunosuppression may worsen the prognosis.[24 25] For autoinflammatory diseases, the results should be interpreted with caution due to the very low number of patients (n=13). The use of higher continual doses of corticosteroids in these populations could have led to a poor outcome.

Within the current cohort, RMD treatments had a variable association with COVID-19 severity and mortality. We assessed the association of each medication separately because the number of different medications was too high to compare to a single reference group and also because of possible overlap between medications, such as conventional synthetic DMARD and biologic DMARD (bDMARD) combination therapy. Studies of patients with RMD and IBD showed that long-term corticosteroid use increased the risk of severe COVID-19 infection and death.[8 11] In contrast, two other studies, CHIC[26] and

Table 7 Association between rheumatic disease treatments and survival*

	Survivors (n=617)	Non-survivors (n=58)	OR (95% CI)†	P value	aOR (95% CI)†‡	P value‡
Rheumatic or inflammatory disease treatments§						
Corticosteroid	172 (27.9)	39 (68.4)	5.59 (3.11–10.05)	<0.001	2.64 (1.36–5.12)	0.004
Daily prednisone doses ≥10 mg or equivalent	50 (29.4)	21 (53.8)	2.80 (1.38–5.70)	0.005	2.91 (1.28–6.59)	0.011
NSAIDs	73 (11.9)	0	NA	NA	NA	NA
Colchicine¶	20 (3.2)	4 (7.0)	2.45 (0.75–6.50)	0.10	8.21 (1.60–37.97)	0.009
Hydroxychloroquine¶	52 (8.4)	2 (3.5)	0.48 (0.10–1.47)	0.28	0.93 (0.16–3.55)	0.92
Methotrexate	237 (38.5)	12 (21.1)	0.43 (0.22–0.82)	0.011	0.34 (0.16–0.70)	0.003
Leflunomide	27 (4.4)	0	NA	NA	NA	NA
Sulfasalazine	9 (1.5)	0	NA	NA	NA	NA
Mycophenolate mofetil/mycophenolic acid¶	14 (2.3)	2 (3.5)	1.87 (0.36–6.32)	0.38	14.20 (2.26–70.24)	0.002
Azathioprine	8 (1.3)	1 (1.8)	NA	NA	NA	NA
IgIV	6 (1.0)	1 (1.8)	NA	NA	NA	NA
Biologics						
Anti-TNF¶	194 (31.5)	4 (7.0)	0.18 (0.06–0.44)	<0.001	0.74 (0.22–2.01)	0.58
Anti-IL-6R¶	25 (4.1)	1 (1.8)	0.62 (0.07–2.43)	0.58	0.50 (0.05–2.38)	0.47
Rituximab	27 (4.4)	7 (12.3)	3.05 (1.27–7.36)	0.013	4.04 (1.35–12.04)	0.012
Anti-IL-17a	25 (4.1)	0	NA	NA	NA	NA
Anti-IL-1	6 (1.0)	2 (3.5)	NA	NA	NA	NA
Abatacept¶	17 (2.8)	1 (1.8)	0.91 (0.10–3.71)	0.92	0.58 (0.06–3.09)	0.59
JAK inhibitor¶	18 (2.9)	2 (3.5)	1.46 (0.29–4.77)	0.59	1.36 (0.23–5.61)	0.71
Other biologic	16 (2.6)	0	NA	NA	NA	NA

Values are presented as frequency (percentage) unless otherwise indicated.
Not applicable (NA) when <10/617 patients or 0 non-survivors.
*Total number of survivors and non-survivors as presented excludes 19 patients whose status at day 21 was unknown at the time of data cut-off.
†ORs were calculated for non-survivors, using survivors as reference.
‡Adjusted for age and sex.
§Two patients with missing information for treatments.
¶Penalised logistic regression (Firth method) was used due to low number of patients (n<5) in an analysed group.
aOR, adjusted OR; IgIV, immunoglobulin intravenous; IL, interleukin; NSAIDs, non-steroidal anti-inflammatory drugs; TNF, tumour necrosis factor.

RECOVERY,[27] recently demonstrated that methylprednisolone 250 mg or dexamethasone 6 mg were beneficial when patients with COVID-19 develop a severe form (cytokine storm syndrome), respectively. These studies and ours suggest that the beneficial or aggravating effect of corticosteroids is a matter of timing. Conversely, anti-TNFα therapies were associated with a lower frequency of severe infection or mortality and also with less frequent hospitalisation. These findings are consistent with a previous study that found lower odds of hospitalisation with bDMARD/targeted synthetic DMARD monotherapy, driven largely by anti-TNF therapies.[8] Of note, similar observations have been made outside the scope of SARS-CoV-2, suggesting a beneficial effect of bDMARDs on the risk of sepsis after serious infection or a fatal outcome.[28] Methotrexate use significantly reduced mortality and was not associated with the risk of severe disease, yet we caution against causal inference regarding drug effects given significant potential for residual confounding, notably indication bias. Interestingly, IL-6 inhibitors did not appear to affect COVID-19 severity or related death in our study. However, the number of patients taking anti-IL-6 agents or JAK inhibitors was small and may have been insufficient to demonstrate other underlying effects. Likewise, the small number of patients treated with colchicine, mycophenolate mofetil, azathioprine, and rituximab (less than 10 patients with severe disease or death) does not allow for conclusions on a potential risk. Furthermore, potential indication bias exists since these drugs are mostly prescribed in patients with autoinflammatory, systemic autoimmune diseases, and vasculitis, all of which were associated with a higher frequency of severe infection in our cohort. Finally, as patients with active or very active iRMD tend to be more heavily medicated and we were unable to obtain information about disease activity, we cannot rule out that the higher frequencies identified with some treatments could be confounded by indication. To further explore these results, ancillary studies will be performed, with the potential merging of data with GRA and EULAR cohorts. Similarly, treatment with agents such as HCQ did not appear to have a positive impact on the frequency of severe disease or death.[29] Our study shows that patients previously treated with HCQ can develop COVID-19, consistent with a report of severe COVID-19 in patients with lupus taking HCQ.[30] We also collected information about the antiviral and immunomodulating therapies, notably HCQ, used by French clinicians to manage COVID-19. Our study is informative, but not built to inform on potential efficacy of antiviral and/or immunomodulating therapies in COVID-19 management.

Despite communication to all French paediatric rheumatology centres, the RMD COVID-19 cohort contained only 13 children that displayed minor symptoms. This strengthens the previous reports on the lack of severe COVID-19 in children with rheumatic diseases.[31] In addition, no iRMD COVID-19 paediatric case fulfilled the criteria for the recently recognised SARS-CoV-2-related paediatric inflammatory multisystem syndrome, a post-infection disease.[32] This latter observation could suggest that inflammatory diseases in children are not a risk factor for this specific syndrome.

There are several limitations to the current study. The first limitation is that no formal sample size calculation was performed for primary and secondary objectives and we cannot exclude a lack of adequate statistical power to detect significant differences. Moreover, due to the small number of events, multivariate analysis was not performed for death. The mortality rate in our cohort (8.3%) was similar to a previous report (7.2%).[23]

Though the current study analysed a large patient population assessed within a single country, the impact of selection bias on the observed frequency of death cannot be dismissed. During the beginning phases of the pandemic, immense pressure on the French medical system precluded PCR testing in all patients and focused confirmatory efforts on subjects with the most severe disease. Despite this shortcoming of unconfirmed diagnosis, our cohort includes a substantial ambulatory subgroup with mild disease. Since the French RMD COVID-19 cohort is an observational multicentre cohort study, we cannot rule out that all highly suspected/confirmed symptomatic patients with COVID-19 were enrolled by comparison to LICORNE registry of all suspected/confirmed patients with COVID-19 admitted at the Lille University Hospital. A potential selection bias in favour of inclusion of more patients with severe iRMD COVID-19 could explain the observed non-significant higher mortality in hospitalised population with iRMD compared with a cohort with non-iRMD. Furthermore, the care provided for patients of LICORNE registry may be different than that delivered to patients from the French iRMD COVID-19 cohort. Indeed, even if all patients come from the same country, discrepancies could exist in the care delivered to patients across the country, with respect to the type of hospital (secondary or tertiary care, academic, non-academic), resources available (including ICU beds and ventilators), the availability of alternative care and palliative care facilities, and the treatment approach itself, especially at the beginning of the pandemic. Moreover, within countries, another variable is the differential effect of the pandemic over time across the country. Nevertheless, an increased risk of death has recently been shown in 19 patients with RA/systemic lupus erythematosus/psoriasis-COVID-19 with an adjusted HR of 1.19 (1.11–1.27).[6]

In conclusion, the present study assesses the frequency of mild, moderate and severe COVID-19 and mortality in a large cohort of patients with rheumatic, autoinflammatory and autoimmune diseases being treated in France. In addition to monitoring the evolution of COVID-19 severity and outcomes, we confirmed the impact of comorbidities within the population with iRMD and generated preliminary data on the effects of anti-rheumatic therapies on disease prognosis following SARS-CoV-2 infection. We observed a higher frequency of death in the hospitalised population with iRMD compared with a cohort with non-iRMD from hospitalised patients with similar comorbidities, although the difference did not reach statistical significance. Furthers studies are warranted to confirm these results.

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Clinical course of COVID-19 in a cohort of 342 familial Mediterranean fever patients with a long-term treatment by colchicine in a French endemic area

The novel COVID-19 pandemic caused by SARS-CoV-2 is responsible for many deaths worldwide. Severe or life-threatening disease induce an exaggerated inflammatory response known as the 'cytokine storm', raising the question of the susceptibility and severity of SARS-CoV-2 infection in patients displaying innate immunity disorders such as familial Mediterranean fever (FMF). Furthermore, FMF patients take a long-term therapy with colchicine, which has been tested in SARS-CoV-2-infected patients with conflicting results.[1]

To tackle this question, we conducted a survey on SARS-CoV-2 infection in FMF patients followed in Paris area. In that meantime, the official French rate of infection in Paris area was 11% of the whole population.[2] FMF patients were identified from the juvenile inflammatory rheumatism (JIR) cohort, an international multicenter data repository and consented to the study. For the purpose of the study, we included only patients fulfilling the international FMF criteria, with a genetic confirmed FMF diagnosis,[3] and followed up in the French national autoinflammatory centre in Paris area.

Identified patients (n=627) were invited to answer a short questionnaire in consultation by phone or email about a possible SARS-CoV-2 infection during the time span ranging from March until end of May 2020; 342 patients answered the survey SARS-CoV-2 infection, diagnosis had been retained if the patient displayed clinical symptoms with a positive SARS-CoV-2 reverse transcriptase (RT)-PCR or serology or a typical chest CT scan. Overall, 27 FMF patients (7.8% of the responders; sex ratio 1:1) contracted the virus and 315 did not. All but one of the FMF-COVID$^+$ patients were taking daily colchicine since a median time of 23 years, mostly 1 mg/day table 1. Four received in addition an interleukin 1 (IL-1) inhibitor. Clinical symptoms of COVID-19 were consistent with those described previously.[4]

Out of the 27 FMF-COVID$^+$ patients, 7 patients were admitted in hospital (25%), displayed and six required oxygen therapy 3 (11%) developed acute respiratory distress syndrome and went to intensive care unit for mechanical ventilation and haemodialysis (online supplemental table). Two patients died (7%) but had respectively three and four comorbidities for severe SARS-CoV-2 infection (see online supplemental table). The third patient, 40 years old, suffered from hypertension and obesity. Patient older than 65 years accounted for 17% of the whole cohort, 75% were hospitalised and required oxygen; one died. Out of the three AA amyloidosis patients, two were hospitalised and one died. No additional antiviral treatment was administrated. At the end of the first epidemic wave in Paris area, the five survivors after hospitalisation went back home. None of them showed clinical signs of FMF attacks during SARS-CoV-2 infection.

The profile of our patients with a severe or life-threatening SARS-CoV-2 infection was like the general population. Severe SARS-CoV-2 infection was seen only in patients displaying known risk factors such as advanced age, chronic kidney disease, hypertension, vascular disease obesity and lung dysfunction. Our study suggests that the dysfunction of the innate immune system of FMF does not seem to be a risk factor in itself. However, preventive effect of long-term colchicine intake cannot be concluded as it was reported in a large cohort of patients with continuous colchicine therapy.[1]

Here, two out of four SARS-CoV-2-infected-FMF patients receiving IL-1 inhibitors died; of note, such patients usually display more advanced FMF including AA amyloidosis. Our study design did not allow us to conclude as to the responsibility of the drug or the underlying condition in this particular observation, despite recent publications showing efficacy of anakinra in severe SARS-CoV-2 infection.[5] None of the non-infected-FMF patients died in the same period. Notwithstanding, our preliminary conclusion is that FMF patients receiving a long-term

Table 1 Clinical course of 27 patients displaying COVID-19 in a cohort of 342 familial Mediterranean fever patients with a long-term treatment by colchicine in a French endemic area

Sex ratio	1
Age years, median	33 (17–87)
Comorbidities	
Age >65 years old	6
Hypertension	4
Cardiovascular disease	2
Diabetes	1
Chronic obstructive pulmonary disease	4
Chronic kidney disease	2
AA amyloidosis	3
Treatment regimen	
Colchicine	26/27
Colchicine 1 mg/day	15
Colchicine 1.5 mg/day	5
Colchicine 2 mg/day	5
Colchicine 2.5 mg/day	1
Interleukin 1 inhibitor	4
TNF alpha inhibitor	1
Signs and symptoms	
Fever	17
Cough	11
Asthenia	11
Shortness of breath	12
Myalgia	9
Anosmia	10
Dysgeusia	7
Headache	8
Diarrhoea	2
Interstitial pneumonia in CT scan	6
Nasal RT-PCR	14
Serology	12
Admission to hospital	7
Oxygen therapy	6/7
Nasal cannula	4/7
Invasive mechanical ventilation	3/7
Prognosis	
Admission to intensive care unit	3
Discharged	4
Died	2
Outcome	
Total recovery	19
Recovery with persistent asthenia	5
Recovery with persistent dyspnoea	1

TNF, tumour necrosis factor.

treatment with daily colchicine have no additional risk factor for severe SARS-CoV-2 infection compared with the general population.

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Correction notice This article has been corrected since it published Online First. The author, Dr Ackermann, has been added to the author list.

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Do autoantibody-responses mature between presentation with arthralgia suspicious for progression to rheumatoid arthritis and development of clinically apparent inflammatory arthritis? A longitudinal serological study

Several nested case-control studies have shown that autoantibody-response maturation in rheumatoid arthritis (RA) precedes clinical arthritis development.[1–3] This suggests a role in disease triggering. However, nested case-control studies have, similar to case-control studies, the disadvantage that controls are selected and that prospective data from non-progressing patients in a similar predisease stage are absent. The phase preceding clinically apparent inflammatory arthritis (IA) can be distinguished into an asymptomatic and symptomatic (ie, clinically suspect arthralgia, CSA) subphase. It is unknown whether autoantibody-response maturation occurs in the symptomatic phase. Likewise, its role in progression to clinical arthritis is undetermined; if autoantibody-response maturation relates to disease development, maturation is expected to be more pronounced in patients with CSA that progress compared with patients with CSA that do not. To better understand the relation between autoantibody-response maturation in time and development of clinical arthritis (RA/IA), we performed a longitudinal study on autoantibody-response maturation in patients with CSA that did and did not progress.

In serum from 147 patients with CSA, we determined with in-house ELISAs the presence and levels of IgM, IgG, IgA anti-citrullinated, anti-carbamylated and anti-acetylated protein antibodies (ACPA, anti-CarP, AAPA), resulting in nine autoantibody measurements per patient per timepoint. Autoantibody-response maturation was defined as increase in number of autoantibody reactivities or isotypes, and/or increase in autoantibody levels. Patients with CSA with paired samples at first presentation at the outpatient clinic and at IA development (n=55) or else after 2 years (n=92) were selected. Analyses were repeated with the outcome RA (the subgroup of patients with IA that fulfilled the 2010 or 1987 criteria at the time of IA development). Detailed description of methods and baseline characteristics is shown in the online supplemental file.

In patients negative for all autoantibodies at baseline, 17% of patients that progressed to IA became positive, compared with 6% of 'non-progressors' (figure 1A, p=0.12). In patients with ≥1 autoantibody reactivity at baseline progressing to IA,

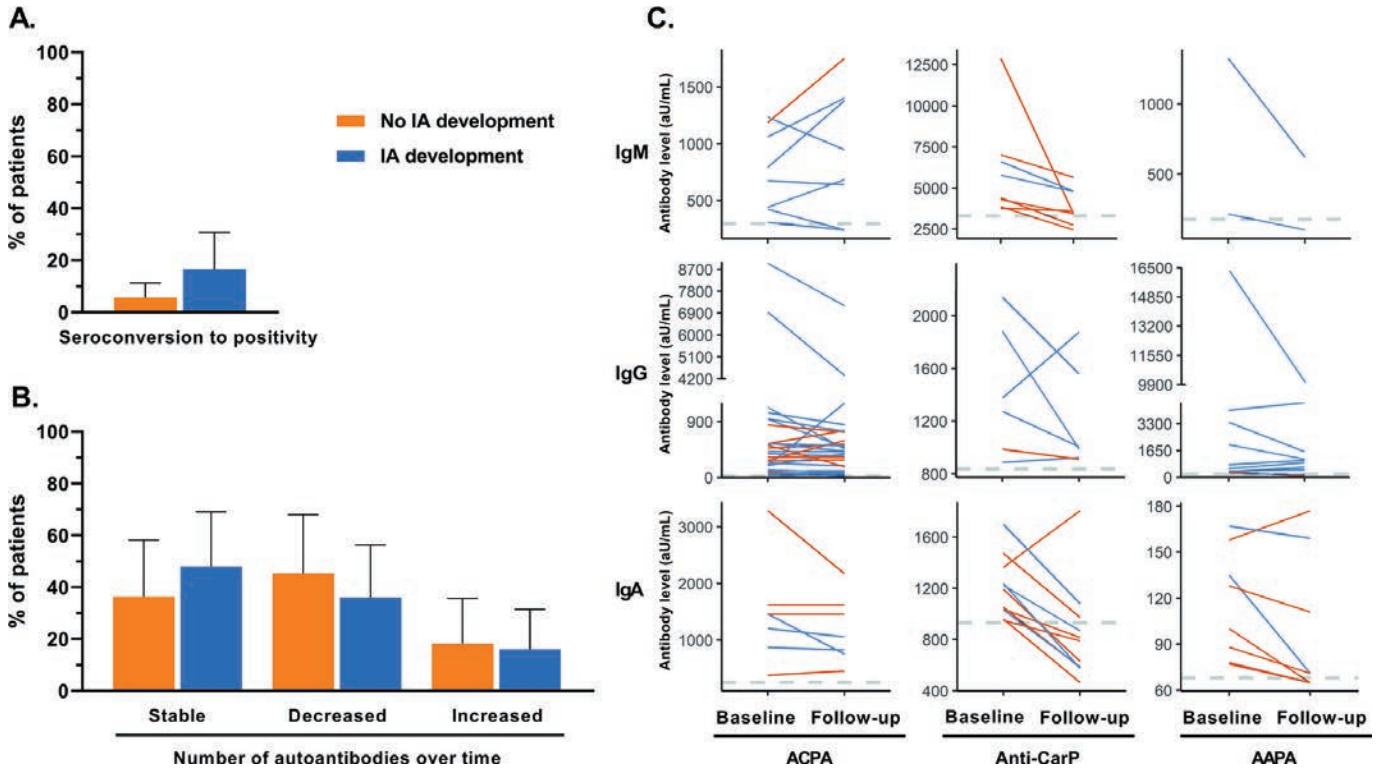

Figure 1 Changes in autoantibody response over time: (A) percentage of patients with seroconversion to positive in patients negative for all autoantibodies at baseline, (B) percentage of patients that has an increasing, decreasing or stable number of positive measurements over time in patients positive for ≥1 autoantibody reactivity at baseline, (C) autoantibody levels over time in patients positive for the respective autoantibody at baseline. All results are shown separately for patients with clinically suspect arthralgia that did and did not progress to clinically apparent inflammatory arthritis (IA). The mean time between first presentation and IA development was 5.6 months (SD 9.2). In patients that did not progress the second serum sample was obtained after 2 years. (A) Autoantibody negativity at baseline was defined as negative for the nine studied measurements (n=100), (B) autoantibody positive was defined as at least one (out of nine) positive measurement at baseline (n=47). Error bars in (A) and (B) represent 95% CI. Dashed grey horizontal lines in (C) indicate the cut-off values for each autoantibody. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; AAPA, anti-acetylated protein antibodies.

the median number of autoantibody reactivities was 1.0 (IQR 1.0–3.5, max. 6) at baseline and 1.0 (IQR 1.0–4.0, max. 6) at IA development (p=0.29). In patients with non-progressing CSA with ≥1 autoantibody reactivity at baseline, this was 1.0 (IQR 1.0–2.0, max. 4) at baseline and 1.0 (IQR 0.0–2.3, max. 5) after 2 years (p=0.07). As shown in figure 1B, an increase in the number of autoantibody reactivities was infrequent (16% in progressors, 18% in non-progressors (p=1.00)). Most changes in autoantibody positivity were explained by fluctuations around the cut-off (data not shown). Levels of autoantibodies did not significantly change over time (p values ranging 0.21–1.00) both in progressors and non-progressors (figure 1C). Similar results were found with the outcome RA (online supplemental figure S1), though remarkably, the number of autoantibody reactivities in patients not progressing to RA significantly decreased over time (1.0 (IQR 1.0–2.0) at baseline and 1.0 (IQR 0.0–2.0) after 2 years, p=0.015). Finally, when evaluating number of autoantibody reactivities and autoantibody-level changes within the entire study population (instead of within patients with ≥1 autoantibody reactivity at baseline), no significant increases were found (online supplemental figure S2).

To the best of our knowledge, this is the first study evaluating multiple isotypes and three anti-modified protein autoantibodies over time in CSA. Our data indicate that the presence and levels of IgM, IgG and IgA ACPA, anti-CarP and AAPA did not significantly increase over time, and that this was similar for patients with CSA that did or did not develop IA.

Autoantibody maturation in terms of cross-reactivity, affinity maturation and involvement of individual B-cell clones was not studied here, which is a limitation. We did not observe changes in isotype-usage over time, indicating that isotype switching was infrequent in both groups (online supplemental figure S3, online supplemental table S4). Although we cannot exclude that the results of this study would be different with a larger sample size (especially in patients with CSA autoantibody-negative at first presentation), the current data suggests that autoantibody-response maturation already occurs before presenting with CSA and that it does not increase substantially during progression to IA. Our results on characteristics of the ACPA, anti-CarP and AAPA response expand on previous longitudinal studies showing similar ACPA and RF levels,[4 5] and absence of change in the ACPA antigen-recognition repertoire in ACPA-positive arthralgia.[6] The data together imply that maturation occurs predominantly in the asymptomatic phase, a finding to be confirmed in population-based studies. Moreover, in relation to a multiple-hit model for RA development, our data suggest that autoantibody-response maturation in the CSA phase is not related to the 'final hit' as maturation was similar in patients with CSA not developing RA. These results increase the comprehension of the pathogenesis of RA.

In conclusion, autoantibody-response maturation as measured in this study occurs in the vast majority of patients with CSA before presenting with symptoms and broadening of the autoantibody response is not specific for progression from arthralgia to clinical arthritis.

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Long-term remission of cryopyrin-associated periodic syndrome after allogeneic haematopoietic stem cell transplantation

Cryopyrin-associated periodic syndrome (CAPS) is an inherited inflammatory disorder.[1] CAPS is characterised by skin rash, fever and the inflammations involving the eyes, ears, bones, joints and meninges. The mutations of NLRP3 encoding interleukin-1 (IL-1) inflammasome protein, cryopyrin, lead to the increased IL-1β secretion. The treatment for CAPS includes symptomatic treatments and IL-1β pathway blocking agents.[2–4] Although such agents are effective, they are not curative. Allogeneic haematopoietic stem cell transplantation (HSCT) is widely performed for haematological disorders and some inherited immune disorders. Through allogeneic HSCT, the hosts' haematopoietic and immune cells are completely replaced by the normal donor cells. Therefore, CAPS could be theoretically cured by allogeneic HSCT; however, there have been no reported patients with CAPS who underwent allogeneic HSCT so far.

A 30-year-old woman had a long-standing history of recurrent symptoms/signs such as fever, skin rash, arthralgia, serious headache and uveitis of the neonatal onset. She had presented a hearing loss since her childhood but hearing aids were not required. Her height was 142 cm suggestive of growth retardation in comparison with 158.6±6.0 cm in Japanese women aged 30–39 years, which was referred from Japanese Government Statistics. The diagnosis of CAPS (Muckle-Wells syndrome, MWS) was made at the age of 27 based on the detection of NLRP3 gene mutation (T348M in exon 3) in the peripheral blood cells by Sanger method, when her son with the same symptoms was diagnosed as having MWS by detecting the same mutation. Since CAPS was considered vertically transmitted to her son, the mutation was not assessed in non-haematopoietic tissue. Therefore, gonosomal mosaicism was not completely ruled out. Optic nerve atrophy was not observed. Two years after diagnosis, she developed acute lymphoblastic leukaemia (ALL), which was refractory to chemotherapy. Although the frequency and severity of CAPS decreased after initiating chemotherapy for ALL, she repeatedly had episodes of CAPS such as fever, skin rash and arthralgia accompanied by C reactive protein elevations (above 10 mg/dL). Allogeneic bone marrow transplantation from an unrelated donor was performed after delivering total body irradiation-based myeloablative conditioning. As graft-versus-host disease (GVHD) prophylaxis, tacrolimus in combination with methotrexate was given. She developed chronic GVHD involving the liver and mouth, which was

successfully managed with tacrolimus continuation and initiation of low-dose prednisolone (0.2 mg/kg). Tacrolimus and prednisolone were tapered and discontinued at 21 months and 24 months post-transplant, respectively. After transplantation, she achieved complete remission of ALL and also became completely free of CAPS signs/symptoms, including laboratory data abnormalities. At present, 7 years after transplantation, she is alive in a good performance status without the disease relapses of both ALL and CAPS.

We here report the first case in which allogeneic HSCT seemed to have been curative for CAPS. In our case, allogeneic HSCT was performed primarily aiming at the cure of ALL, and the remissions of both ALL and CAPS were achieved. The patient has been free of CAPS symptoms for the 7 years after transplantation, although there had been repeated episodes of CAPS prior to the transplantation.

Cryopyrin is highly expressed by leucocytes such as neutrophils and monocytes, and chondrocytes.[5 6] Such restricted expressions of cryopyrin in specific cells could be attributable to the characteristic target organs of CAPS. Allogeneic HSCT only eradicates and replaces haematopoietic and immune cells, but not chondrocytes. However, it is of note that the successful outcome of our case suggest that the eradication and replacement of haematopoietic/immune cells through allogeneic HSCT have the potential to induce remission and possibly cure of CAPS at least in MWS. An accumulation of cases like ours is required to further evaluate the potential curativeness of allogeneic HSCT for CAPS.

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Janus kinase inhibitor significantly improved rash and muscle strength in juvenile dermatomyositis

Juvenile dermatomyositis (JDM) is a rare systemic autoimmune vasculopathy characterised by weakness in proximal muscles and pathognomonic skin rashes.[1] Clinically, some patients are refractory to any available treatments or became steroids dependent.[2] The adverse reactions of long-term use of steroids are severe; therefore, more effective and safer medications are urgently needed. JAK inhibitors (JAKi) can reduce interferon (IFN)-induced STAT1 phosphorylation and block the JAK-STAT pathway, demonstrating a therapeutic potential of inflammation control in JDM.[3] The successful uses of JAKi were reported in adult dermatomyositis (DM) and two patients with JDM.[3–5] Here, we want to share the JAKi using experiences of 25 refractory JDM cases who were diagnosed and classified according to Bohan and Peter's criteria and treated between November 2017 and May 2019. Written informed consents were obtained from the guardians of all patients before starting the treatment.

Among 25 cases, 44% (11/25) patients were female, the mean age of onset was 4.6±3.3 years and the mean age to start add-on JAKi treatment was 7.2±4.0 years. The mean disease course of the 25 JDM patients before JAKi treatment is 21.0 months (range: 14.0–36.5). All cases are refractory JDMs, including 32% (8/25) ineffective patients and 68% (17/25) glucocorticoid-dependent cases. After routine treatment fails, they received JAKi for 3–18 months as an off-label use. In subsequent JAKi treatment, 28% (7/25) used tofacitinib, and 72% (18/25) used ruxolitinib. In patients of <25 kg (n=11), the initial dosage was 2.5 mg twice daily, and in patients of ≥25 kg (n=14), the initial dosage was 5 mg twice daily, and one patient required the maximum dosage of 7.5 mg twice daily.

The 25 patients were followed for a median of 7.0 months (range: 3–21 months). Ninety-six per cent (24/25) had rash when

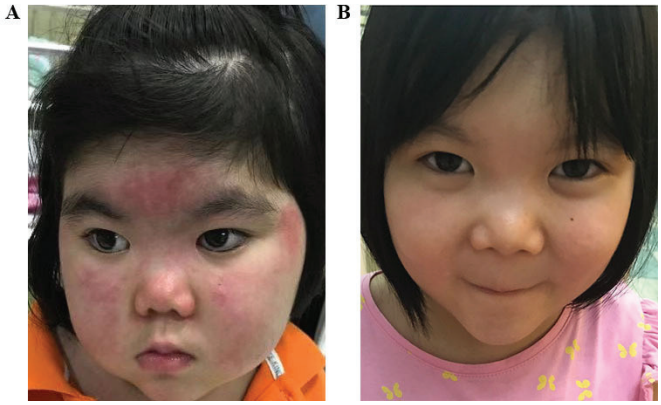

Figure 1 A typical case of a girl >25 kg who received an initial dose of 5 mg twice daily of Janus kinase inhibitor (JAKi) but required the maximum dose of 7.5 mg twice daily. She received this dose for 6 months, and the dose was gradually tapered. In the meantime, glucocorticoids were also tapered, and the patient showed an increased growth rate. (A) The typical skin lesions before treatment with JAKi. (B) Those lesions had mostly disappeared after treatment.

JAKi was added, and all showed improved rashes, including 66.7% (16/24) cases of complete resolution. In patients with rash, rashes started to improve after 1.0 (0.6–2.0) weeks of JAKi and showed obvious improvement after 2.5 (2.0–4.0) weeks of JAKi. No clinically observable rash could be seen after 12.0 (8.0–24.0) weeks of JAKi. The Cutaneous Assessment Tool Binary Method score decreased dramatically from 7.0 (3.0–10.0) to 0.0 (0.0–1.0) ($p<0.001$). figure 1 shows a girl with a long-term refractory rash that disappeared after gradually increasing the JAKi dose to 7.5 mg twice daily. Up to the last follow-up in August 2019, 28% (7/25) patients discontinued glucocorticoids including this girl. There is currently only one case relapsed, the rash disappeared after 8 weeks of JAKi but recurred 4 weeks later and JAKi was stopped at 12 weeks.

Additionally, 10/25 (40%) patients had decreased muscle strength, and 4% (1/25) had continuous high levels of muscle enzymes. After treatment, seven cases improved in childhood myositis assessment scale (CMAS) score (from 18.6±15.0 to 35.7±6.3, $p=0.018$). Two patients did not change in CMAS score (pretreatment/post-treatment score=47) but reported improvement in fatigue and activity tolerance. One patient was unevaluable for CMAS score before JAKi treatment due to joint contracture. As for biochemical indicators, CK and/or LDH were abnormal in 12 patients when JAKi was added. Median CK levels were normal before and after treatment. LDH decreased from 361.5 (306.3–463.3) U/L to 291.0 (275.8–394.8) U/L ($p=0.034$) in 12 patients, but two patients showed LDH increase, from 340 to 395 U/L and from 307 to 420 U/L, respectively. More details of patients' clinical characters and index changes are in the online supplemental material. During our observation period, no increase in the infection rates with Epstein-Barr virus, cytomegalovirus, varicella-zoster virus and tuberculosis occurred as reported by another study.[6] No thromboembolic event was observed as well.

This is the first case series study summarising the JAKi treatment on patients with refractory JDM. In our observation, JAKi improved refractory rash and muscle involvement, helped to reduce or stop glucocorticoid and no obvious side effects were found. Therefore, our study suggested that JAKi might be an idea choice in children with refractory JDM.

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Impact of grouping serial journal articles by disease category: analysis of article placement order in *ARD* 2013–2019

Article placement order within journal issues has the potential to influence perceived research importance, with earlier listed articles being more visible and more likely to be downloaded and cited.[1 2] In an analysis of serial rheumatology journals published between 2013 and 2018, we reported that certain rheumatic diseases were prioritised within journal issues.[1] Articles about rheumatoid arthritis were preferentially ordered at the front of issues, while other diseases including connective tissue diseases, crystal arthritis, paediatric syndromes and pain syndromes were consistently ordered towards the back. This prioritisation was evident in journals with disease-specific tables of contents sections within issues, but was not observed in journals that did not group content by disease category.

Prior to 2019, *Annals of the Rheumatic Diseases (ARD)* did not group articles within each issue by disease category. In the first issue of 2019, *ARD* announced 'small but visible structural changes' in which articles would be grouped within each issue by disease category.[3] We set out to determine whether specific rheumatic diseases were prioritised or deprioritised by this change in policy.

We analysed all original research articles published in *ARD* between June 2013 and December 2019. Each article was coded into one of six disease categories: connective tissue disorders, crystal arthritis, osteoarthritis, rheumatoid arthritis, spondyloarthropathies and other rheumatic diseases with small numbers of articles (online supplemental table 1). Each article was assigned a Standard Article Placement Index (SAPI),[1] defined as the order of the article in the issue/total number of articles in the issue (eg, the first article in an issue of 19 articles was given an SAPI of 1/19=0.0526 and the last article 19/19=1). This approach accounts for the variation in article numbers across issues and between disease categories. Cumulative distribution functions of SAPIs were plotted and two-sample Kolmogorov-Smirnov Z tests were conducted to determine whether differences in distribution of SAPIs between articles published in issues with content not grouped by disease category (2013–2018 articles) and issues with content grouped by disease category (2019 articles) were significant. The analysis was also undertaken using articles published in 2018 versus 2019 (online supplemental text and figure S1). All analyses were performed in SPSS (V.25 IBM Corp). $P<0.05$ was considered significant.

A total of 1646 articles were included, with 1471 from issues with content not grouped by disease category (2013–2018 articles) and 175 from issues with content grouped by disease category (2019 articles) (online supplemental table 2). Comparisons between issues with and without content grouped by disease demonstrated a significant difference in SAPI distributions; from January 2019, articles about rheumatoid arthritis were placed more towards the front of issues ($p=0.004$), and articles about connective tissue diseases, crystal arthritis, osteoarthritis and other diseases were placed towards the back of issues (all $p\leq0.034$) (figure 1).

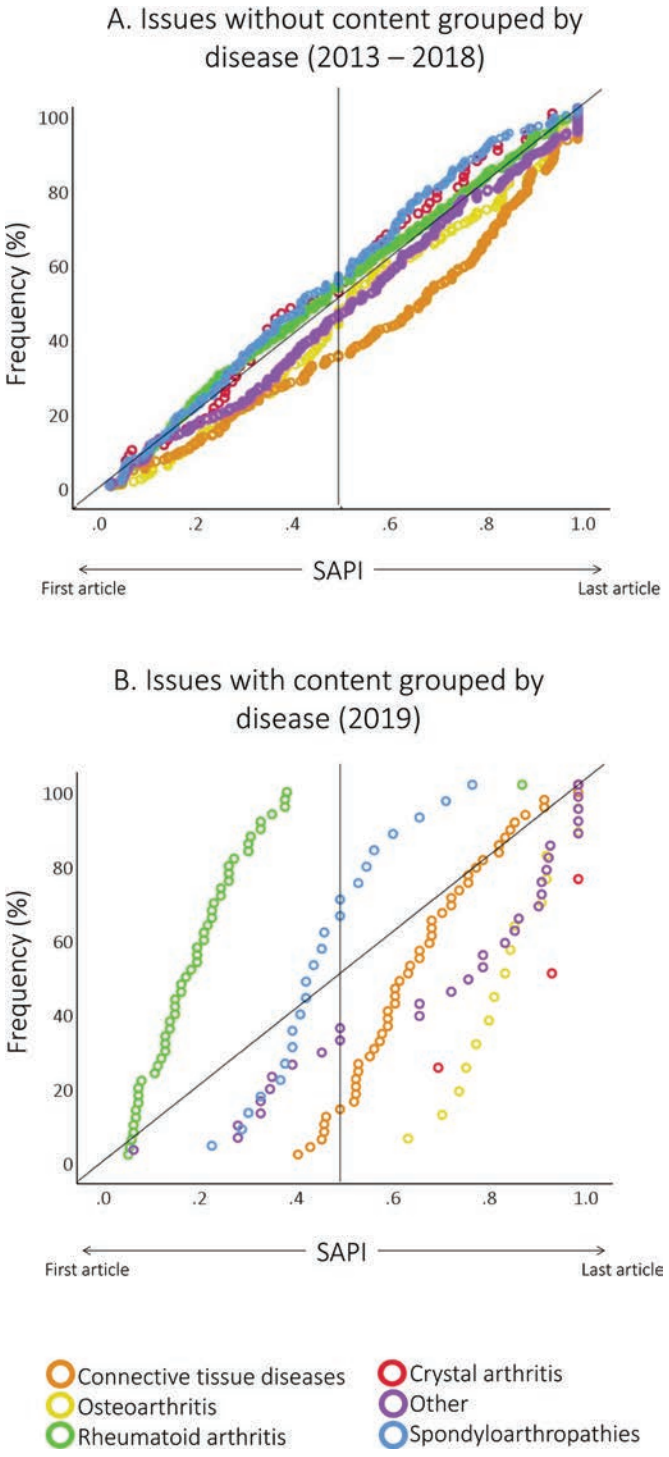

Figure 1 Cumulative density function plots showing distributions of SAPI for each disease category for: (A) Issues with content not grouped by disease; and (B) issues with content grouped by disease. SAPI was defined as the order of the article in the issue/total number of articles in the issue. The score ranged from 0 to 1 with the last article in the issue=1. Left deviated distributions suggest prioritisation towards the front of journal issues. SAPI, Standard Article Placement Index.

In summary, this change in journal policy has increased the prominence of rheumatoid arthritis in *ARD*, and deprioritised

other rheumatic diseases, including connective tissue disease and crystal arthritis. Grouping article content by disease category may improve some aspects of reader experience, but may also reduce readers' exposure to rheumatic diseases that may already be understudied or less well understood. The short time between publication and data extraction limited our ability to analyse the impact on citations or downloads. Although some readers might access digital content using keyword searches, the order in which documents are presented in digital formats influences which are more frequently accessed.[4–6] We encourage the *ARD* editors and publisher to remove grouping of articles by disease category, or to cycle the order of disease category groups for each issue.

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Telerheumatology in COVID-19 era: a study from a psoriatic arthritis cohort

We read with interest the article by Gupta *et al*[1] who reported the management of treatments for rheumatic diseases during COVID-19 pandemic among practitioners in India. In this study, the authors showed that about half of the physicians would reduce the use of biological disease-modifying antirheumatic drugs (bDMARDs) or defer specific drugs such as rituximab.[1] Choices were apparently made considering possible relationships between drug mechanism of action and effect on the viral infection.[1]

During COVID-19 pandemic, telemedicine is emerging as a possible tool for reducing the risk of contagion and viral spread,[2–4] and a useful strategy for the management of chronic diseases.[5 6]

As of 29 April 2020, the outbreak of COVID-19 generated 201 505 confirmed cases in Italy, with 2405 confirmed cases in Naples Metropolitan area.

The objective of our study was to evaluate telemedicine when offered as part of routine care for the follow-up of patients with psoriatic arthritis (PsA), during the COVID-19 pandemic, for reducing risk of contagion and the number of face-to-face visits.

From 9 March 2020, our face-to-face outpatient clinic, devoted to management of patients with PsA under subcutaneous bDMARDs and targeted synthetic DMARDs (bDMARDs and tsDMARDs), was converted to a telerheumatology model over 7 weeks.

Every patient with an existing appointment was called by phone and was asked the consent to perform a live interactive telemedicine visit.

Established patients who were unable or unwilling to perform a video visit were offered the option of a telephone visit.

All the patients accepted of interacting with physicians by live interactive video or telephone visits. These were also supported using secure data transmission of laboratory test and instrumental reports, and email consultations. Patients were also invited to upload high-resolution photographs of suspected active articular and cutaneous manifestations.

In case of severe symptoms and signs, as evaluated via telemedicine, we provided to perform an in-person visit within the same day or the following day.

Within 7 weeks, we completed 105 telerheumatology visits for 105 patients with PsA (51 male and 54 female; mean age: 52.3 years), under bDMARDs (91 cases) or tsDMARDs (14 cases).

In 94 patients, therapy with bDMARDs or tsDMARDs was continued due to effectiveness and safety. In 10 of them, a therapy with non-steroidal anti-inflammatory drugs (NSAIDs) was added, due to monoarticular or entheseal pain in the absence of local swelling and redness, as evaluated by anamnesis and photographs; particularly, in 7 cases, NSAIDs were added to bDMARDs therapy and, in 3 cases to phosphodiesterase 4 inhibitor (PDE4i) therapy. In one patient on methotrexate and anti-tumour necrosis factor-α therapy, methotrexate was withdrawn due to an increase of transaminases. In this case, a strict laboratory evaluation and liver ultrasound were required.

Signs of active arthritis and/or enthesitis were clearly referred by 10 patients and visualised by photographs. These were also associated with increased pain on Visual Analogue Scale (mean: 7.6). Among them, in three cases, a psoriasis worsening was also seen. For all these 10 cases, we decided to perform an in-person visit within the same day or the following day.

In our experience, telemedicine has represented a valuable instrument for PsA care in COVID-19 era. Telemedicine could have a key role for the management of patients with rheumatic disease, in particular for those with comorbidities, because of a higher severity rate in the case of COVID-19.[5 6]

Telemedicine could also represent a valid support to maintain social distance and to help 'flatten the pandemic curve'. However, further studies are need for evaluating this approach.

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Response to: 'Telerheumatology in COVID-19 era: a study from a psoriatic arthritis cohort' by Costa *et al*

Costa has raised a relevant question of switching to online platforms for maintaining continuity of medical care in patients with chronic rheumatic diseases (RDs).[1] A near-total focus of healthcare systems on COVID-19, non-availability of medical consultations and medicines at some places, and delayed infusions have seriously undermined the care of chronic RDs. The unprecedented situation has heralded a felt need for a shift to virtual consulting, ranging from telephonic to instant messaging, email-based and video consultations.

In our survey, 114 of 221 (51.6%) rheumatologists in India had adopted virtual consultations in March 2020, with half of them (57) delivering patient care over WhatsApp, and 22.8% (26) and 27.1% (31) resorting to emails and video consultations.[2] Merely 10% of physicians were continuing their clinics at the time of the survey. The choice of the platform might differ, with WhatsApp being more prevalent in certain countries like India and WeChat in China.[3] The delivery of routine care on virtual platforms can prevent the need for travel and minimise personal and administrative costs towards healthcare. Such an approach assumes an even greater relevance in a country like India where a single rheumatologist is available for 40 352 patients with rheumatoid arthritis (RA) vs 1:425 in the USA (still considered to be inadequate).[4] Despite the challenge of learning to operate different online software, the use of technology for patient consultations is rapidly gaining wider acceptance in these times.[5] However, it is essential to be mindful of patient rights, including privacy, especially in light of the recent incidents of technological failure.[6]

Notably, Costa observed that the same treatment was continued in 89.5% of patients with psoriatic arthritis (psA), signifying that the renewal of prescriptions was the predominant requirement. At our unit we provided 199 teleconsultations (RA 31.6%, spondyloarthritis 15.6%) over the last fortnight, wherein over half (51.2%, 102) were advised to continue the same treatment, while routine investigations were awaited for another 8.5%. Admission was advised in 4.0%, immunosuppressants (IS) discontinued in 3%, and 3.5% were advised to start a new IS, respectively (table 1). Titration of the dose of IS and non-steroidal anti-inflammatory drugs (NSAIDs) for managing symptoms was required by one in five patients (20.6%, 41). Notably, certain RDs that may require closer monitoring (vasculitis and myositis) were underrepresented in the cohort but over-represented among admissions, the reverse being true for RA and spondyloarthritis. Thus, a triage algorithm based on the type of RD, disease activity, age, and comorbid conditions may be designed to prioritise medical care.

Besides, utility of teleconsultations in recent times could signal a shift towards patient-reported outcome measures in future. In countries with established infrastructure, linking the app-based services with the hospital information system can provide an organised record base of future reference.[3 7] While the importance of real interaction and formal physical examination cannot be overemphasised, teleconsultations can tide over the crises. At the same time, logistics such as reimbursement can be sorted out at the federal level. Further, teleconsultation-based services have the potential to be extended to practitioners at the primary healthcare level by specialists placed at higher centres for guidance in the management of complex diseases when the patients cannot be referred due to travel restrictions.

Thus, the successful use of telemedicine for managing psA by Costa and recent insights at our centre argues for greater exploration of this digital tool for a decentralised approach towards seamless patient care.

Table 1 Experience with teleconsultations at a rheumatology tertiary care centre in India (n=199)

Diagnosis	N (%)
Rheumatoid arthritis	63 (31.6)
Spondyloarthritis	34 (17)*
Systemic lupus erythematosus (SLE)	28 (14)
Vasculitis	7 (3.5)†
Systemic sclerosis	9 (4.5)
Sjogren's syndrome	4 (2)
Inflammatory myositis	4 (2)
Juvenile idiopathic arthritis (JIA)	10 (5)
Connective tissue disease-interstitial lung disease	3 (1.5)
Osteoarthritis and soft-tissue rheumatism	9 (4.5)
Miscellaneous ‡	16 (8)
Unclear	12 (6)
Tele consult advice	
Continue the same treatment	102 (51.2)
Infusion	4 (2.0)
Admission	8 (4.0)§
Consult local doctor or another specialist	15 (7.5)
Intervention	54 (27.1)
Stopped drug	6 (3.0)
Start new drug	7 (3.5)
Dose titration	41 (20.6)
Methotrexate	10
Sulfasalazine	3
NSAIDs	19
Glucocorticoids	7
Others	2
Investigations awaited	17 (8.5)

*22 ankylosing spondylitis, 7 psoriatic arthritis, 5 reactive arthritis.
†1 Behcet's disease, 3 granulomatosis with polyangiitis, 2 polyarteritis nodosa, 1 Takayasu's arteritis.
‡One each of fibrosing mediastinitis, common variable immunodeficiency (CVID), leprosy, drug reaction with eosinophilia and systemic symptoms, peripheral symmetric gangrene, pyoderma gangrenosum, and eosinophilic fasciitis, 5 each of gout and sarcoidosis.
§three dermatomyositis, 1 SLE, 1 polyarteritis nodosa, 1 JIA, 1 systemic-onset JIA, 1 CVID.

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Correction notice This article has been corrected since it published Online First. Reference 5 has been corrected.

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work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Telemedicine will not keep us apart in COVID-19 pandemic

We read with interest the letter by Bozzalla Cassione *et al*[1] about the role of telemedicine in their clinic during COVID-19 time. Telemedicine represents a useful tool not only in regions with limited access to healthcare[2] but also in different settings like quarantine, when healthcare personnel became essential.

Since the Italian National lockdown decision[3] and the WHO announcement of the COVID-19 pandemic,[4] enormous demand to handle the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection disease challenged the Italian healthcare system. Indeed, in the Fondazione Policlinico Universitario A. Gemelli (FPG) Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) in Rome, the delegated taskforce (Gemelli against COVID-19) gradually opened 14 COVID-19 dedicated wards, in accordance to the progressive increase of serious cases. Concomitantly, several clinics were remodulated to optimise the staff use and to avoid patient exposure to the hospital environment.

The Division of Rheumatology of the FPG-IRCCS is a high-flux rheumatological centre, with almost 16 000 visits performed in the last year for chronic inflammatory arthritis (38%), connective tissue diseases (CTDs) (34%) and other rheumatic diseases (27%) (ie, osteoarthritis and fibromyalgia) from all Italian regions. Before the spread of COVID-19, our rheumatology service was organised to routinely provide several general (for first and follow-up visits) and dedicated outpatient clinics, that is, an early arthritis clinic, two biological clinics for patients on biological disease modificed antirheumatic drugs (bDMARDs) or targeted synthetic DMARDs (tsDMARDs), disease-specific clinics (for psoriatic arthritis, spondyloarthropathies, undifferentiated peripheral inflammatory arthritis, systemic sclerosis, systemic lupus erythematosus, systemic vasculitis, Sjögren syndrome, idiopathic inflammatory myopathies and juvenile idiopathic arthritis), muskuloskeletal ultrasound examination, intra-articular injection and infusion services, osteoporosis service (clinical as well as for dual-energy X-ray absorptiometry scan) and a biopsy unit. The telemedicine approach was limited only to deal with blood testing remote examination or to handle urgent matters.

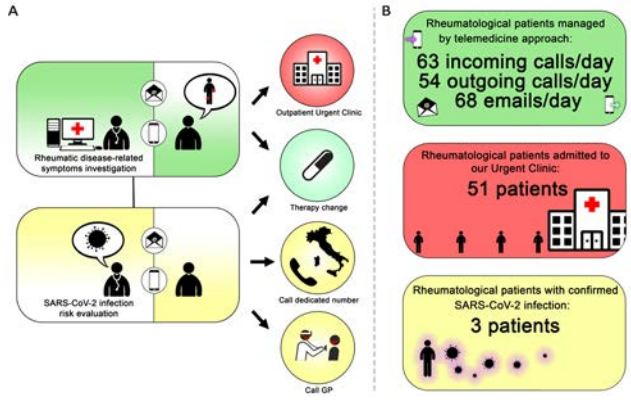

Figure 1 Graphical representation of telemedicine protocol approach at the Division of Rheumatology of the Fondazione Policlinico Universitario A. Gemelli IRCCS. (A) During each phone clinical interview, the patient's general status and the presence of concomitant rheumatological disease-related symptoms were investigated (green box). Then, the presence of any symptoms of infection and a possible contact with a suspected/confirmed SARS-CoV-2-infected individual were explored (yellow box); in the presence of any elements suggesting SARS-CoV-2 infection, the patient was invited to call his/her GP or the official regional dedicated phone number (yellow circles), and the therapy was modified accordingly (green circle). Indeed, in case of disease-related symptoms, the therapy was adjusted and, if necessary, the patient was invited to attend the urgent clinic of our division (red circle). (B) In the period between 9 March and 9 May 2020, by telemedicine approach, we managed a mean of 117 calls/day (63 incoming and 54 outgoing calls/day) and 68 emails/day (green box), recognised 51 critical patients who were invited to attend our urgent clinic (red box) and succeeded to identify three patients with rheumatological diseases with confirmed SARS-CoV-2 infection (yellow box). GP, general practitioner; SARS-CoV-2, severe acute respiratory syndrome coronavirusSevere Acute Respiratory Syndrome Coronavirus 2.

By the COVID-19 diffusion in Italy, our division was promptly and fully reorganised not only in terms of the active clinic number but also in terms of space logistics to fulfil the new requirements for social distance and patient protection. Therefore, during the first phase, only the infusion clinic for patients with chronic inflammatory arthritis and CTDs was maintained to avoid treatment discontinuation. Moreover, an urgent clinic was activated and an official protocol for telemedicine practice was immediately implemented to screen urgent non-postponable appointments, to conduct a virtual consultation asking not to attend rheumatology service in person and still to reassure patients (figure 1). Each phone clinical interview aimed to investigate patients' general status and presence of concomitant rheumatological disease-related symptoms, and to assess the presence of any symptoms of infection in the last month, on a possible contact with a suspected/confirmed SARS-CoV-2-infected individual. In the presence of any elements suggesting SARS-CoV-2 infection, the patient was invited to call his/her general practitioner (GP) or the official dedicated number[5] and the therapy was modified if required. Furthermore, regardless of SARS-CoV-2 infection, in case of disease-related symptoms, the therapy was adjusted and, if necessary, the patient was invited to attend our urgent clinic.

Furthermore, we activated a unique official mobile number for all our patients to give an answer to any questions, doubts or just to give comfort, and every clinic continued to use its own email address with the same aims. Finally, official mobile number and email addresses were spread on social media through the Italian patients' associations.

Our experience demonstrated how it is possible to help rheumatological patients by telemedicine despite dramatic fast changes in daily life. Actually, thanks to the reduction of new COVID-19 cases and within national lockdown exit plan, our strategy will be to integrate and implement our active telemedicine protocol within the next clinical practice organisation.

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Response to: 'Telemedicine will not keep us apart in the COVID-19 pandemic' by Perniola *et al*

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We thank Perniola *et al* for their interest in our paper and the circumstantial details on their outpatient activity.[1] Our service is a referral rheumatology department with more than 100 outpatients evaluated every day. Since the first Italian subject has been diagnosed with COVID-19 and transferred to our Hospital on 21 February 2020, a prompt reorganisation of our department and of the entire hospital facilities has been carried out in order to create a COVID-19 hub hospital and deal with the emergency at best.[2] Starting 24 February 2020, we have severely restricted the access to our outpatient clinic. Nevertheless, we have maintained the administration of intravenous therapies (biologics, antimetabolites and prostanoids) and the evaluation of patients requiring urgent interventions (eg, patients suffering from organ or life-threatening conditions and/or disease flares). Follow-up visits have been initially taken over by phone contact.[3] Subsequently, a proper telemedicine platform has been implemented by our institution, allowing a direct visual interaction with patients and the possibility of sharing test results, pictures and medical records in real-time during the visit. The use of this platform was preferred over a simple phone-consultation and/or unprotected email interaction because it was integrated into the official medical record software in use at our institution and also for data security. In fact, patients had to undergo a three-step authentication in order to access the platform and shared data were stored in protected servers of our institution and automatically destroyed after 45 days.

We initially experimented with the platform on systemic lupus erythematosus (SLE) patients, who were chosen as a good test sample because of their complexity. Telemedicine served us a double aim: (1) to carry out routine evaluation of our outpatients and their clinical conditions and (2) to retrieve information about exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the clinical course and outcome of COVID-19.[4] Currently, despite the containment measures having been eased, we are continuing to adopt teleconsultations, after an initial telephonic triage, for patients with stable clinical conditions or with symptoms possibly attributable to COVID-19 who do not require hospitalisation.

In our experience, telemedicine is allowing to guarantee most of the planned visits in safe conditions for chronic patients already in our outpatient circuit. A similar approach has been carried out at our hospital by other specialties, including cardiology. However, a spike in deadly manifestations such as sudden cardiac death has been reported, due to the inaccessibility of routine care or fear to appeal to medical help.[5] In the light of these reports, we have still to evaluate the usefulness of telemedicine in new patients requiring prompt diagnosis and very early referral as in the detection and fast tracking of large vessel vasculitides.[6] It is likely that we will be able to better balance the consequences of such delays that occurred during the COVID-19 pandemic, in the future and we should get ready to manage them in the best possible way.

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COVID-19 pandemic: an opportunity to assess the utility of telemedicine in patients with rheumatic diseases

We read with interest the letter published by Bozzalla Cassione *et al*,[1] in which authors evaluated 165 patients with systemic lupus erythematosus using telemedicine as the follow-up method. As in Italy, the high infectivity and the risk of collapse of intensive care units led to the Spanish government to announce on 14 March the strict confinement and prohibition of social mobility to ensure a decrease in COVID-19 contagion rates. As a consequence, physical consultations of rheumatology outpatients have been replaced by phone consultations to prevent the risk of contagion.[2] One of the most important concerns that limits the care quality of rheumatic patients in Spain is the pressure of healthcare, since the number of patients is excessive and human resources are limited. This epidemic outbreak has proven to be a great opportunity to test phone consultations in assisting rheumatic patients. The rheumatology department of Reina Sofía University Hospital in Córdoba (Spain) conducted a survey among rheumatic patients that was disseminated via patient organisations and social media throughout the national territory between 25 April and 5 May. The objective of this survey was to evaluate the patients' level of satisfaction with the phone consultation and the profile of patients who considered this type of consultation to be useful for future implementation.

In this survey, the following data were collected: sex, age, diagnosis, current treatment and disease status (pain, stiffness, fatigue and depression in visual analogue scales ranging from 0 to 100). We also asked patients whether they underwent a phone consultation with their rheumatologist during the pandemic, the patients' level of satisfaction with this consultation (0–100 scale) and their opinion of the utility of phone consultation in the future.

On 5 May, a total of 644 patients completed the survey, of which 244 (37.9%) underwent a phone consultation during confinement. The mean level of satisfaction of this consultation was 64.7±35.8. Among the 244 patients who received a phone consultation, 220 patients answered the following question: 'Do you think that phone consultation could be useful in the monitoring of your rheumatic disease?'. A total of 116 (52.7%) opined 'yes' and 104 (47.3%) answered 'no'. The characteristics of patients who considered the phone consultation to be useful in comparison with those who thought that would not be useful are shown in the table 1.

These results showed that neither gender nor age were associated with good acceptance of phone consultation, although young patients showed a trend towards better satisfaction with this type of assistance. We also found a similar prevalence of diagnosis between patients who considered useful phone consultation and those who did not. We expected to find that patients under biological disease-modifying antirheumatic drugs would be more prone to feeling unsatisfied with a phone consultation due to their need for tight control of their disease; however, interestingly, there were no differences in opinions on phone consultations with regard to treatment intake. The only difference found between satisfied and unsatisfied patients was the level of symptomatology. Patients who considered the phone consultation to be useful showed lower levels of axial pain (52.4±32.8 vs 63.7±29.8), peripheral stiffness (47.2±29.4 vs 56.1±29.0) and axial stiffness (47.6±32.7 vs 62.1±29.5) than did patients who did not find it useful.

Based on this survey, it seems that there is no specific profile of patients who considered a phone consultation to be useful, since neither the diagnosis nor the treatment intake was associated with this opinion. However, these results suggest that the status of the disease in terms of activity is the most important factor in patients' acceptance of a phone consultation for their monitoring; to a lesser extent, young age was another important

Table 1 Comparison of clinical characteristics between patients who considered phone consultation useful and those who did not

	Overall population N=220	Patients who considered phone consultation to be useful n=116	Patients who did not consider phone consultation to be useful n=104	P value
Gender (female)	159/218 (72.9%)	86/115 (74.8%)	73/103 (70.9%)	0.517
Age	46.6 (13.6)	44.9 (14.3)	48.4 (12.6)	0.059
SpA or PsA	110/220 (50.0%)	53/116 (45.7%)	57/104 (54.8%)	0.177
Rheumatoid arthritis	47/220 (21.4%)	23/116 (19.8%)	24/104 (23.1%)	0.557
Autoimmune diseases	30/220 (13.6%)	18/116 (15.5%)	12/104 (11.5%)	0.280
Osteoarthritis or osteoporosis	11/220 (5.0%)	8/116 (6.9%)	3/104 (2.9%)	0.173
Fibromyalgia	14/220 (6.4%)	8/116 (6.9%)	6/104 (5.8%)	0.732
Other diagnosis	8/220 (3.6%)	6/116 (3.6%)	2/104 (1.9%)	0.392
NSAID use	130/220 (59.1%)	67/116 (57.8%)	63/104 (60.6%)	0.671
csDMARD use	83/220 (37.6%)	48/116 (41.4%)	35/104 (33.7%)	0.238
bDMARD use	95/220 (43.2%)	47/116 (40.5%)	48/104 (46.2%)	0.399
VAS peripheral pain	51.6 (29.7)	48.6 (30.5)	54.9 (28.6)	0.116
VAS axial pain	57.9 (31.8)	52.4 (32.8)	63.7 (29.8)	0.011
VAS peripheral stiffness	51.5 (29.5)	47.2 (29.4)	56.1 (29.0)	0.032
VAS axial stiffness	54.7 (31.9)	47.6 (32.7)	62.1 (29.5)	0.001
VAS fatigue	57.1 (29.9)	54.5 (29.2)	59.8 (30.5)	0.208
VAS anxiety	50.4 (30.1)	46.9 (30.5)	54.3 (29.4)	0.098
VAS depression	49.4 (31.4)	46.5 (31.8)	52.5 (30.8)	0.194

Univariate comparisons using chi-square or T-test.
Results are reperesented as mean and standard deviation (continous variables) or as absolute frequency and percentage (qualitative variables).
bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying anti-rheumatic drug; NSAID, Non-steroidal anti-inflammatory drugs; PsA, psoriatic arthritis; SpA, spondyloarthritis; VAS, visual analogue scale (0–100 scale).

factor. The results from this survey will be helpful in the design of a model of telemedicine for patients with chronic rheumatic diseases, in which on-site consultation could be alternated with phone supervision during periods of low disease activity.

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Response to: 'COVID-19 pandemic: an opportunity to assess the utility of telemedicine in patients with rheumatic diseases' by Lopez-Medina *et al*

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We thank Lopez-Medina *et al*[1] for their comment on our paper and for sharing their experience with phone consultations.[2] Telemedicine, in the past years, has been progressively implemented into medical practice. However, it has not been able to fully take root into routine medical care yet. The COVID-19 pandemic provided the opportunity to take a further step towards the integration between virtual and traditional medical assistance in many medical specialties including rheumatology. The restriction rules, taken over by numerous countries, together with the necessity of assuring a proper continuity of care, have forced us to adopt telemedicine tools in our routine involving chronic patients. To overcome legal matters of privacy and data protection, we have recently set up a telemedicine software provided by our institution that allows us to have visual interaction with the patient and to share files in a password-protected virtual room. This approach has revealed a useful help with a major response rate by patients, achievable thanks to the broad internet coverage and connectivity with an increasing percentage of people owning a smartphone nowadays in Italy. As expected, we have observed high response rates among the younger population, with the older ones frequently needing support from other family members (G Zanframundo *et al*, submitted). Furthermore, visual contact may overcome the barriers of a simple phone call. Telemedicine perfectly fits for stable, long-standing conditions, and it can be useful for intermediate follow-up visits. This would markedly reduce the burden on medical resources, better balancing population medical needs and human resources in our health system, highly stressed by COVID-19. A role for a tele-rheumatological triage to better identify those patients needing urgent or specialist evaluation could be another potential benefit. It could greatly refine outpatient clinic access, reducing the workload on third-level referral centres, in turn improving medical care. Furthermore, an increased implementation of digital and cloud-based medical visits and prescriptions might propel specialist–specialist and specialist–general practitioner interactions for the benefit of the patient. Lastly, telemedicine might take advantage of the development of remote medical technologies. There is an increasing interest in the aid that wearable devices may provide to the global care of patients as already described in chronic inflammatory arthritides and virtually applicable in every rheumatological condition.[3] However, despite having represented an enormous help during the COVID-19 pandemic, telemedicine bears major caveats that must be carefully addressed and adapted to our new postpandemic routine. Indeed, certain rheumatological conditions require prompt diagnosis and rapid treatment initiation with a regular and objective follow-up. The treat-to-target approach in early rheumatoid arthritis is one clear example.[4] In conclusion, we believe that this novel approach may be a rich opportunity in rheumatology when properly and timely used, taking into account the intrinsic limits of a telemedicine assessment in different patients at different times.

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Concerns and needs of patients with systemic lupus erythematosus regarding hydroxychloroquine supplies during the COVID-19 pandemic: results from a patient-centred survey

We read the letter by Mathian *et al* with great interest.[1] In their paper, the authors report on the course of COVID-19 in 17 patients with systemic lupus erythematosus (SLE). The data suggest that patients with SLE on hydroxychloroquine (HCQ) are not protected from COVID-19 infection but have a high level of comorbidities, which potentially renders them more susceptible to a severe course. HCQ, an essential drug for patients with SLE,[2] has been advocated for prophylaxis and treatment of COVID-19 by many. Subsequently, drug shortages have ensued, which has led to discussions on scientific reporting[3] and ethics of treatment allocation[4] because withdrawing HCQ in SLE is associated with flares.[5] Rheumatologists are involved in this pandemic as counsellors for physicians unfamiliar with repurposed antirheumatic drugs used in COVID-19 but also face the concerns and needs of their chronically ill patients. These discussions also need to involve patients' views. In SLE, this is particularly important.

Table 1 Self-reported survey respondents' characteristics

	N (%)
Demographic characteristics	
Age	
18–30	45 (12.2%)
31–40	87 (23.6%)
41–50	99 (26.9%)
51–60	90 (24.5%)
60–70	33 (9%)
>70	14 (3.8%)
Gender	
Female	347 (94%)
Male	22 (6%)
Organ manifestations	
Arthritis	225 (61%)
Skin	120 (32.5%)
Lupusnephritis	127 (34.4%)
Pulmonary involvement	51 (13.8%)
Heart	54 (14.6%)
Serositis	55 (14.9%)
NPSLE	57 (15.4%)
Haematological	94 (25.5 %)
Other	*
Concomitant medications	
Prednisone	197 (53.4%)
MTX	40 (10.8%)
AZA	74 (20%)
MMF	47 (12.7%)
Cyclosporine	6 (1.6%)
Tacrolimus	6 (1.6%)
Belimumab	40 (10.8%)
Cyclophosphamide	2 (0.5%)

*Other organ manifestations: antiphospholipid antibody syndrome (APS), n=8 (2.1%); myalgia, n=7 (1.9%); fatigue, n=3 (0.8%)
AZA, azathioprine; MMF, mycophenolate mofetil; MTX, methotrexate; NPSLE, neuropsychiatric lupus erythematosus.

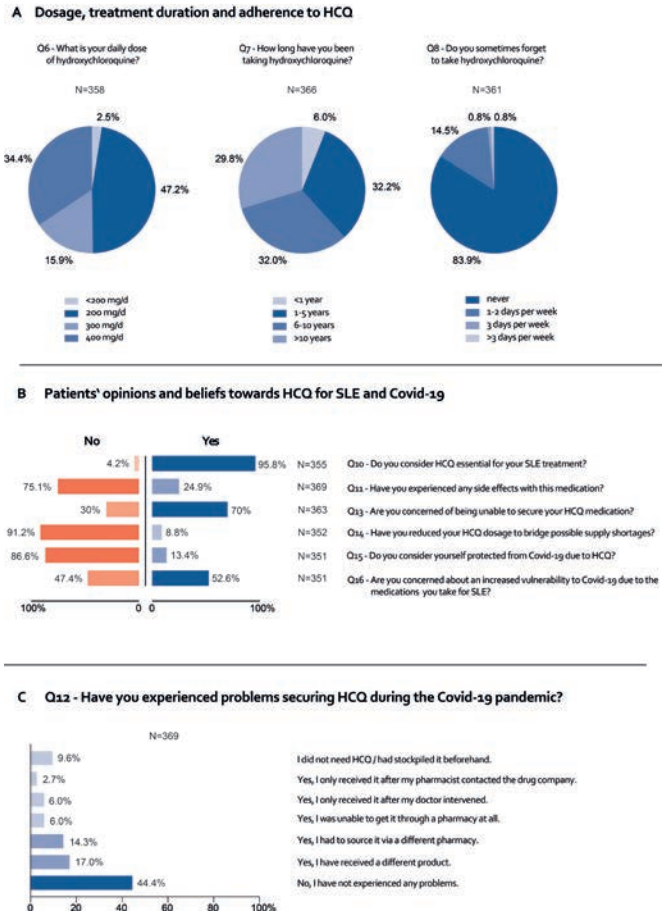

Figure 1 Survey results of 369 respondents with complete results. (A) Self-reported dosage, treatment duration and adherence to hydroxychloroquine (HCQ) are reported. (B) Patients' general opinions and beliefs concerning HCQ in systemic lupus erythematosus (SLE) and COVID-19. (C) Potential supply issues reported by the patients. Q, question number.

To gain insights into supply chains of HCQ, we conducted a survey (online supplementary table S1) to investigate the current situation among patients with SLE in Germany. We received 554 responses; 185 were excluded based on prespecified answers to questions 1 and 5 or incomplete data. The self-reported characteristics of the respondents are shown in table 1. In short, 347 (94%) were women, and 75% of the respondents were between 31 and 60 years of age. SLE manifestations included arthritis (n=225, 61%), nephritis (n=127, 34.3%), skin (n=120, 32.5%) or haematological abnormalities (n=94, 25.5%), among others. Medications included prednisone (n=197, 53.4%), azathioprine (n=74, 20%), mycophenolate mofetil (n=47, 12.7%), methotrexate or belimumab (n=40, 10.8%, respectively).

The survey questions relating to dose, treatment duration and adherence to HCQ (figure 1A) revealed that almost half (47.4%) of respondents reported a daily intake of 200 mg. Treatment duration was 1–5 years, 6–10 years and more than 10 years in about a third each. The vast majority (83.9%) stated they never forget their intake. Furthermore, 95.8% of patients considered HCQ essential for their SLE treatment (figure 1B). 70%expressed concerns about being unable to receive prescriptions; 8.8% reduced their daily dose to overcome potential supply issues. Importantly, 86.6% saw no benefit regarding an impending

COVID-19 infection, while half of the patients expressed concerns of increased vulnerability because of their SLE. One question specifically addressed supply issues (figure 1C): here, about 45% reported different types of supply issues, 44.4% had not experienced any problems at all and almost 10% had stockpiled HCQ beforehand.

Overall, our data represent the first surveyed report in patients with SLE regarding HCQ supplies during the COVID-19 pandemic. Our cohort showed a typical distribution of organ manifestations and treatment profiles, which support their representativeness.

On 3 April 2020, the German Federal Institute for Drugs and Medical Devices issued a statement for the security and reliability of HCQ supplies.[6] It reiterates that any off-label use should only be conducted in clinical trials. In practical terms, prescriptions of HCQ in Germany have to include an in-label diagnosis justifying its use. This potentially ensures continued supplies for patients who depend on the drug and prevents off-label use, including for COVID-19. Nevertheless, supply issues were reported commonly in our survey.

Patients with SLE and caregivers are facing challenges with the improper use of essential drugs, and healthcare policies need to take this into account. As politics differ regionally, it may prove informative to investigate patients' concerns globally and put emphasis on their needs. Ultimately, even during global crises, vulnerable populations need to be protected. The data reported by Mathian *et al* and our presented survey data suggest that patients with SLE are particularly vulnerable.

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Contributors MP collected and analysed data and wrote the manuscript. GC distributed the survey link among the self-help groups, designed the survey questionnaire, analysed data and wrote the manuscript. PK conceived the study, designed the survey questionnaire, analysed data and wrote the manuscript.

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Response to: 'Concerns and needs of patients with systemic lupus erythematosus regarding hydroxychloroquine supplies during the COVID-19 pandemic: results from a patient-centred survey' by Plüß *et al*

We thank Plüß *et al* for their interest in our study reporting on the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19) in a case series of patients with systemic lupus erythematosus (SLE) under long-term treatment with hydroxychloroquine (HCQ).[1 2] Plüß *et al* highlight a major point with respect to the consequences of the SARS-CoV2 outbreak on patients with SLE, in particular the difficulties that the latter have experienced in securing HCQ supplies for a licensed indication as a consequence of the off-label use of this drug to treat COVID-19 in the general population.

Plüß *et al* report that 70% of patients with SLE in Germany expressed concerns regarding the inability to receive their prescriptions for HCQ which was furthermore underscored by the observation that 46% also reported HCQ supply issues, including 17% of patients having received a different product instead of HCQ. A report by Fragoulis *et al* confirmed that HCQ shortage in Greece was a considerable problem for patients with systemic rheumatic diseases, with 54% of patients who discontinued treatment with HCQ being compelled to do so because of drug shortage.[3] This has most likely created a very annoying situation for those patients particularly adherent to their treatment. Indeed, in the survey conducted by Plüß *et al*, almost all the patients expressly stated that HCQ was essential for their treatment, while four out of five confirmed to never forget taking this medication.

Fear of a shortage of HCQ, although fortunately often temporary, has arisen since the start of the pandemics, despite a lack of proven preventive or curative efficacy of HCQ against SARS-Cov-2 infection at that time, except in a few clinical studies marked by numerous methodological flaws,[4 5] as well as attempts of public authorities in many countries, such as France and Germany, to secure the prescription of HCQ for patients suffering from rheumatic diseases.

However, data emphasising ineffectiveness of HCQ for the treatment against SARS-Cov-2 are now plentiful. Long-term treatment with this drug does not seem to prevent COVID-19 in patients with SLE[1 6 7] or rheumatic diseases[8] as reported by us and others. Additionally, several large observational studies have reported that the administration of HCQ to patients hospitalised for COVID-19 was associated with neither a lower, nor an increased, risk of transfer to an intensive care unit, intubation or death.[9–11] Moreover, results from a multicentre, randomised controlled trial showed that administration of HCQ did not lead to a significant higher probability of negative conversion and alleviation of symptoms than standard of care in patients with mild to moderate COVID-19.[12] Finally, in a randomised, double-blind, placebo-controlled trial across the USA and parts of Canada it was confirmed that HCQ did not prevent COVID-19 manifestations when used as postexposure prophylaxis within 4 days after viral exposure.[13] Together, the majority of studies at present demonstrate that there is no longer any reason to use HCQ in the battle against COVID-19 except in clinical trials. As a consequence, we can only hope that off-label use of HCQ for the treatment of COVID-19 will dramatically decrease and that patients with SLE will again have unrestricted access to HCQ for the treatment of their disease.

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Clinical course of COVID-19 in patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine

As obstetricians with a maternal–foetal medicine practice taking care of pregnant women treated with hydroxychloroquine (HCQ) in the prevention of systemic lupus erythematosus (SLE) flare, we read with interest the recent report of clinical data collected through the COVID-19 Global Rheumatology Alliance registry.[1] Indeed, in the current epidemic, a number of cases of severe acute respiratory syndrome have occurred worldwide in pregnant women and have jeopardised both mother and fetus and have sometimes led to extreme prematurity, confirming reports from previous coronavirus outbreaks.[2] Hence, any safe treatment with a potential for prevention of severe forms of COVID-19 would be of great interest. As HCQ used in the prevention of SLE flare has a good safety profile during pregnancy, and its continuation is even recommended in pregnant patients with SLE by the American College of Obstetricians and Gynaecologists,[3] this drug could be a good candidate.

From the global physician-reported registry, Konig *et al*[1] identified 80 patients with SLE and COVID-19 and concluded that patients with SLE on baseline therapy with HCQ are not universally protected from COVID-19. The authors report that patients were predominantly female and less than 65 years of age, with a similar proportion of severe forms whether or not they were treated 'with an antimalarial prior to onset of COVID-19'. However, no information was given about comorbidities that could explain progression of the disease in each group.

Indeed, the authors refer to another recent report by Mathian *et al*,[4] who also tackle this issue of the possible protective effect of HCQ against COVID-19 in such context. However, in this series of 17 patients, Mathian *et al*[4] report an important proportion of major comorbidities as compared with recent large-scale studies assessing the prognosis in SLE populations of patients[5]: obesity and chronic kidney disease are present in 10 (59%) and 8 patients (48%), hypertension in 6 (35%), venous thrombosis in 4 (24%), arterial thrombosis in 3 (18%), cerebrovascular disease in 3 (18%), coronary heart disease or cardiovascular disease in 2 (12%), chronic obstructive lung disease in 2 (12%) and malignant tumour in 1 (6%).

In addition, in this short series,[4] two patients (12%) were administered prednisone greater than or equal to 10 mg/day, and 7 (41%) had an immunosuppressant treatment, which raises other questions regarding the effect of these drugs on the course of COVID-19; also, the proportion of patients with anticoagulant treatment was only 29%, whereas a history of thrombosis existed in 35% of cases, and the involvement of thromboses in the progression of COVID-19 is now well documented.[6]

As all these factors and comorbidities may have an important impact on the progression of COVID-19 and must be taken into account, it is difficult for us to really consider from such data that HCQ has no protective effect.

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Response to: 'Clinical course of COVID-19 in patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine' by Carbillon *et al*

We thank Carbillon *et al* for their correspondence.[1] The use of hydroxychloroquine (HCQ) in pregnant women with systemic lupus erythematosus (SLE) is not controversial.[2 3] Similar to its primary role in the prophylaxis and treatment of SLE, discontinuation of HCQ in pregnancy has been linked to increased disease activity and glucocorticoid use in women with lupus.[4–6] Given its benefit and preferable safety profile, the continuation of baseline HCQ therapy in pregnant women with lupus is recommended to maintain disease remission,[2 3] regardless of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) status. In contrast, there is currently no evidence to suggest that baseline use of HCQ in pregnant women with lupus is protective of SARS-CoV-2 infection or severe COVID-19.

The authors adequately summarise our findings that patients with lupus—even if they are using an antimalarial such as HCQ as baseline therapy—can develop SARS-CoV-2 infection and severe COVID-19 at similar frequency as patients not on antimalarials.[7] We agree that unequal distribution of comorbidities and disease-modifying antirheumatic drug therapy have to be considered as sources of confounding, and statistical correction for such variables may be informative as sample size increases. In a recent publication by the COVID-19 Global Rheumatology Alliance examining 600 patients with rheumatic disease with COVID-19, 22% were taking antimalarials prior to hospitalisation.[8] No significant association between baseline antimalarial use and hospitalisation was observed after adjusting for sex, age over 65 years, smoking status, underlying rheumatic disease, comorbidities, conventional synthetic disease-modifying antirheumatic drug (csDMARD) monotherapy, biological disease-modifying antirheumatic drug (bDMARD)/targeted synthetic disease-modifying antirheumatic drug (tsDMARD) monotherapy, csDMARD–bDMARD/tsDMARD combination therapy (excluding antimalarials), use of non-steroidal anti-inflammatory drugs and glucocorticoid dose (OR=0.94, 95% CI 0.57 to 1.57; p=0.82).[8] The null effect remained in additional models controlling for disease activity. In light of these findings, but also acknowledging the innate limitations of observational and physician-reported data, patients with lupus on HCQ do not appear to be protected from severe COVID-19. We await the results of ongoing randomised controlled trials to clarify whether HCQ has any role in the prophylaxis or treatment of COVID-19.

Adding to these clinical data, we provide a pharmacokinetic rationale why antiviral properties of HCQ at doses commonly prescribed in lupus (400 mg daily or less) are not expected to be protective of SARS-CoV-2 infection.[7] Importantly, this does not preclude potential benefits of HCQ for the hypercoagulable state observed in some patients with COVID-19. While HCQ has been shown to be protective against arterial and venous thrombosis in SLE,[9 10] extrapolating these benefits to the coagulopathy of COVID-19 is premature. Ongoing controlled trials of HCQ in patients without lupus will likely be informative to explore potential antithrombotic benefits for COVID-19 coagulopathy.

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Monitoring of patients with systemic lupus erythematosus during the COVID-19 outbreak

The emergence and spread of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the resulting COVID-19 disease, have had a tremendous impact on public health and the world economy. Thus far, COVID-19 does not appear to be more severe in immunocompromised patients, but relevant data are scarce.[1] Conversely to what was initially thought, patients with systemic lupus erythematosus (SLE) are also involved.[2 3] In an article by Mathian *et al*,[4] some severe forms of COVID-19 infection were described in 17 patients with SLE, particularly in those with renal failure or obesity. This greater susceptibility might arise from dysregulation of ACE2 and interferon expression.[5] At the same time, hydroxychloroquine (HCQ), the reference standard-of-care treatment for SLE, has emerged as a potential treatment option for COVID-19. HCQ has shown the potential to inhibit the viral replication in vitro, and several clinical trials are under way to evaluate its clinical efficacy.[6] Building on the report of Mathian *et al*,[4] we would like to share our experience from another French university centre to further the discussion of SLE patient outcomes during the COVID-19 outbreak. Here we aim to address COVID-19 disease progression in patients with SLE and the potential beneficial effects of HCQ treatment by monitoring patients during the first weeks of the COVID-19 outbreak.

Our observational cohort study included all adult patients with a confirmed SLE diagnosis, according to the 2019 European League Against Rheumatism/American College of Rheumatology criteria, followed by the clinical immunology and nephrology units at the Montpellier University Hospital, who consulted during the past year. We conducted interviews with each patient to investigate symptoms suggestive of infection or to identify confirmed cases of COVID-19. The period concerned is from 1 February (shortly after the first COVID-19 cases in France) to 24 April (end of data collection). Official containment measures were implemented on 17 March in France.

The study included a total of 120 patients. The main characteristics of our cohort are summarised in table 1. Seven patients were not included because they either did not answer the phone calls (n=6) or refused to participate (n=1). Only three patients reported contact with confirmed patients with COVID-19, and all three remained asymptomatic. Thirty-six (30.0%) patients reported symptoms of infection. However, no cases were definitively confirmed. One patient was hospitalised and eventually died from an inhalation pneumopathy, but SARS-CoV-2 infection was excluded by two negative nasal swabs and incompatible chest CT findings. Eight (6.7%) patients reported symptoms highly suggestive of COVID-19. None of these patients was hospitalised, and no severe outcomes were reported. Only two of these patients had comorbidities. The percentage of patients reporting symptoms of COVID-19 infection did not differ between those exposed to HCQ (6.9%) and those who were not (6.3%).

While no preventive role of HCQ was observed in the study, the statistical power was insufficient. The strength of the present study is that the data are likely highly representative since ≥90% of patients with SLE are followed at least once a year by our departments and nearly 95% participated in the study. Contrary to suggestions that COVID-19 disease progression may be exacerbated in patients with SLE because of the higher comorbidity prevalence,[4] we found no severe forms of COVID-19 infection among the patients with SLE we followed. The younger age and fewer associated comorbidities in our cohort could explain this difference.

Our study has several limitations, mainly its small sample size and the absence of COVID-19 confirmation by reverse

Table 1 Clinical characteristics and outcomes of patients with SLE during the COVID-19 outbreak according to hydroxychloroquine treatment status

	Hydroxychloroquine		
	Exposed (n=72)	Not exposed (n=48)	P value
General characteristics			
Female sex, n (%)	66 (91.7)	44 (91.7)	1.0
Age, years, mean (SD)	42.4 (12.2)	51.8 (12.8)	<0.01
Lupus characteristics			
Disease duration, years, mean (SD)	15.3 (10.5)	21.7 (13.9)	0.014
Current or history of:			
Cutaneous involvement, n (%)	57 (79.2)	34 (70.8)	0.30
Articular involvement, n (%)	69 (95.8)	42 (87.5)	0.15
Nephritis, n (%)	30 (41.7)	25 (52.1)	0.26
Serositis, n (%)	21 (29.2)	8 (16.7)	0.12
sAPL, n (%)	11 (15.3)	7 (14.6)	0.92
Current treatments			
Steroids, n (%)	32 (44.4)	18 (37.5)	0.45
Prednisone dose, mg/day, mean (SD)	9.7 (7.3)	9.8 (13.2)	0.52
Mycophenolic acid, n (%)	15 (20.8)	19 (39.6)	0.026
Sirolimus/tacrolimus, n (%)	4 (5.6)	14 (29.2)	<0.01
Methotrexate, n (%)	7 (9.7)	4 (8.3)	1.0
Anticoagulants, n (%)	7 (9.7)	8 (16.7)	0.26
ACE inhibitors/ARBs, n (%)	16 (22.2)	22 (45.8)	<0.01
Comorbidities			
Tobacco use, n (%)	38 (52.8)	29 (60.4)	0.41
Daily smokers, n (%)	24 (33.3)	10 (20.8)	0.14
Past smokers, n (%)	14 (19.4)	19 (39.6)	0.016
Hypertension, n (%)	8 (11.1)	22 (45.8)	<0.01
Diabetes, n (%)	3 (4.2)	7 (14.6)	0.087
Obesity (BMI >30 kg/m^2), n (%)	2 (2.8)	4 (8.3)	0.22
Renal failure, n (%)	4 (5.6)	16 (33.3)	<0.01
Cardiovascular diseases, n (%)	4 (5.6)	10 (20.8)	0.011
Pulmonary diseases, n (%)	4 (5.6)	6 (12.5)	0.20
Malignancy, n (%)	2 (2.8)	2 (4.2)	1.0
Kidney transplants, n (%)	3 (4.2)	14 (29.2)	<0.01
Survey reporting			
Contact with a known COVID-19 patient, n (%)	3 (4.2)	0 (0.0)	0.27
Symptoms of infection, n (%)	20 (27.8)	16 (33.3)	0.52
Negative reverse transcription PCR analysis, (n)	3	2	1.0
Highly suggestive of COVID-19 (n)	5	3	1.0
Previous contact with symptomatic patients (n)	4	3	1.0
Secondary symptoms in contacts (n)	2	2	1.0
Fever (n)	5	2	0.70
Dry cough (n)	3	3	0.68
Headache (n)	4	2	1.0
Anosmia/dysgeusia (n)	1	2	0.56
Diarrhoea (n)	1	2	0.56
Symptom duration, days, mean (SD)	6.4 (2.5)	10 (4)	0.16
Symptomatic treatment (n)	5	2	0.70

ARBs, angiotensin II receptor blockers; BMI, body mass index; sAPL, secondary antiphospholipid syndrome; SLE, systemic lupus erythematosus.

transcription PCR or serological tests. The criteria to define probable COVID-19 cases may also be questionable, although some of the symptoms to predict infection were derived from a recent review.[7] Unfortunately, in France, no study has directly estimated the prevalence of confirmed COVID-19 cases on the basis of clinical symptoms. The only estimation, recently issued by the Pasteur Institute, is indirect.[8] Their report of a 4.4% (2.8%–7.2%) prevalence in low-risk regions is similar to our observation. Reported infection rates underestimate the actual prevalence, as they do not take into account asymptomatic and untested patients. A report by the National Health Agency on 29 April found 6389 confirmed cases out of 73 608 tested (8.7%) in the Occitanie region, yielding a global prevalence of 0.11% in the area.[9] Among those testing positive, 3260 (51.0%) required hospitalisation and 360 (5.6%) died. Finally, none of the 303 patients admitted to our hospital for severe COVID-19 during the study period suffered from SLE.

The availability of a larger cohort of patients and combining this type of follow-up with serological determination of SARS-CoV-2 infection will be valuable to better understand the impact of COVID-19 in patients with SLE.

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Response to: 'Monitoring of patients with systemic lupus erythematosus during the COVID-19 outbreak' by Holubar *et al*

We thank Holubar *et al* for their interest in our study reporting on the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19) in a case series of patients with systemic lupus erythematosus (SLE) under long-term treatment with hydroxychloroquine.[1 2] Their results showing a low incidence of symptoms of viral infections in a population of patients with SLE corroborates those from Favalli *et al*,[3] suggesting that the impact of COVID-19 in patients with SLE is rather low. However, as acknowledged by Holubar *et al*, the prevalence of COVID-19 in the general population is very low as well, and even lower in regions, such as Occitanie in Southern France where the study of Holubar *et al* was carried out between 1 February and 24 April.[1 2] By 11 May, Salje *et al* estimated that only 4.4% (range 2.8–7.2) of the general French population had been infected and that this proportion was likely to be even lower, that is, 1.9% (range 1.2–3.3) in Occitanie, as compared with 9.9% (range 6.6–15.7) in Ile-de France, including Paris, and 9.1% (range 6–14.6) in Grand Est, the two most affected regions of the country.[4] A recent study also shows that even in one of the epicentres of the outbreak, the prevalence of anti-SARS-CoV-2 antibodies was very heterogeneous.[5] In the latter study, the infection attack rate (IAR) based on specific antibody detection ranged from 25.9% (95% CI 22.6 to 29.4) in a sample of 661 participants, including pupils, their parents and siblings as well teachers and non-teaching staff involved in a cluster of COVID-19 that took place in a high school in the Oise department to 3.0% (95% CI 1.1 to 6.4) in samples from two nearby blood donors centres.[5] Therefore, given the low IAR of SARS-CoV-2 in general, it is currently impossible to draw any meaningful conclusions on the incidence and severity of COVID-19 in patients with SLE.

It is also seems important to mention that the reporting of symptoms suggestive of an infection cannot by any means replace the use of reliable markers of infection, particularly in regions with a low IAR. Whereas anosmia and ageusia may have a high positive predictive value for SARS-CoV-2 infection in epicentres of the outbreaks, this is certainly not the case outside these regions. This consideration is also applicable to all other symptoms suggestive of infection such as dry cough, fever, diarrhoea, and so on, and only the systematic use of reliable tests such as viral detection by real-time reverse transcription-PCR analysis and/or the detection of anti-SARS-CoV-2 antibodies should be used to confirm COVID-19 positive cases. In addition, a chest CT scan suggestive of SARS-CoV-2 pneumonia will also allow, in the context of a COVID-19 outbreak, to confirm the diagnosis of SARS-CoV-2 infection.

We agree with Holubar *et al* that only studies of a large cohort of patients with SLE, infected with SARS-CoV-19, will permit to better understand the impact of COVID-19 on this population, although they will undoubtedly be difficult to conduct. Meanwhile, the relatively low IAR observed during the pandemic in France indicates that establishing protective herd immunity will be a lengthy process.[4 5] Therefore, in the absence of a reliable preventive and curative treatment, it is likely that patients with SLE will have to experience continued uncertainty as to whether or not they are at risk to develop a severe form of COVID-19.

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Case series of acute arthritis during COVID-19 admission

The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has changed life significantly worldwide. Rheumatologists also had to get used to the new context, prioritising remote over in-person consultations or solving doubts and giving advice to our immunocompromised patients. Besides, international collaborations have provided opinions concerning decisions that may affect people with rheumatic diseases; for instance, a major provision and use of hydroxychloroquine for COVID-19 might lead to a shortage for patients with lupus or rheumatoid arthritis, as Graef and colleagues point out in their recent viewpoint.[1]

In areas of higher impact of the outbreak, as occurred in Spain, some of us have collaborated with colleagues from respiratory medicine and infectious diseases within multidisciplinary teams to face COVID-19. A collateral advantage has been the opportunity to evaluate the musculoskeletal manifestations of COVID-19, to date just described as non-specific joint pain,[2] while no cases of arthritis have been reported at diagnosis or during the infection.

Up to 30 April 2020, in Alicante General University Hospital, 306 patients with proven COVID-19 have been admitted. Eighty-one (26.4%) complained of muscle and joint pain at presentation. No patient had evident arthritis at admission, but four (1.3%) developed acute arthritis during hospitalisation. Here we present their relevant features (table 1).

All patients had a history of recurrent acute arthritis in different locations (knee, first metatarsophalangeal joint or ankle). Three had a previous diagnosis of gout, but presence of crystals was only studied in one (patient 4). Treatment with allopurinol was variable, and none received daily colchicine.

COVID-19 was diagnosed in three of them by reverse transcriptase PCR (RT-PCR) for SARS-CoV-2 in nasopharyngeal aspirates. Patient 4 was repeatedly tested negative in RT-PCR but confirmed by SARS-CoV-2 IgM and IgG detection.[3] All patients received hydroxychloroquine; tocilizumab (in three cases) and pulses of methylprednisolone (in two cases) were necessary because of severe pneumonia. Interestingly, all episodes of acute arthritis occurred despite those treatments. The synovial fluid analysis allowed definitive diagnoses, and flares successfully resolved with our standard approach (corticosteroids and colchicine).

Joint and muscle pain are common in acute viral illnesses. It also seems to occur in COVID-19,[2] but the occurrence of arthritis has not been confirmed to date. Here we report four cases of acute arthritis developed during COVID-19 admissions, all due to crystal-proven flares (gout and calcium pyrophosphate disease). It remains essential to check every arthritis by polarised microscopy, even during the SARS-CoV-2 pandemic. Viral detection in synovial fluid had not been tested to date, though reported low rates of viraemia make it unlikely.[4 5] We managed to have synovial fluids tested for SARS-CoV-2 by RT-PCR in the three cases, being all negative.

Rheumatologists, at multiple levels and from different perspectives,[1] may be of great value during the COVID-19 pandemic.

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Table 1 Clinical features and management of four patients with acute arthritis during COVID-19 admission

Patient	1	2	3	4
Age (years)/gender	71/male	61/male	64/male	45/male
Days from COVID-19 symptom onset to arthritis	8	19	8	27
Days from admission to arthritis	3	17	7	21
COVID-19 management	Hydroxychloroquine	Hydroxychloroquine, azithromycin, tocilizumab, pulses of methylprednisolone	Hydroxychloroquine, azithromycin, lopinavir–ritonavir, tocilizumab	Hydroxychloroquine, tocilizumab, pulses of methylprednisolone
Known inflammatory arthritis	Gout, on allopurinol 100 mg/day	Gout, on allopurinol 100 mg/day (irregular)	Previous recurrent arthritis, not studied or treated	Crystal-proven gout, on allopurinol 300 mg/day
Allopurinol stopped during admission	No	No	–	Yes
Involved joints	First MTP	Ankle	Both knees	Knee and ankle
SF characteristics	ND	Glucose: 38 mg/dL Leucocytes: 137 534/µL (95% PMN)	Glucose: 94 mg/dL Leucocytes: 1362/µL (77% PMN)	Glucose: 38 mg/dL Leucocytes: 39 065/µL (96% PMN)
Polarised light microscopy	MSU crystals	MSU crystals	CPP crystals	MSU crystals
SF culture	ND	Negative	Negative	Negative
SF RT-PCR for SARS-CoV-2	ND	Negative	Negative	Negative
Acute arthritis management	► Intra-articular triamcinolone plus mepivacaine. ► Colchicine.	► Oral prednisone. ► Colchicine.	► Intra-articular triamcinolone plus mepivacaine.	► Colchicine.

CPP, calcium pyrophosphate; MSU, monosodium urate; MTP, metatarsophalangeal; ND, not done (insufficient amount of fluid was obtained); PMN, polymorphonuclear; RT-PCR, reverse transcriptase PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SF, synovial fluid.

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Response to: 'Case series of acute arthritis in COVID-19 admission' by López-González *et al*

We read the comment on our article by López-González *et al* with great interest.[1 2] The authors detail the presentation of four cases of acute arthritis in patients hospitalised with COVID-19 and underlying gout (three cases) or recurrent arthritis of unknown origin (one case). Although we await further reports, there have been anecdotes of newly diagnosed inflammatory arthritis in the context of COVID-19 infection, perhaps representing either a viral-associated arthritis or a reactive arthritis.[3 4] However, as the authors note, common causes of acute inflammatory arthritis must continue to be considered in the differential diagnosis—these include crystal-associated arthritis, such as gout or pseudogout. Acute illnesses, including infection, are well-established risk factors for gout and pseudogout flares; inpatient gout flares are known to complicate admissions for heart failure, pneumonia and acute kidney injury.[5] These comorbidities are either associated with or features of severe COVID-19 infection and so may explain the presentations of acute inflammatory arthritis detailed in their report.[6]

The thorough workup completed by the authors highlights some of the current gaps in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing methods. While reverse transcription (RT)-PCR testing was negative in all three of the synovial fluid samples in these patients with documented COVID-19 infection from nasopharyngeal swab, no molecular testing method has been validated yet to detect SARS-CoV-2 in synovial fluid. Thus, the clinical significance of the synovial fluid cultures and RT-PCR results is currently unknown. Despite these uncertainties, we commend the authors' efforts in providing the first report of SARS-CoV-2 nucleic acid testing in synovial fluid.

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Treatment adherence of patients with systemic rheumatic diseases in COVID-19 pandemic

We read with great interest the preliminary German Society of Rheumatology recommendations for the management of patients with autoimmune inflammatory rheumatic diseases (AIRD) during the COVID-19 pandemic.[1] As other regulatory bodies suggest[2 3] patients should not discontinue their anti-rheumatic treatment because of fear.[1]

Herein, we investigated to which extent patients with AIRD altered their treatment during COVID-19 pandemic and whether there are any factors that affected their decision. We telephone-interviewed (14 April 2020–22 April 2020), 500 consecutive AIRD-patients followed-up in our centre and recorded the following parameters: age, sex, cohabitation, region of residence (urban, semiurban, rural), level of education (first, second, third), employment status, disease duration, current treatment and presence of co-morbidities (hypertension, hyperlipidemia, coronary heart disease, diabetes mellitus, chronic obstructive pulmonary disease (COPD), depression, anxiety).

Specific questions referred to the COVID-19 pandemic period in Greece, starting on 26 February 2020, with predefined answer-options, were asked: discontinuation of any medication received for AIRDs, possible reasons that led to drug discontinuation (including fear of immunosuppresion and lack of resources/drug shortage), whether advise was received from a clinician or other sources, symptomatology compatible with COVID-19 infection, subjective assessment (on a five-point Likert scale) of disease activity and a questionnaire to detect nocebo behaviour (cut-off score: 15).[4] Univariate and binary logistic regression analyses were performed using 'discontinuation of medication due to fear of infections', 'discontinuation of medication due to lack of resources/drug shortage' and 'consultation by a clinician' as dependent variables, in three different models.

We interviewed 500 patients (73.2% female, mean (±SD) age: 53.7±15.3 years, disease duration: 10.0±9.4 years) with various AIRDs: inflammatory arthritis: 52.4%, connective tissue diseases: 33% (systemic lupus erythematosus: 16%, systemic sclerosis: 11%, anti-phospholipid syndrome: 3.6%, Sjogren's syndrome: 1.2%, polymyalgia rheumatica: 1.2%), vasculitis: 9.4%, auto-inflammatory diseases: 5.2%. Of them, 65.8% were cohabiting with another person, 83.6% were living in urban area, 47.6% and 38.2% had a second and third level of education, respectively and 6% were unemployed. Half (46.6%) of our patients were on steroids, 73.4% were on conventional disease modifying anti-rheumatic drugs (cDMARDs), 6.8% on targeted DMARDs and 43.8% on biologics (bDMARDs).

Collectively, 11/500 (2.2%) discontinued AIRD treatment due to fear of immunosuppression; all but two were on bDMARDs. Nineteen (3.8%) patients stopped their treatment because of lack of resources/shortage of drug; 7/19 (36.7%) were on treatment with hydroxychloroquine. Noteworthy, 53.8% (7/13) of patients who discontinued treatment with hydroxychloroquine did so because of drug shortage. Additionally, 13/500 (2.6%) and 30/500 (6.0%) discontinued their medication due to a respiratory infection or for other reasons (eg, side-effects), respectively. In total, 124 (24.8%) patients received advice about possible modification of their treatment. All but three, were guided by a clinician.

Therapy withdrawal due to fear of immunosuppression was associated with underlying COPD in univariate analysis (p=0.022), and with unemployment (OR, 95% CI: 9.19, 1.30 to 64.7, p=0.03) and COPD (OR, 95% CI: 27.53, 3.17 to 239.1, p=0.003) in regression analysis. Treatment discontinuation due to lack of resources/drug shortage was not associated with any parameter tested. Decision about consulting a clinician was associated with unemployment status, COPD and male gender (p=0.001, p=0.03 and p=0.03, respectively) in univariate analysis. Regression analysis confirmed these findings: unemployment (OR, 95% CI: 3.55, 1.58 to 7.93, p=0.002); COPD (OR, 95% CI: 3.93, 1.11 to 13.95, p=0.03); male gender (OR, 95% CI: 1.82, 1.13 to 2.93, p=0.01).

COVID-19 infection symptomatology was reported in 39 patients, two of whom were tested and found negative. For most patients (66%) the disease remained stable during the pandemic. Ninety-three (18.6%) patients reported improvement and 77 (15.4%) deterioration from their last visit. Treatment discontinuation due to fear of infections or lack of resources/shortage of drugs was not associated with a disease exacerbation (p=0.472). Nocebo behaviour was detected in 10.2% of the patients.

In conclusion, discontinuation rate due to fear of immunosuppression in our cohort was low, mostly observed in patients on bDMARDs. Hydroxychloroquine shortage was a considerable problem for our patients. Special consideration should be given to patients with certain social or clinical characteristics, such as unemployment status and COPD.

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Response to: 'Treatment adherence of patients with sytemic rheumatic diseases in COVID-19 pandemic' by Fragoulis *et al*

We read with interest the study of Fragoulis *et al*[1] about treatment adherence and behaviour changes of patients with autoimmune inflammatory rheumatic diseases (AIRD) in the context of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/COVID-19 pandemic. In their study, only 11 out of the 500 patients with AIRD interviewed had discontinued antirheumatic treatment solely due to fear of immunosuppression, for example, for fear of an increased risk for SARS-CoV-2 infection. This is reassuring to note as interruption of clinically efficacious therapy in AIRD is associated with an increased risk of relapse[2] which might lead to the necessity of intensifying immunosuppressive therapy, possibly beyond the original level. For this very apprehension and also for the accumulating cautious impression that patients with rheumatological diseases might not have a worse prognosis during COVID-19,[3 4] we highlighted the importance of the recommendation to not generally interrupt or reduce immunosuppression in the current COVID-19 pandemic by placing it as our first statement in the preliminary recommendations of the German Society of Rheumatology for the management of patients with inflammatory rheumatic diseases during the SARS-CoV-2/COVID-19 pandemic.[5]

Fragoulis *et al* report that discontinuation of medication was not associated with an exacerbation of the underlying rheumatic disease.[1] However, this view has to be taken with some caution as the interviews were conducted about 2 months after the COVID-19 pandemic had started in Greece on 26 February 2020 and it is very well conceivable that not all patients who had discontinued their medication at some time after the start of the pandemic already had experienced reactivation of their AIRD when interviewed. While reactivation of inflammatory rheumatic diseases may occur within 2 weeks when treatment with JAK inhibitors is interrupted,[6] it may take several weeks to months for the different biologicals.[2] In light of the fact that the pandemic is ongoing and vaccination will not be available for a considerable time, patients who interrupt their antirheumatic treatment will be at risk for reactivation of their AIRD while still unprotected against SARS-CoV-2. If the patients from Fragoulis' cohort would continue to be off antirheumatic therapy, it would be worth to follow them longitudinally. In order to gain further insight into current treatment of patients with AIRD, it would also be informative to know how many of the patients identified in the study had been advised to do so by a physician or even by their rheumatologist.

Despite the general recommendation to not stop antirheumatic medication in patients with AIRDs, several reasons and situations do exist where interruption of immunosuppressive therapies is advisable in the context of COVID-19. These situations and the preferred actions are illustrated in the current guidelines of national and international societies.[5 7 8] As specific evidence for SARS-CoV-2 is currently still low, most of the guidance in these recommendations is based on analogies to other viral infections and common thoughts on providing care and caution. The association of particular social characteristics with discontinuation of medication for non-clinical reasons as found by Fragoulis is worrisome in this regard. Rheumatology societies should be encouraged to increase their efforts to educate physicians and patients with a particular focus on the risk of unjustified discontinuation of therapy solely because of fear for infection with SARS-CoV-2.

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